ENRICHMENT OF AU-RICH ELEMENT CONTAINING mRNAs DURING INTESTINAL CELL EPITHELIAL-MESENCHYMAL TRANSITION: ROLES, MECHANISMS, AND SIGNIFICANCE

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To Luke, my ever supportive and inspiring husband

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LIST OF ABBREVIATIONS

AR = amphiregulin
ARE = adenylate/uridylate rich element
AU-rich = adenylate/uridylate rich
BMP = bone morphogenic protein
COX-2 = cyclooxygenase 2
CRD-BP = coding region determinant binding protein
CRC = colorectal cancer
CUGBP2 = CUG triplet repeat, RNA binding protein 2
EGF = epidermal growth factor
EGFR = epidermal growth factor receptor
ELAV = embryonic-lethal, abnormal vision, Drosophila
ELISA = enzyme linked immunosorbant assay
EMT = epithelial-mesenchymal transition
ERK = extracellular-regulated kinase
HB-EGF = heparin-binding epidermal growth factor
hnRNP A/B = heterogenous nuclear ribonuclear protein A/B
hnRNP L = heterogenous nuclear ribonuclear protein L
HuR = Hu antigen R
Id2 = inhibitor of DNA binding 2
IPA = Ingenuity Pathway Analysis
IPTG = isopropyl-1-thio-β-D-galactopyranoside
JNK = c-jun N-terminal kinase
KEGG = Kyoto Encyclopedia of Genes and Genomes
6-keto-PGF1α = 6-keto-prostaglandin F1α
MAPK = mitogen activated protein kinase
MEK = MAPK/ERK kinase
MET = mesenchymal to epithelial transition
mRNA = messenger ribonucleic acid
NSAID = non-steroidal anti-inflammatory drug
PAI-1 = plasminogen activator inhibitor 1
PGI_2 = prostaglandin I2
PI3K = phosphatidylinositol-3 kinase
PLCγ = phospholipase Cγ
qRT-PCR = quantitative reverse transcription polymerase chain reaction
RIE = rat intestinal epithelial
RNA = ribonucleic acid
RNABP = RNA binding protein
RT-PCR = reverse transcription polymerase chain reaction
SDS-PAGE = sodium dodecyl sulfate polyacrylamide gel electrophoresis
TβRI = transforming growth factor β type I receptor
TβRII = transforming growth factor β type II receptor
TGF-α = transforming growth factor α

TGF-β = transforming growth factor β
TNFα = tumor necrosis factor α
VEGF = vascular endothelial growth factor
UTR = untranslated region
YAMC = young adult mouse colonocyte

CHAPTER I

INTRODUCTION

There were nearly 148,000 new cases of colorectal cancer in the U.S. in 2006 and over 55,000 deaths due to this disease. The five-year survival rate for localized colorectal cancer (stage 1) is around 90%, but more than 60% of cases present with stage III or IV disease. For patients with tumors that have spread to regional lymph nodes, the five-year survival rate drops to around 68%; the 5-year survival rate plummets to a dismal 10% for those patients with distant metastatic disease (American Cancer Society, *Cancer Facts and Figures,* 2006). The genetic alterations occurring in the conversion of normal intestinal epithelium to malignant carcinoma are well characterized (Vogelstein et al., 1988). Tumor cell interaction with its microenvironment and mutations in oncogenes or tumor suppressor genes play important roles in regulating cancer cell growth and behavior. The interactions of these events and conditions, especially their cooperation, are of particular importance during tumor development and progression. Several cellular processes are altered when a normal cell converts to a cancer cell. Among these are increased growth and proliferation, which can be affected by activating mutations in oncogenes and disruption of growth inhibitory pathways. These events lead to the increased expression of tumor promoting factors, such as cyclooxygenase 2 (COX-2) and vascular endothelial growth factor (VEGF), which aid the development and progression of tumors. A better understanding of the molecular mechanisms regulating gene expression in carcinogenesis will aid us in developing successful therapeutic strategies.

Transformation and EMT

In order for cancer cells to metastasize, they must invade adjacent tissues, gain access to vascular or lymphatic channels, survive transit, extravasate, and finally colonize a distant organ or tissue. Cancer cells acquire the capacity to invade and metastasize through activation of oncogenes and the loss of tumor suppressors; however the underlying molecular mechanisms involved in cellular invasiveness and metastasis are incompletely understood. The majority of cancers are carcinomas, which arise from epithelial cells. Normal epithelial cells are well organized, immobile, polarized cells with complex cell-cell and cell-matrix junctions. Enhanced growth, disruption of cell adhesion, and induction of neovascularization are crucial steps toward metastasis.

Epithelial to mesenchymal transition (EMT) is a process whereby epithelial cells lose polarity, acquire a mesenchymal phenotype, and exhibit invasive behaviors. Although EMT is a normal, tightly controlled and reversible event during embryonic development and in response to injury, it can contribute to the progression of carcinomas. EMT is characterized by a loss of cell-cell junctions, in part through loss of the adherens junction protein E-cadherin, disruption of cell-matrix interactions mediated by altered integrin expression and increased expression of matrix dissolving proteolytic enzymes, and rearrangement of the cytoskeleton. These events result in the acquisition of a fibroblastoid phenotype, accompanied by an increase in mesenchymal markers such as vimentin and α-smooth muscle actin, and increased cell motility and invasiveness (Zavadil and Bottinger, 2005).

Transforming growth factor-β

TGF-β signaling and growth inhibition

A balance of autocrine and paracrine growth promoting signals and growth inhibitory signals tightly regulate cell growth and epithelial architecture. Transforming growth factor β (TGF-β) is normally a potent inhibitor of epithelial cell growth (Filmus et al., 1992; Kurokowa et al., 1987) and functions through activation of the Smad signaling pathway, initiating transcription of target genes and potently inhibiting cell growth (Shi and Massague, 2003). The three human isoforms of TGF-β, TGF-β_1, TGF-β_2 and TGF-β_3, are highly conserved and bind the same heteromeric receptor complex composed of the type I and type II receptors (TβRI and TβRII) both of which have serine/threonine kinase activity (Kurokowa et al., 1987). The TβRII binds TGF-β ligand, associates with and phosphorylates the TβRI, which then phosphorylates and activates downstream effectors known as Smad proteins, the mammalian homologues of the *C. elegans* Sma and *Drosophila* Mad proteins (Figure 1). Phosphorylated Smad2 and Smad3 in turn bind Smad4, and the complex translocates to the nucleus, where it interacts with several different transcription factors to initiate transcription of target genes (Massague, 1998). TGF-β can activate other signaling pathways independently of Smad signaling, including the mitogen-activated protein kinases extracellular-regulated kinase (Erkinheimo et al.) and c-jun N-terminal kinase (JNK), phosphatidylinositol-3 kinase (PI3K), and Rho GTPases (Massague, 1998).

Growth inhibition appears central to TGF-β's role as a tumor suppressor. Reduced

expression of TGF-β or TβRI increases the malignancy of colon carcinoma cells (Wang et al., 1996; Wu et al., 1993). TβRII is mutated and inactivated in colon cancers with or

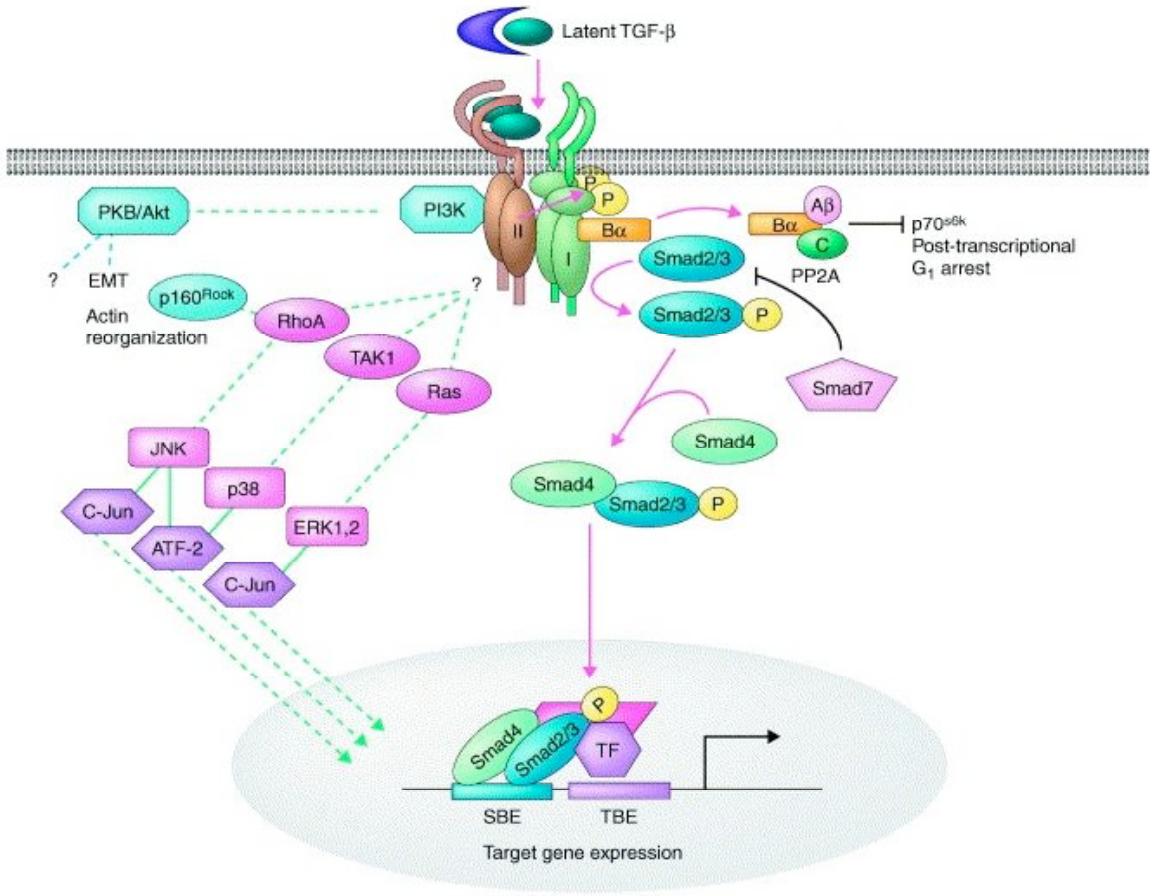


Figure 1: The TGF-β signaling pathway. TGF-β ligand binds TβRI and TβRII, recruiting the receptor-Smads (Smad2 and Smad3), which then bind Smad4 and the complex translocates to the nucleus and activate gene transcription. TGF-β also activates Smad-independent pathways such as Ras, TAKI, RhoA, and PI3 kinase. Illustration from Wakefield and Roberts, (2002) Curr. Opin. Gen. & Dev. 12:22.

without microsatellite instability (Grady et al., 1999; Markowitz et al., 1995). In addition, mutations in Smad4, occurring in 30% of colorectal cancers and 50% of pancreatic cancers, are associated with more aggressive tumors (Hahn et al., 1996; Takagi et al., 1996). The tumor suppressor activity of TGF-β occurs partially though its regulation of key genes involved in the regulation of the cell cycle. The TGF-β activated Smad complex interacts with the transcription factor SP-1 to increase expression of $p21^{Cip1}$, $p15^{Ink4b}$, and $p27^{Kip1}$, potent negative regulators of the cyclins and cyclin-dependent kinases involved in cell cycle progression (Feng et al., 2000; Massague et al., 2000; Pardali et al., 2000). In intestinal epithelial cells, TGF-β causes cell cycle arrest at the G1/S transition (Ko et al., 1994; Ko et al., 1995) and down regulates cyclin D1 expression (Ko et al., 1998). Furthermore, intestinal epithelial cells overexpressing cyclin D1 become refractory to the growth inhibitory actions of TGF-β (Ko et al., 1998).

TGF-β signaling alterations in cancer

During tumorigenesis and neoplastic transformation, the growth inhibitory response to TGF-β is often lost. In this case, TGF-β promotes growth, increases cell motility, and promotes transformation. TGF-β can induce an epithelial to mesenchymal transition in keratinocytes, melanoma cells, and mammary epithelial cells (Cui et al., 1996; Janji et al., 1999; Miettinen et al., 1994). *In vivo* data support the view of TGF-β switching from a tumor suppressor to a tumor promoter. TGF-β expression is up-regulated in more than 90% of colorectal cancers (Derynck et al., 1987). While TGF-β acts as a tumor suppressor in early stage tumors (Engle et al., 1999), increased expression of TGF-β in colorectal tumors correlates with depth of tumor invasion and advanced

tumor stage (Xiong et al., 2002). Furthermore, TGF-β has been shown to promote progression of late stage tumors by enhancing invasion and metastasis (Takenoshita et al., 2002). Increased levels of TGF-β in plasma or the primary tumor also correlate with advanced tumor stage (Robson et al., 1996; Shim et al., 1999) and predict liver metastases in patients who have undergone surgical resection of a primary tumor (Tsushima et al., 2001). A high level of TGF-β expression in primary tumors is an independent risk factor for recurrence (Friedman et al., 1995) and is also associated with shorter survival (Robson et al., 1996).

Activation of Ras signaling switches TGF-β to a growth promoter

Ras oncogene

The *Ras* proto-oncogene is a central component of many signal transduction pathways regulating cellular growth and differentiation. The Ras family of GTPases is composed of three highly conserved members, K-, H-, and N-Ras, with considerable functional overlap. Ras proteins are activated by diverse extracellular signals, the best characterized is via tyrosine kinase receptors such as the epidermal growth factor receptor and G protein-coupled receptors. Ras activates multiple downstream effectors including the classical mitogen activated protein kinase (MAPK) cascade, Raf-MEK (MAPK/ERK kinase)-ERK1/2, and the PI3K-Akt pathway (Giehl, 2005) (Figure 2). Conserved oncogenic mutations of *Ras* genes at codons 12, 13, or 61 locks Ras in an active, GTP-bound state, triggering constitutive activation of its downstream effectors. This activation

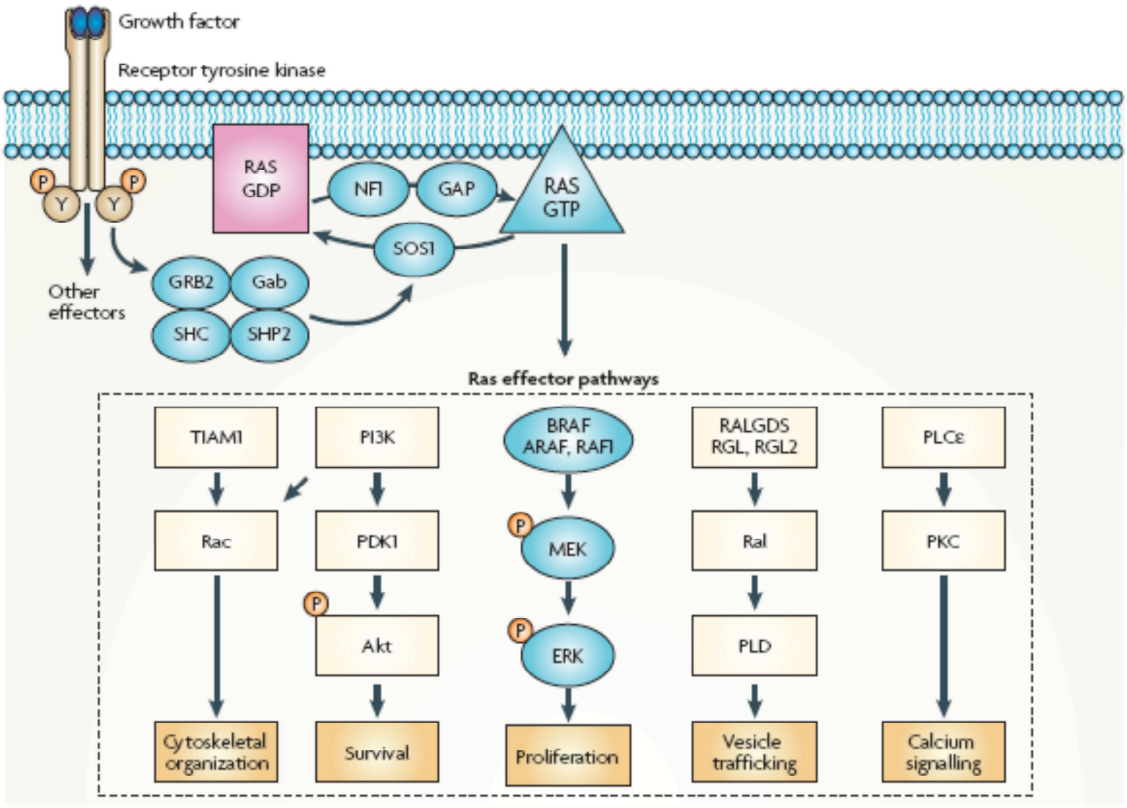


Figure 2: The Ras signaling pathway. Growth factor binding to tyrosine kinase receptors activates Ras through adaptors such as growth-factor-receptor bound protein 2 (GRB2), SH2-containing protein (SHC), and son-of-sevenless 1 (SOS1), which increase Ras-guanosine diphosphate (GDP) exchange for guanosine triphosphate (GTP). This activation of Ras effects multiple signaling pathways as depicted here, such as Raf-MEK-ERK and PI3K-PKD1 (3-phosphoinositide-dependent protein kinase 1)-Akt signaling, which control proliferation and survival, respectively. Illustration from Schubbert et al., (2007) Nat. Rev. Cancer 7:295.

of Ras results in changes in expression of many autocrine growth factors and their receptors, such as members of the TGF-β and epidermal growth factor (EGF) protein families, and increases the expression of several factors with known tumor-promoting activity including COX-2 (Sheng et al., 2000) and VEGF (Rak et al., 2000). Expression of activated H-Ras in intestinal epithelial cells also leads to profound changes in cell behavior including an increase in invasiveness, a resistance to growth inhibition by TGF-

β, and altered expression and localization of cell junction proteins like E-cadherin and β-catenin leading to transformation to a malignant phenotype (Fujimoto et al., 2001; Sheng et al., 2000). Constitutive expression of oncogenic Ras, through these changes in gene expression and cell behavior, drives cells to form tumors in nude mice (Sheng et al., 1997).

Mutations in oncogenes or tumor suppressor genes, in combination with tumor cell interaction with its microenvironment, play important roles in regulating cancer cell growth and behavior. Ras is mis-regulated in at least one third of all human cancers (Bos, 1989) and K-Ras is mutated in 50% of colorectal malignancies and 90% of pancreatic cancers (Kinzler and Vogelstein, 1996), while H-Ras is commonly mutated in bladder cancer (Visvanathan et al., 1988) and mutations in N-Ras are observed in 25% of acute leukemias (Bos et al., 1987). Furthermore, activation of the downstream MAP kinase, MEK, occurs in more than 70% of colorectal tumors (Lee et al., 2004b), implicating Ras activation as a central regulator of carcinogenesis.

Cooperation between oncogenic Ras and TGF-β

In normal epithelial cells, homeostasis is achieved by a balance of growth promoting signals, like epidermal growth factor, and growth inhibitory signals, such as TGF-β. We and others have found that transforming events, such as Ras activation, contribute to both the resistance of epithelial cells to growth inhibition by TGF-β as well as to the tumor promoting effects of TGF-β signaling (Cui et al., 1996; Filmus et al., 1992; Fujimoto et al., 2001; Saha et al., 2001). The interactions between the Ras and TGF-β signaling cascades are well described in keratinocytes and mammary epithelial

cells. Oncogenic H-RasV12 transformed keratinocytes are refractory to TGF-β-mediated growth inhibition and have reduced TGF-β-Smad signaling, due to decreased ligand and TβRII expression and nuclear accumulation of Smad2/3 (Game et al., 1992; Kretzschmar et al., 1999). TGF-β cooperates with Ras in EpRas mammary epithelial cells to induce a spindly phenotype, loss of cell-cell junction integrity associated with cytoplasmic localization of E-cadherin and β-catenin, decreased expression of epithelial markers, increased expression of mesenchymal markers, and increased invasion (Oft et al., 1996). Blocking TGF-β signaling with dominant negative TβRII expression in EpRas cells blocks EMT, invasion, tumor growth, and metastasis (Oft et al., 1998). TGF-β stimulates Ras-dependent ERK activity and increases motility and invasion in transformed keratinocytes (Santibáñez et al., 2000). Furthermore, restoration of low levels of TβRII expression in colon cancer cell lines increases cell invasiveness but high TβRII expression restores the growth inhibitory effects of TGF-β (Oft et al., 1998). Expression of Smad7, which inhibits TGF-β-Smad signaling, induces oncogenic Ras expressing keratinocytes to form carcinomas instead of papillomas (Liu et al., 2003). TGF-β and Ras cooperate to increase invasion and migration in breast cancer cells and TGF-β-induced EMT requires active MEK/ERK signaling (Kim et al., 2005a; Xie et al., 2004). These studies indicate that activation of oncogenes, such as Ras, induces a switch in the TGF-β response in epithelial cells from tumor suppression to tumor promotion.

The mechanisms by which oncogenic Ras and TGF-β cooperate to induce EMT and invasion are not completely understood. We have shown that oncogenic Ras induces degradation of Smad4 in the rat intestinal epithelial cells, expression of which is necessary for TGF-β-induced growth suppression (Saha et al., 2001). Oncogenic Ras also

inhibits the TGF-β-induced nuclear accumulation of Smad2 and Smad3 by inducing phosphorylation of Smad2/3 at several ERK consensus sites in the region linking the DNA binding domain and the transcriptional activation domain (Kretzschmar et al., 1999). In colon cancer cells that both lack Smad4 expression and harbor an activating K-Ras mutation, expression of Smad4 together with a Ras-resistant mutant form of Smad3 lacking inhibitory MAPK phosphorylation sites restores the TGF-β anti-proliferative response (Calonge and Massague, 1999). These studies indicate that oncogenic Ras inhibits the growth inhibitory Smad-mediated responses of TGF-β.

We previously reported that conditional oncogenic Ha-RasV12 expression causes morphological changes consistent with EMT, actin reorganization, increased vimentin expression, decreased E-cadherin expression, and increased invasiveness of rat intestinal epithelial cells (RIE:iRas cells) (Fujimoto et al., 2001). TGF-β enhances EMT in RIE:iRas cells transformed by oncogenic Ras, further decreasing E-cadherin expression, increasing nuclear localization of β-catenin, and increasing cell invasiveness (Fujimoto et al., 2001; Sheng et al., 2000) (Figure 3). Interestingly, while blocking TGF-β signaling with dominant negative TβRII expression decreases the growth inhibitory effect of TGF-β regardless of RasV12 expression, dnTβRII also decreases TGF-β induced invasion in Ras transformed cells, demonstrating that both growth suppressing and tumor promoting activities of TGF-β act through TβRII (Fujimoto et al., 2001).

Additionally, TGF-β treatment of oncogenic Ras expressing cells was shown to synergistically induce COX-2 mRNA and protein expression through a mechanism of increased COX-2 mRNA stability involving the AU-rich element (ARE) region of the

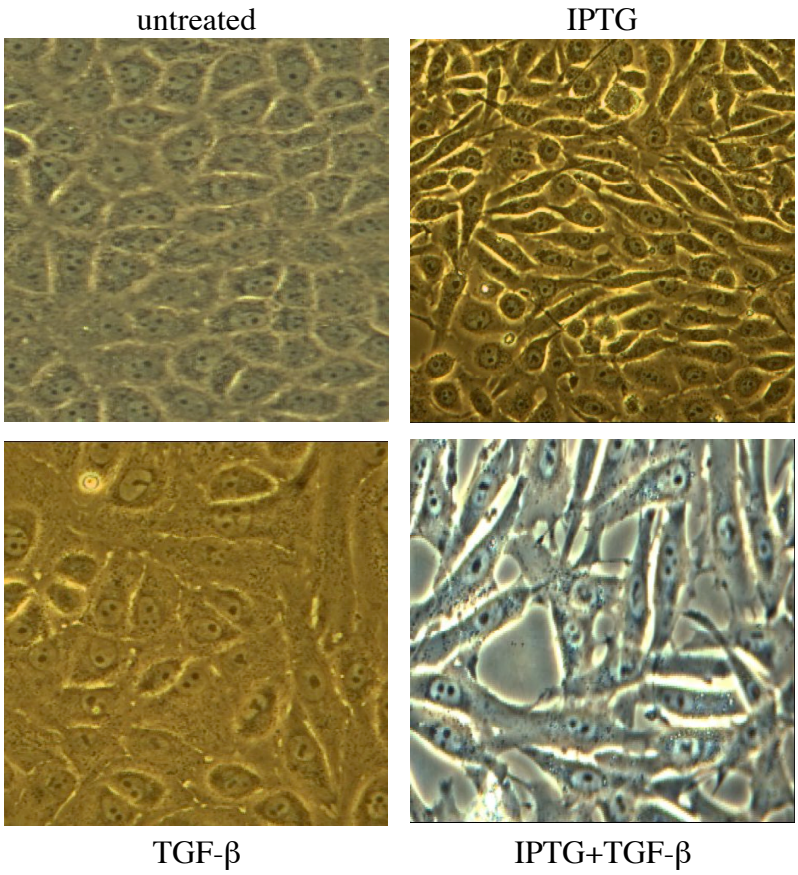


Figure 3: Oncogenic Ras expression and TGF-β treatment induce epithelial-mesenchymal transition in RIE:iRas cells. Cells were treated for 72 hours with or without 5 mM IPTG to induce RasV12 expression or treated with 3 ng/ml TGF-β. Phase contrast micrographs show phenotypic transformation (100x).

COX-2 3'untranslated region (UTR) (Roman et al., 2002; Sheng et al., 2000). Interestingly, increased COX-2 expression and mRNA stability in colon cancer cell lines is associated with increased tumor growth *in vivo* (Dixon et al., 2001). Taken together these results suggest that post-transcriptional gene regulation is an important mechanism for regulating gene expression that contributes to cancer progression and that Ras and TGF-β collaboratively regulate mRNA stability of selected target genes.

Summary

In addition to unrestrained growth, Ras and TGF-β collaborate to induce epithelial to mesenchymal transition (EMT), increase cell motility and invasiveness, and influence the expression of genes involved in cell-cell and cell-matrix interactions, like E-cadherin, integrins, and intermediate filaments, as well as genes involved in tumorigenesis, such as COX-2 (Fujimoto et al., 2001; Sheng et al., 2000). In this study, the collaborative effects of Ras and TGF-β were examined by gene expression profiling. Global gene expression patterns induced by Ras transformation alone, TGF-β treatment alone, and the combination of Ras transformation and TGF-β treatment were examined by oligonucleotide microarray in order to characterize changes in gene expression related to the cooperative effect on malignant behavior that occurs with TGF-β treatment of Ras transformed cells. A pivotal role for EGFR signaling during EMT was examined, as well as a global mechanism of post-transcriptional regulation of gene expression. Furthermore, access to microarray data from 65 human colorectal cancer (CRC) patients, through the GI SPORE, afforded a unique opportunity to examine data from human CRC samples. Analysis of the gene expression changes associated with EMT in the well-controlled intestinal epithelial cell line were used to inform the human CRC data, demonstrating the utility of RIE:iRas cells to model colorectal cancer and implicating post-transcriptional gene regulation as a novel mechanism involved in carcinoma progression.

CHAPTER II

MATERIALS AND METHODS

Reagents

TGF-β and TGF-α were purchased from R&D Systems (Minneapolis, MN). EGF was purchased from Sigma-Aldrich (St. Louis, MO). TNF-α and amphiregulin were gifts from Robert Coffey, Vanderbilt University. U0126, LY294002, EKI-785-785, and SB203580 were purchased from Calbiochem (San Diego, CA). A pharmacological inhibitor of TGF-β type I receptor (LY364947 or TRKi) was provided by Eli Lilly (Indianapolis, IN). NS398 was from Cayman Chemical Co. (Ann Arbor, MI) and celecoxib from Pharmacia and Upjohn (New York, NY).

Cell culture

Rat intestinal epithelial cells (RIE-1) cells were maintained in DMEM containing 10% FBS as described previously (Saha et al., 1999). RIE cells stably expressing inducible Ha-RasG12V cDNA under the control of the Lac operon (RIE:iRas) were derived and maintained as previously described (Sheng et al., 2000) in DMEM (Invitrogen, Carlsbad, CA) containing 10% FBS, 400 μg/ml G418, and 150 μg/ml hygromycin B. Transcriptional expression of Ha-RasG12V was induced by treatment with 5 mM isopropyl-1-thio-β-D-galactopyranoside (IPTG; Sigma-Aldrich, St. Louis, MO). RIE-H-RasV12 were kindly provided by Dr. Robert Coffey (Vanderbilt

University). Cells stably transfected with human H-RasV12 are maintained in DMEM containing 10% FBS and 400 μg/ml G418 (Gangarosa et al., 1997).

Young adult mouse colonocytes (YAMC), YAMC cells expressing Ha-RasG12V (YAMC-Ras), and YAMC cells derived from a COX-2 -/- mouse (YAMC-COX-2-null) were kindly provided by Dr. Robert Whitehead (Vanderbilt University). Cells were maintained as previously described (D'Abaco et al., 1996) in permissive conditions at 33ºC in RPMI medium containing 5% FBS and 5 U/ml murine interferon-γ (Roche, Indianapolis, IN). For all experiments, cells were cultured under non-permissive conditions at 37ºC without interferon-γ.

LIM1863 cells were a gift from Dr. Robert Whitehead (Vanderbilt University). Cells were grown as organoids and maintained in RPMI 1640 (Invitrogen, Carlsbad, CA) supplemented with 5%FBS and 1% Insulin-Transferrin-Selenium (Invitrogen, Carlsbad, CA) as described (Whitehead et al., 1987).

Human Colorectal tissue collection and processing

65 patients were recruited at the Vanderbilt Ingram Cancer Center at Vanderbilt University Medical Center (VUMC) and the Tennessee Valley Healthcare System Veterans Affairs Medical Center (VAMC) in Nashville, TN between 2003 and 2005. All patients were diagnosed with adenoma or colorectal adenocarcinoma (stages I-IV) according to current AJCC guidelines Patients utilized for this study were further selected based on the diagnosis of adenoma (n=5 for Affymetrix analysis and n=5 for ABI analysis), stage 1 (n=10), stage 2 (14), stage 3 (n=17), or stage 4 colorectal adenocarcinoma (n=19 for Affymetrix analysis and n=6 for ABI analysis). Informed

consent was obtained from each patient and all protocols and procedures were approved by the Institutional Review Board at VUMC and the Nashville VAMC.

Tissue specimens from all colon and rectal tumors were obtained in the operating suite or at the time of endoscopic biopsy, respectively, and a representative specimen was sent to pathology to confirm the diagnosis of adenoma or adenocarcinoma. The remaining specimen was immediately flash frozen in liquid nitrogen, transported to the laboratory, and stored at -80°C. Representative quality assessment slides were obtained to verify the diagnosis and quality of all tissue sent to the laboratory.

RNA was purified from adjacent biopsy and surgical specimens with a confirmed diagnosis of adenoma or adenocarcinoma using the RNeasy® kit from Qiagen (Valenica, CA) according to manufacturer's protocol, except β-mercaptoethanol was not added to lysis buffer. RNA was eluted with 10mM Tris/DEPC H_2O at pH 8.0 samples were submitted to the VMSR. RNA quality was assessed with an Agilent 2100 bioanalyzer (Foster City, CA).

Western blots

Cells were treated, washed 2x in PBS, then lysed in RIPA buffer (150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS, 50 mM Tris-HCl (pH 8.0), 2 mM EDTA, 1 mM PMSF, 3 μg/ml aprotinin, 10 μg/ml leupeptin, 250 μM vanadate, and 10 mM NaF) and stored at -80°C until use. Protein samples were separated by SDS-PAGE (20 to 40 μg/lane) and transferred to PVDF. The blots were blocked in PBS containing 0.1% Tween-20 and 5% BSA or milk and incubated overnight in primary antibody at 4°C, rinsed, then incubated for 1 hour in horseradish-peroxidase conjugated secondary

antibody (Santa Cruz Biotechnology, Santa Cruz, CA), and developed with ECL (Amersham, Piscataway, NJ). Mouse anti-phospho-ERK, rabbit anti-ERK, and mouse anti-β-actin antibodies were purchased from Sigma (St. Louis, MO). Rabbit anti-phospho-Smad 2, rabbit anti-Akt, mouse anti-phospho-Akt(ser473), rabbit anti-p38 MAPK, mouse anti-p38 MAPK, and mouse anti-phospho-EGFR(tyr1068) antibodies were purchased from Cell Signaling Technology (Danvers, MA). Mouse anti-Smad2 antibody was purchased from Invitrogen (Carlsbad, CA). Goat anti-COX-2 and mouse anti-HuR (3A2) were purchased from Santa Cruz Biotechnology (Santa Cruz Biotechnology, Santa Cruz, CA). Rabbit anti-EGFR was purchased from Upstate Biotechnology (Lake Placid, NY). Mouse anti-phospho-tyrosine (clone 4G10) were obtained from BD Transductions Laboratories (Franklin Lakes, NJ).

Microarray

RIE:iRas cells were plated at $0.5x10^6$ cells per 10cm plate, allowed to attach overnight, then cells left untreated or treated for 72 hours with 5mM IPTG (for H-RasV12 induction), 3ng/ml TGF-β or IPTG and TGF-β together in DMEM containing 0.5% FBS. RNA was collected from 3 (IPTG and TGF-β) or 4 (untreated and IPTG+TGF-β) independent cultures and purified using the RNeasy® kit from Qiagen (Valenica, CA) according to manufacturer's protocol, except no β-mercaptoethanol was added to lysis buffer. RNA was eluted with 10mM Tris/DEPC H_2O at pH 8.0 samples were submitted to the Vanderbilt Microarray Shared Resource (VMSR, www.vmsr.net). RNA quality was assessed with an Agilent 2100 bioanalyzer (Foster City, CA), then hybridized to Affymetrix Rat Genome 230 2.0 GeneChip Expression arrays (Santa Clara,

CA) according to manufacturer's instructions. Data sets were normalized and background subtracted by the Robust MultiChip Analysis (RMA) method (Irizarry et al., 2003). For each replicate, samples treated with IPTG and/or TGF-β were compared with the matched untreated sample. Gene expression increases or decreases were defined by a log2 ratio (treated/untreated) greater than 1 or less than –1, respectively, using GeneSpring software (Agilent Technologies, Santa Clara, CA). Synergistic regulation is defined as IPTG and TGF-β together showing a more than additive increase or decrease in expression compared to IPTG and TGF-β alone in at least two of three replicates. Significance of differentially expressed genes was determined using the Benjamini-Hochberg multiple testing comparison with a p-value <0.05.

RNA isolated from human CRC samples (5 adenomas and 19 stage 4 colon adenocarcinomas) were hybridized to Affymetrix U133 Plus 2.0 GeneChip Expression arrays (Santa Clara, CA) according to manufacturer's instructions. Analysis of microarray results was performed as described for the rat arrays using the Affymetrix analysis functions in Bioconductor (http://www.bioconductor.org). Genes differentially expressed between adenomas and stage 4 adenocarcinomas were identified by a two-sample t-test and probes with a q-value<0.05 were selected for further analysis (Storey and Tibshirani, 2003).

Network and functional analysis

Rat microarray data were analyzed through the use of Ingenuity Pathway Analysis (Ingenuity® Systems, www.ingenuity.com). Functional analysis of differentially regulated genes identified the biological functions and/or diseases that were most

significant ($p<0.05$) to the data set. Fischer's exact test was used to calculate a p-value determining the probability that each biological function and/or disease assigned to the data set is due to chance alone.

The WebGestalt toolkit (http://bioinfo.vanderbilt.edu/webgestalt) was used for the orthologous mapping, boolean operation, and functional annotation of the differentially expressed gene sets (Zhang et al., 2005). Rat and human Affymetrix probe set IDs were uploaded into WebGestalt for the analyses. Rat genes were mapped to human orthologs to allow a direct comparison with the human gene sets.

Identification of AU-rich elements

Human and rat RefSeq mRNAs with complete 3′UTR were processed to computationally extract their 3′UTRs; the ARE motifs were searched as previously described (Bakheet et al., 2001). Rat genes from the synergy list containing AREs were identified by a second independent approach, for those with incomplete 3′UTR or missing 3′UTR from rat GenBank mRNA records, AREs were defined from the human sequence homologs since AREs are conserved among mammalian species (Khabar et al., 2005). This was accomplished by using BLAST to human mRNA records and retrieval of data from HomoloGene database at NCBI. In order to assess the statistical significance of ARE gene representation, we used the stringent ARED-Organism (Halees et al., 2008), which is based on the HomoloGene database, to examine the lists of differentially expressed rat genes (individual, combined, and synergistic conditions) and human adenoma compared to adenocarcinoma or each individual stage. The Chi-squared test was used to determine statistical significance.

Real time RT-PCR

RIE:iRas cells were plated at $0.5x10^6$ cells per 10cm plate, allowed to attach overnight, then cells left untreated or treated for 72 hours with 5mM IPTG (for H-RasV12 induction), 3ng/ml TGF-β or IPTG and TGF-β together in DMEM containing 0.5% FBS. RNA was isolated and purified via RNeasy® kit (Qiagen, Valenica, CA) according to manufacturer's instructions. For time course experiments, RNA was isolated from RIE:iRas cells treated for 0, 24, 48, 72, or 96 hours with IPTG and/or TGF-β under similar conditions as above.

cDNA synthesis was performed using 300ng RNA with SuperScript III Reverse Transcriptase from Invitrogen (Carlsbad, CA) and one gene specific primer (COX-2, Wnt5a, Spp1, CUGBP2, TGF-α, or hnRNP A/B) at 50ºC. Primers were designed in our laboratory and ordered from IDT Technologies (Coralville, IA); see Table 1 for sequences. Real Time SYBR Green/Fluorescein PCR Master Mix was obtained from SuperArray (Frederick, MD). PCR reactions were prepared according to SYBR Green Master Mix protocol (SuperArray) and analyzed using a Bio-Rad iCycler under the following conditions: 95 °C for 13:30m (1 cycle), 95 °C for 15s, 55 °C for 30s , 72 °C for 30s (45 cycles); 72 °C for 7m (1 cycle), melt curve 50 °C for 10s and increase temperature by 0.4°C for each cycle (100 cycles). Real Time PCR was performed on a minimum of three biological replicates. All samples were set up in quadruplicate with efficiencies for each well calculated from the slopes of the exponential phase of the log transformed reaction curves. Fold changes were calculated according to the methods of Schefe et al. (Schefe et al., 2006). Reactions using phosphomannomutase 1 (Pmm1) housekeeping gene were used to normalize all data sets (Rubie et al., 2005). Analysis of

variance (ANOVA) with Bonferroni correction was used to examine differences between mean fold change in gene expression among 3 treatment groups. Statistical analyses were performed using Kaleidagraph version 4.03.

For Itgb1, Itga5, and Axin2, real time PCR was performed similar to the above procedure with the following changes. RNA was DNase treated with RQ1 RNase-Free DNase (Promega, Madison, WI) according to manufacturer's instructions prior to purification. Xpress-Ref Universal Rat RNA (SuperArray) was obtained for standards. cDNA synthesis was performed using1.5μg RNA with M-MLV Reverse Transcriptase (Promega) and Oligo(dT) primers according to manufacturer's guidelines. Primers were ordered from SuperArray. Standard curves were constructed using cDNA from Universal Rat RNA in at least 6 serial dilutions. All reactions were run in triplicate. Standard curves were used to derive starting quantities of cDNA in each unknown.

Table 1: Real time PCR primers

COX-2	Forward	5'- CCACTTCAAGGGAGTCTGGA -3'
	Reverse	5'- AAGGGCCCTGGTGTAGTAGG -3'
Wnt5a	Forward	5'- TGAATAACCCTGTTCAGATGTCA -3'
	Reverse	5'- TGTACTGCATGTGGTCCTGA -3'
Spp1	Forward	5'- GACCCATCTCAGAAGCAGAA -3'
	Reverse	5'- TTCGTCAGATTCATCCGAGT -3'
CUGBP2	Forward	5'- ATGCAACAGCTCAACACTGC -3'
	Reverse	5'- CAGCGTTGCCAGATTCTGTA -3'
TGF-α	Forward	5'- CACTCTGGGTACGTGGGTG –3'
	Reverse	5'- CACAGGTGATAATGAGGACAGC –3'
hnRNP A/B	Forward	5'- GAGGTGTACCAGCAACAGCA –3'
	Reverse	5'- AGTAGTTGCCGTAGCCCTGA –3'

Luciferase-reporter assay

RIE-H-RasV12 cells ($1x10^5$/well) were transiently transfected with pGL3-basic firefly luciferase reporters (250ng) with or without the VEGF promoter and co-

transfected with a renillia luciferase reporter (15ng) to control for transfection efficiency. Full length VEGF promoter-reporter construct was a gift from Dr. Keping Xie (Shi et al., 2001). Plasmids were combined with Lipofectamine Plus (Invitrogen, Carlsbad, CA) transfection reagent, according to manufacturer's instructions, and incubated in Opti-mem media (Invitrogen, Carlsbad, CA). After six hours, cells were treated with or without 3ng/ml TGF-β in complete DMEM with 10% FBS for 48 hours. Dual luciferase assay was performed according to manufacturer's instructions (Promega, Madison, WI). All transfections and treatments were performed in triplicate wells for each of seven independent experiments. Firefly luciferase activity was normalized to renilla luciferase activity for each sample. Average VEGF-promoter luciferase units were normalized to pGL3-basic for each experiment. Statistical analysis was performed by t-test using Kaleidagraph version 4.03.

TGF-α RIA

RIE:iRas cells were treated with or without 5 mM IPTG and 3 ng/ml TGF-β for 24 hours in DMEM with 0.5% FBS. Media were collected and cells washed twice in PBS then lysed in 1 ml lysis buffer (25 mM Tris-HCl (pH 8.0), 50 mM NaCl, 0.5% sodium deoxycholate, 0.5% NP-40, 0.02% sodium azide, 2 mM PMSF, 5 μg/ml pepstatin, 5 μg/ml leupeptin, and 5 μg/ml aprotinin) on a rocker at 4C for 1 hour. RIA was performed as described (Russell et al., 1993) to measure TGF-α levels in both the conditioned media and lysates. Representative wells were trypsinized and cells were counted with a hemacytometer to normalize the data. TGF-α was measured in LIM1863 cells as described above except cells were grown for 48 hours in RMPI with 5% FBS and 1%

ITS. LIM1863 organoids in suspension were pelleted, media collected from both suspension and adherent cells, and cells lysed.

EGFR IP/western

Cells were treated and lysed in RIPA buffer, as above for Western blots. Lysate (500μg) was incubated overnight at 4°C with 4μg of rabbit anti-EGFR (Upstate Biotechnology). Protein G-sepharose (50μl of packed beads) was added and incubated overnight at 4°C. Immunoprecipitations were washed 3 times in PBS and beads resuspended in 2x Laemmli sample buffer. SDS-PAGE and immunoblot analysis were performed as above using mouse anti-phospho-EGFR(tyr1068) antibody (Cell Signaling Technology).

Matrigel invasion assay

A modified Boyden chamber assay was performed using Transwells (8 μm pore size, 12 mm diameter) from Costar (Cambridge, MA) and Matrigel (BD Biosciences). Each Transwell insert was first coated with 2.5 mg/ml Matrigel diluted in serum-free media. RIE:iRas cells ($5x10^4$) or LIM1863 cells (approximately $3x10^4$) were washed with serum-free media twice, re-suspended in 0.2% bovine serum albumin serum-free medium, then seeded in Transwell inserts, and grown in the presence of 10% fetal bovine serum media in the lower chamber. Both cell lines were treated with DMSO or 10μM EKI-785 and either with or without 5 mM IPTG and 3 ng/ml TGF-β (RIE:iRas) or with and without 2 ng/ml TGF-β and 10 ng/ml TNF-α in both the upper and lower chambers. After 72 hours of incubation, cells that had not invaded were removed with cotton swabs,

and the cells that had invaded to the lower surface of the inserts were rinsed with PBS, fixed in 4% formaldehyde for 30 min at room temperature, mounted on glass slides with DAPI mounting media, and the invaded cells were counted under a light microscope (200x). Five random fields on each insert were counted to determine the number of cells invaded. The invasion assay was performed in triplicate. Analysis of variance (ANOVA) with Bonferroni correction was used to examine differences between treatment groups. Statistical analyses were performed using Kaleidagraph version 4.03.

RNA extraction and Northern blots

Total cellular RNA was extracted from RIE:iRas cells using Trizol (GibcoBRL, Carlsbad, CA), following manufacturer's instructions. RNA was separated on a formaldehyde-agarose gel (10 μg/lane) and transferred to Hybond N (Amersham, Piscataway, NJ). Membranes were hybridized for 16 hours at 42°C with a mouse $VEGF_{165}$ or HuR cDNA probe labeled with [α-^{32}P]-dCTP by random primer extension (DECAprime II kit, Ambion, Austin, TX). Membranes were then washed and mRNA levels examined by autoradiography and quantified by phosphor-imaging. Background was subtracted from VEGF mRNA levels and RNA loading was normalized to 18S ribosomal RNA visualized with ethidium bromide and quantified with a BioRad Gel Doc (Hercules, CA). Where indicated, RIE:iRas cells were treated for 24 hours with TGF-β, IPTG, or both and then transcription was inhibited by treatment with 5 μg/ml actinomycin D (Sigma-Aldrich, St. Louis, MO) for up to 4 hours. Mouse $VEGF_{165}$ cDNA was a gift from Charles Lin (Vanderbilt University, Nashville, TN). VEGF mRNA half-life was estimated from a log-linear plot of VEGF/18S ratio versus time and least squares

regression analysis. VEGF mRNA stability was modeled on a log scale via multiple linear regression with effects for time, TGF-β, IPTG, and interactions between treatments and between treatments and time, controlling for differences in three biological replicates.

VEGF ELISA

RIE:iRas or YAMC cells were treated with TGF-β and/or IPTG for 24 hours in serum-containing media, conditioned media were collected and cells lysed in RIPA buffer (150mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS, 50mM Tris-HCl pH 8.0, 2mM EDTA, 1mM PMSF, 3μg/ml aprotinin, 10μg/ml leupeptin, 0.25mM vanadate and 10mM NaF). Media and lysates were stored at -80°C until use. Samples were thawed and an ELISA performed using the rat/mouse VEGF Duoset kit (R&D Systems, Minneapolis, MN), according to manufacturer's instructions. VEGF concentration (ng/ml) was normalized to total protein levels in cell lysates as determined by BCA protein assay (Pierce, Rockford, IL). ANOVA was used to examine differences between mean VEGF protein expression levels among 4 treatment groups. Statistical analyses were performed using SAS version 9.1 and R version 2.1.1. VEGF dose response was modeled via multiple linear regression with terms for linear, quadratic, and interactions between log IPTG and log TGF-β concentration.

Prostaglandin analysis

RIE:iRas cells were pretreated for 15 minutes with 10μM NS398, 10μM celecoxib, or an equal volume of DMSO vehicle in DMEM with 0.5% FBS, then 5mM

IPTG and/or 3ng/ml TGF-β were added for an additional 24 hours. Prostaglandins ($PGF_{1\alpha}$ and PGE_2) in conditioned media were quantified by the mass spectrometric method developed by the Morrow laboratory, as previously described (DuBois et al., 1994).

Immunofluorescence

RIE:iRas cells (1 x 10^5 cells) were grown on cover slips and were subsequently left untreated or incubated with 5mM IPTG, 5 ng/ml TGF-β, or both IPTG and TGF-β for 24 hours. The cells were fixed in 4% paraformaldehyde for 15 minutes at RT, washed in PBS, and permeabilized with 0.2% Triton X-100 in PBS containing for 5 minutes. Cells were then blocked with 1% bovine serum albumin (BSA) and 10% horse serum in PBS for 30 min at RT. HuR expression was detected with an anti-HuR monoclonal antibody (3A2; 1:250) diluted in PBS containing 3% BSA. The cells were incubated with the anti-HuR antibody for 1 hour at RT, washed, and then incubated with anti-mouse FITC-conjugated secondary antibody (1:100, Vector Labs, Burlingame, CA) for 1 h at RT. Images were obtained using a Zeiss Axiovert 200 inverted microscope equipped with an AxioCam Mrc5 camera. Anti-HuR and anti-tubulin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-nucleoporin was purchased from BD Biosciences (San Diego, CA).

Nuclear and cytoplasmic fractionation

RIE:iRas cells were plated at 500,000 cells per dish in p60 plates and subsequently treated with IPTG, TGF-β, or both for 24 hours. The cells were then trypsinized,

centrifuged, washed with PBS, centrifuged, and 250 μl cytoplasmic lysis buffer (10mM HEPES pH 8, 3mM $MgCl_2$, 4 mM KCl, 0.2% NP-40, 10% Glycerol, 0.1 mM DTT) was added to each cell pellet. Cells were incubated on ice 15 minutes then centrifuged at 10,000 rpm for 2 minutes. Supernatant (cytoplasmic fraction) was transferred to new tubes. Remaining pellets were washed with 100 μl cytoplasmic lysis buffer, centrifuged again, and nuclei were lysed in 150 μl RIPA with protease inhibitors. Western blots were performed on nuclear and cytoplasmic fractions as described above.

HuR siRNA

RIE-1 cells were transfected with varying concentrations of control siRNA or siRNA against HuR (Ambion, Austin, TX) using siQuest reagent (Mirus Bio, Madison, WI), according to manufacturers instructions. After 24 hours of transfection, media was changed and cells were grown for another 24 hours. Media were then collected for VEGF expression analysis by ELISA and cell lysates were analyzed for HuR expression by SDS-PAGE, as described above. VEGF production was normalized to total cell protein and is expressed relative to control siRNA. Three independent experiments were performed.

CHAPTER III

EFFECTS OF ONCOGENIC RAS AND TGF-β ON GLOBAL GENE EXPRESSION

Introduction

Although cooperation between activated Ras and TGF-β signaling in the process of EMT has been well established, the molecular mechanisms by which changes in cellular behavior occur are not well understood. The cooperative interaction between oncogenic Ras and TGF-β activity could occur at several different levels, from transactivation of immediate downstream effectors, such as Smad2/3/4 and Raf-MEK-ERK, to interaction between secondary gene products, such as COX-2 . Rat intestinal epithelial cells stably transfected with an inducible activated H-RasG12V construct (RIE:iRas cells) were utilized to examine the interaction between oncogenic Ras and TGF-β signaling. In these cells, oncogenic Ras expression induces EMT and invasion, which is further augmented by treatment with TGF-β (Fujimoto et al., 2001; Sheng et al., 2000). In order to validate that this phenotypic transformation is specific for Ras activation and TGF-β treatment, both immediate downstream effectors (i.e., Smads and MEK) as well as targets further downstream, such as COX-2, were examined in the RIE:iRas cells.

Results

Ras and TGF-β specifically activate downstream effectors in RIE:iRas cells

The first goal of this study was to verify that oncogenic Ras and TGF-β specifically activate downstream signaling as expected and then to determine whether RasV12 and TGF-β together augment the activation of their immediate downstream effectors.

TGF-β signaling. TGF-β-induced activation of its primary downstream effector, Smad2, was examined by western blotting for phosphorylated Smad2. Treatment of RIE:iRas cells for 24 hours with TGF-β induced robust phosphorylation and activation of Smad2 in the presence or absence of Ras induction (Figure 4A), while oncogenic Ras alone did not activate Smad2. An inhibitor of the kinase activity of TGF-β type I receptor (TβRI), which blocks its ability to activate downstream effectors, was used for two purposes: first, to confirm the specificity of TGF-β treatment and second, to determine whether oncogenic Ras expression induces expression of TGF-β in the RIE:iRas cells and whether this TGF-β then acts alone or in concert with RasV12 to activate downstream signaling. Inhibition of TβRI activity with LY364947 (TRKi) blocks TGF-β-induced phosphorylation of Smad2 (Figure 4A). When RIE:iRas cells were treated with TRKi to inhibit TGF-β signaling, TGF-β-induced expression of COX-2, a known downstream effector of TGF-β (Sheng et al., 2000) was completely blocked and the cooperative induction of COX-2 by Ras and TGF-β together was reduced to the level of COX-2 expression induced by Ras alone (Figure 4A).

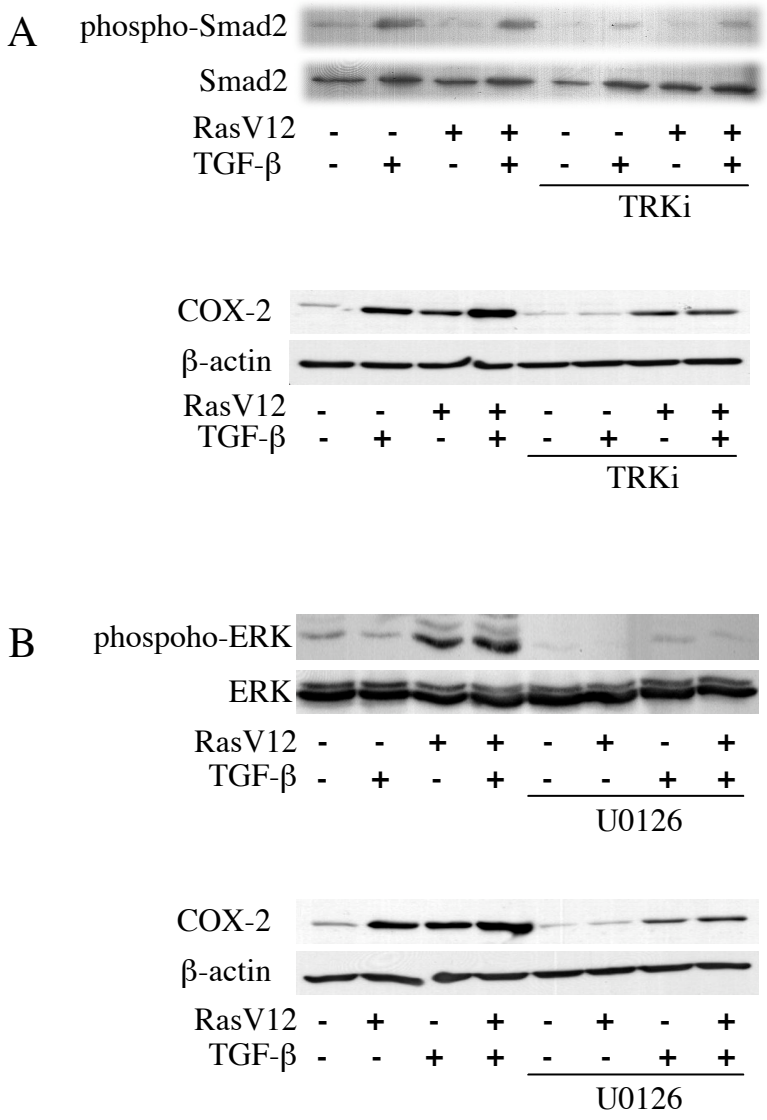


Figure 4: Ras and TGF-β activate their downstream effectors in RIE:iRas cells. RIE:iRas cells were pretreated for 15 minutes with **(A)** a TGF-β receptor kinase inhibitor (2μM LY364947/TRKi) or **(B)** a MEK inhibitor (10μM U0126), then treated for 24 hours with 5mM IPTG to induce RasV12 and/or 3ng/ml TGF-β. Western blotting was performed for phospho-Smad2, total Smad2, phospho-ERK, total ERK, COX-2 and β-actin.

Ras/MEK/ERK signaling. Phosphorylation and activation of the MEK/ERK signaling pathway by oncogenic Ras and TGF-β was examined by western blot using antibodies specific for phosphorylated ERK or total ERK. After treatment of RIE:iRas cells for 24 hours with IPTG, TGF-β or IPTG and TGF-β together, ERK was

phosphorylated in the RasV12 expressing cells. Although TGF-β alone transiently activates ERK within the first few hours of treatment (data not shown), MEK/ERK was not active after 24 hours of TGF-β treatment alone in these cells (Figure 4B). No marked difference in ERK phosphorylation was seen with RasV12 and TGF-β together compared to RasV12 alone. Pretreatment of RIE:iRas cells with U0126, a MEK inhibitor, blocked both basal and Ras-induced phosphorylation of ERK. Inhibition of MEK/ERK signaling with U0126 blocked Ras-induced and slightly reduced TGF-β-induced COX-2 expression in the presence or absence of oncogenic Ras (Figure 4B). These results show that oncogenic Ras and TGF-β do not appear to cooperate through augmented activation of immediate downstream effectors.

Characterization of Oncogenic Ras and TGF-β cooperation and global gene expression

Gene expression profiling was used to identify the gene expression changes that occur during Ras and TGF-β-induced EMT. Examination of gene expression patterns, molecular pathways, and biological functions have the potential to identify important molecules, signaling pathways, and novel mechanisms that mediate the cooperative interaction between oncogenic Ras and TGF-β.

Microarray analysis reveals a cooperatively regulated gene signature. In order to assess global changes in gene expression related to the cooperative interaction of Ras and TGF-β in transformation, we conducted microarray analysis of RIE:iRas cells at 72 hours post induction of H-RasV12 expression and/or TGF-β treatment. At this time point, the cells have undergone the molecular and morphological changes associated with EMT and acquired an invasive phenotype (Fujimoto et al., 2001). RNA from RIE:iRas cells left

untreated, induced to express oncogenic Ras with IPTG treatment, treated with TGF-β, or after both Ras induction and TGF-β treatment together were hybridized to a 28,000 gene rat-specific oligonucleotide microarray and analyzed. At least three independent biological replicates were analyzed for each condition. Each treatment group yielded both

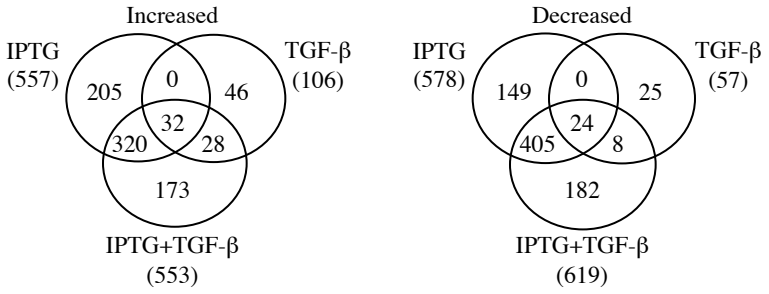


Figure 5: Summary of microarray results. Venn diagrams demonstrating the distribution of genes upregulated or downregulated in RIE:iRas cells after treatment with 5mM IPTG to induce RasV12 expression, 3ng/ml TGF-β treatment or combined IPTG and TGF-β treatment for 72 hours. The total number of genes with altered expression above 2-fold ($p<0.05$, Benjamini-Hochberg multiple testing comparison) in at least two of three biological replicates is given for each treatment.

unique and overlapping gene signatures (Figure 5). Analysis of the gene expression profile containing the set of genes that showed a more than additive, or synergistic, change with combined RasV12 expression and TGF-β treatment over either treatment alone was conducted next. We found that RasV12 induction and TGF-β treatment significantly ($p<0.05$, Benjamini-Hochberg multiple testing comparison) induced the expression of 553 transcripts, of which 194 showed a synergistic increase. Similarly, Ras activation and TGF-β treatment together significantly ($p<0.05$, Benjamini-Hochberg multiple testing comparison) downregulated 619 transcripts, including 185 that were synergistically decreased. In total, Ras activation and TGF-β treatment synergistically regulated 379 transcripts on the oligonucleotide arrays. After removal of ESTs (expressed

sequence tags) and taking into account genes with multiple probes, this represents 191 unique, annotated rat genes. A complete list of synergistically regulated genes is found in Appendix Table 5. Oncogenic Ras expression and TGF-β treatment together display a novel gene expression profile that was distinct from either stimulus alone, indicating the cooperative regulation of a cohort of genes, collectively referred to hereafter as the "Ras and TGF-β signature".

Ras and TGF-β target genes involved in cell growth and movement. The Ras and TGF-β gene signature was subsequently subjected to WebGestalt and Ingenuity Pathway Analysis (Pertovaara et al.) to examine the signaling pathways, cellular and molecular functions, and biological processes in which these genes are involved based on the published literature. This analysis demonstrated that one interaction network was linked through TGF-β as a central node and contained 29 genes involved in tissue morphology, cell cycle, and cellular development. Another network contained H-Ras as a central node for 13 genes with known roles in cellular movement, cancer and cell morphology.

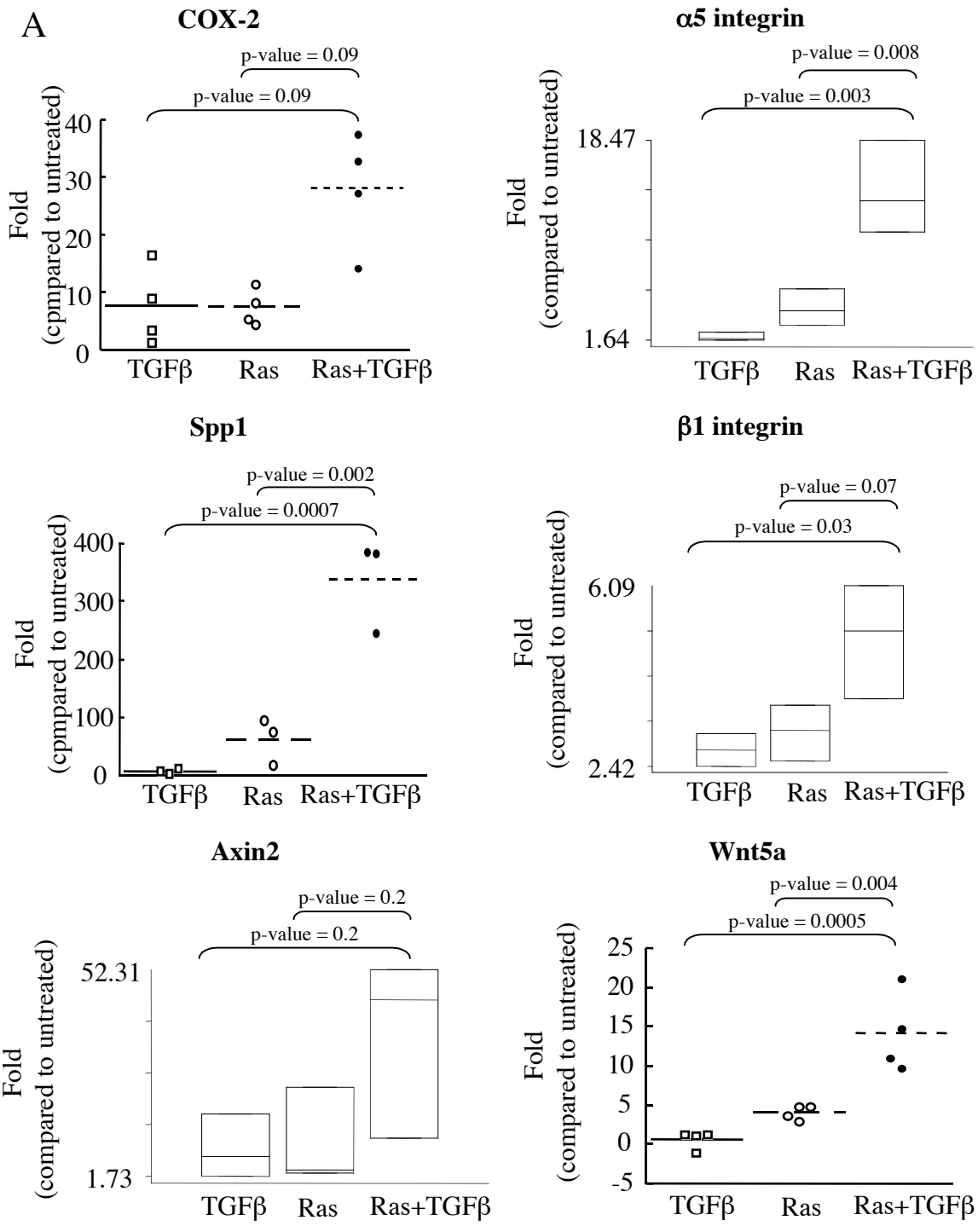
The 379 synergistically regulated probe IDs were loaded into WebGestalt and map to 189 human orthologs with annotation for gene identity and function. Overall, the Ras and TGF-β signature was significantly enriched for genes associated with cell movement (27% or 52 genes; p-value<0.002), development (31% or 59 genes; p-value<0.002), and cell growth and proliferation (42% or 80 genes; p-value<0.002). The Ras and TGF-β signature also contains several transcription factors (*Zfp36l1, Fosb, Tcf7, Dmrt2, Klf4, Stat1, Id2, Smarca2*), extracellular matrix-related genes (*Col5a3, Col14a1, Timp2, Adamts1, Mmp13, Mmp10, Lamb3, Lamc2, Itga5*) as well as genes involved in cell adhesion and cytoskeletal organization (*Ctnnal1, Marks, Fhl3, Tns4, Frmd4a,*

Map1b, Ocln, Cav1, Cadm1), angiogenesis (*Vegfa, Edn1, Fgf1, Sphk1*), and several members of the TGF-β (*Id2, Bmp2, Inhba, Tgfb1, Bmp4, Fst, Pai1*) and Wnt (*Wnt5a, Fzd1, Axin2, Wnt2*) signaling pathways. In addition, the Ras and TGF-β signature is enriched for several genes with known roles in cancer (67 genes; p-value<0.002).

Validation. We have previously shown that oncogenic Ras and TGF-β cooperate to synergistically increase COX-2 expression (Sheng et al., 2000). Independent and quantitative validation of the Ras and TGF-β signature was conducted in RIE:iRas cells for both mRNA and protein expression. The synergistic upregulation of several genes implicated in tumor progression, such as COX-2, osteopontin (Spp1), integrin α5 and integrin β1 were validated by quantitative real-time RT-PCR (Figure 6A). Additional validation at the protein level was determined by western blot for Id2 and Follistatin, two genes involved in TGF-β/BMP signaling during EMT and development (Figure 6B). For each of these genes, oncogenic Ras expression combined with TGF-β treatment resulted in synergistic regulation of gene expression levels compared to each condition alone. The synergistic regulation of genes involved in EGFR signaling as well as regulation of the angiogenic factor VEGF, both of which play important roles during EMT and cancer progression, is described in detail in the following sections.

Examination of the Ras and TGF-β signature from transformed RIE:iRas cells revealed changes in several genes involved in TGF-β superfamily signaling. As previous studies from our lab have shown, Ras and TGF-β cooperate to increase the expression of TGF-β1 ligand and the TGF-β target gene *Pai-1* (Fujimoto et al., 2001; Sheng et al., 2000). Oncogenic Ras and TGF-β also cooperatively regulated *Bmp-1, Bmp-2, Bmp-4,*

Id2, follistatin, and *inhibinβA* (Appendix Table 5). The synergistic increase in secreted Follistatin was confirmed at the protein level by western blotting of RIE:iRas conditioned media after either 24 or 72 hours of induction of Ras expression and TGF-β treatment (Figure 6B). The observed increase in follistatin mRNA levels in RIE:iRas cells could regulate EMT by blocking BMP-2 (increased 28-fold) signaling and encourage the retention of an epithelial phenotype. RIE:iRas cells undergoing Ras and TGF-β-mediated EMT also showed a decrease in Id2 expression (Appendix Table 5), which was confirmed by RT-PCR and decreased Id2 protein was observed after both 24 and 72 hours of treatment (Figure 6A-B). These Ras and TGF-β-induced changes in follistatin and Id2 expression we see in the RIE:iRas cells are consistent with a model of TGF-β-induced EMT and BMP-induced mesenchymal to epithelial transition (Lee et al.), respectively.


A
COX-2
p-value = 0.09
p-value = 0.09
Fold
(cpmpared to untreated)
40
30
20
10
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TGFβ
Ras
Ras+TGFβ
α5 integrin
p-value = 0.008
p-value = 0.003
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(compared to untreated)
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1.64
TGFβ
Ras
Ras+TGFβ
Spp1
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p-value = 0.0007
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300
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100
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TGFβ
Ras
Ras+TGFβ
β1 integrin
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p-value = 0.03
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TGFβ
Ras
Ras+TGFβ
Axin2
p-value = 0.2
p-value = 0.2
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(compared to untreated)
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1.73
TGFβ
Ras
Ras+TGFβ
Wnt5a
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p-value = 0.0005
Fold
(compared to untreated)
25
20
15
10
5
0
-5
TGFβ
Ras
Ras+TGFβ
Id2
Cyclophilin

B

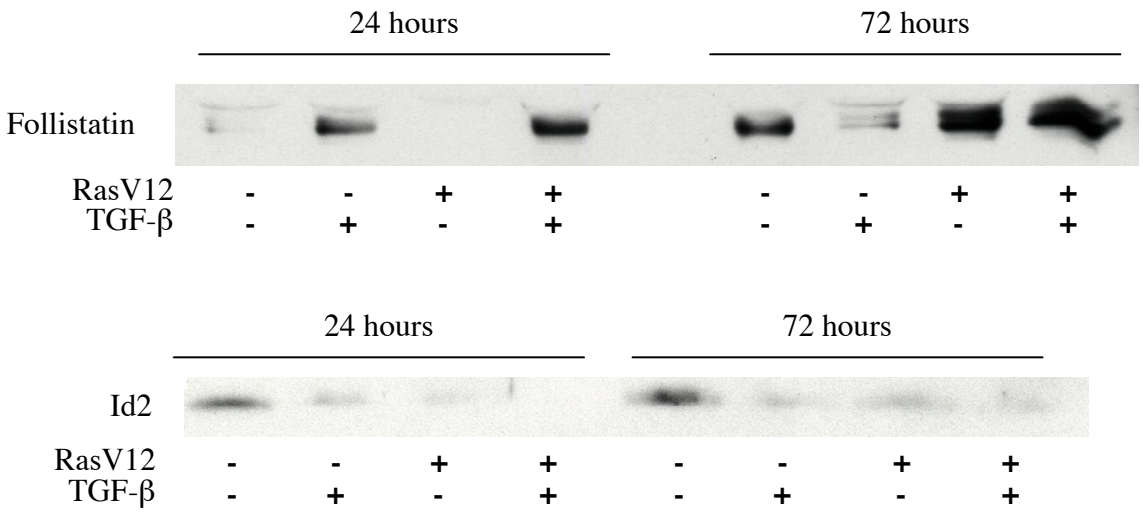


Figure 6: Validation of synergistic gene expression. (A) RNA was isolated from RIE:iRas cells treated for 72 hours with 5mM IPTG, 3ng/ml TGF-β, or IPTG and TGF-β together and expression of COX-2, Spp1, α5 integrin, β1 integrin, Axin2, and Wnt5a quantified by real time RT-PCR. Changes in gene expression under treated conditions were calculated relative to untreated samples and all values were normalized to the housekeeping gene Pmm1. Dotted lines show mean expression for each treatment. Significance was determined by ANOVA with Bonferroni correction. RT-PCR for Id2 and cyclophilin. **(B)** Western blotting for Follistatin in conditioned media or Id2 in cell lysates from RIE:iRas cells treated for 24 or 72 hours with or without 5 mM IPTG and 3 ng/ml TGF-β.

Summary

Treatment with specific inhibitors of TGF-β receptor tyrosine kinase activity or MEK signaling demonstrate the specificity of TGF-β to activate downstream signaling in RIE:iRas cells through activation of the primary effector Smad2 and increased expression of the COX-2 target gene and show that induction of RasV12 expression in RIE:iRas cells specifically activates downstream signaling through MEK and ERK to increase the expression of target genes such as COX-2. Taken together, these results indicate that the cooperation between oncogenic Ras and TGF-β does not occur at the level of primary signal transduction through ERK and Smads since the combination of Ras activation and TGF-β treatment does not modify the activation of ERK or Smad2 compared to either alone. Rather, these data suggest that Ras and TGF-β signaling events intersect further downstream. Microarray analysis of gene expression patterns indicate that oncogenic Ras expression and TGF-β treatment together display a novel gene expression profile that would not have been predicted from either stimulus alone. The synergistic regulation of a set of genes, many of which have known roles in cellular functions such as mitogenesis, migration and invasion, is associated with oncogenic Ras and TGF-β-induced EMT. Detailed examination of the molecular mechanisms involved in the synergistic regulation of specific genes, pathways, and gene families will provide insights into the molecular events that contribute to the oncogenic process.

CHAPTER IV

EGFR SIGNALING IS NECESSARY FOR TGF-β-INDUCED EMT

Introduction

Growth factor signaling has long been known to play a crucial role in cancer development and progression. As tumors develop, cell growth becomes independent of exogenous growth factors through mechanisms including increased autocrine growth factor production and increased growth factor receptor expression, enhancing the sensitivity of the tumor cells to low concentrations of host- or tumor-derived factors. Increased signaling driven by growth factors like epidermal growth factor (EGF) has been well characterized to increase cell growth and proliferation and enhance cell survival.

EGFR family and signaling

The EGF receptor (EGFR) family, also known as ErbB tyrosine kinase receptors, is composed of four members: EGFR, ErbB2, ErbB3, and ErbB4 (Figure 7). A diverse array of ligands that are synthesized as transmembrane precursors that are cleaved by metalloproteinases into mature growth factors that bind and activate the ErbB receptors (Dong et al., 1999). Among these, EGF, transforming growth factor α (TGF-α) and heparin binding-EGF (HB-EGF) specifically bind EGFR, while epiregulin, β-cellulin, and amphiregulin (AR) are able to bind both EGFR and ErbB4 (Yarden, 2001). Ligand

binding stimulates homo- or heterodimerization of the receptors, activation of the tyrosine kinase domain, and receptor phosphorylation, allowing the recruitment of adaptors and signaling molecules (Olayioye et al., 2000). EGF receptor stimulation activates signaling pathways such as Ras-MAPK, PI3K-Akt, and PLCγ to promote cellular proliferation, survival, and migration, respectively (Jorissen et al., 2003).

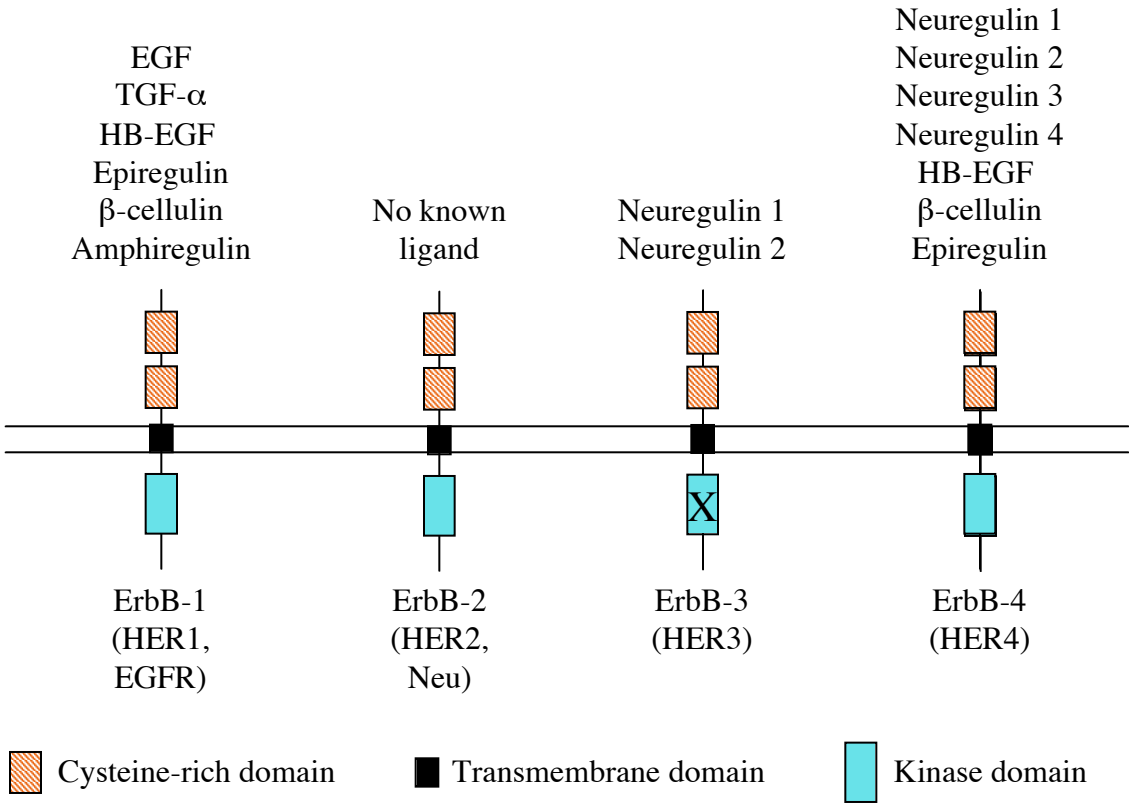


Figure 7: EGF family of receptors and ligands. Illustration shows members of the EGF family of receptors and ligands. Listed above each receptor are known ligands. Epidermal growth factor (EGF). Transforming growth factor α (TGF-α). Heparin-binding epidermal growth factor (HB-EGF).

Role of EGFR in cancer

The epidermal growth factor family of ligands and receptors play a central role during early embryonic development in the pathogenesis and progression of carcinomas in the gastrointestinal tract. TGF-α and EGFR expression is observed in the fetal colon, particularly at the base of fetal human colonic crypts in regions with high proliferative activity (Hormi and Lehy, 1994; Menard and Pothier, 1991). While TGF-α continues to be expressed in adult colonic tissue, EGFR expression decreases to low levels in normal adult colonic mucosa (Borlinghaus et al., 1993; Hormi and Lehy, 1994). However, increased expression of EGFR, TGF-α, and HB-EGF is observed in premalignant, hyperproliferative aberrant crypt foci, along with subsequent activation of Ras and ERK (Cohen et al., 2006) and several EGF receptors and ligands are overexpressed in colon cancer compared to adjacent normal mucosa, including EGFR, ErbB3, AR, TGF-α, and EGF (Ciardiello et al., 1991; Messa et al., 1998; Saeki et al., 1995). Overexpression of ErbB2 and its role in cancer has been extensively characterized, particularly in breast cancer; however, increased ErbB2 expression occurs less frequently in colon cancer (Normanno et al., 2003). Furthermore, increased TGF-α expression may correlate with increased stage and lymph node metastasis in colorectal cancer (Saeki et al., 1995).

Several studies in transgenic mice have demonstrated the importance of EGFR signaling in normal gut development as mice carrying triple null mutations in the EGF-related ligands, AR, EGF, and TGF-α show defects in the gastrointestinal tract, such as reduced proliferation of crypt cells (Troyer et al., 2001). Similarly, EGFR null mice show disorganized crypt formation (Threadgill et al., 1995). The transforming properties of EGF receptors and ligands are also seen in transgenic animals where EGFR

overexpression in the mammary gland induced hyperplasia, but these only progressed to dysplasias and adenocarcinomas in lactating animals (Brandt et al., 2000). TGF-α overexpression also induces marked hyperplasia in several organs, including the liver and gastrointestinal tract (Sandgren et al., 1990). The activated form of ErbB2 however, is able to stimulate mammary tumors when overexpressed (Muller et al., 1988). These studies suggest that overexpression of EGF receptors or ligands, other than activated ErbB2, are not alone able to induce carcinomas and that other events such as proto-oncogene activation are required.

Clinical trials of a monoclonal antibody directed against EGFR show efficacy in about 10% of patients with chemotherapy-resistant metastatic colorectal cancer (Cunningham et al., 2004; Saltz et al., 2004), particularly in patients with increased *EGFR* gene copy number (Moroni et al., 2005). Although EGFR is a promising therapeutic target for colorectal cancer, it may only be effective in a subset of patients. Therefore, a more detailed understanding of the molecular mechanisms of EGFR signaling in the gastrointestinal tract is necessary to take full advantage of EGFR inhibiting therapeutic agents.

EGFR signaling in EMT

Normal epithelial cells are growth inhibited by TGF-β, often undergoing apoptosis, while neoplastic epithelial cells are resistant to these inhibitory effects in part through increased EGFR signaling. TGF-β induces TGF-α transcription in Fet-1 cells, a well differentiated colon adenocarcinoma cell line, that undergo TGF-β-induced growth inhibition but not apoptosis (Lynch et al., 1993). During TGF-β-induced EMT in fetal rat

hepatocytes, TGF-α and HB-EGF expression increases (Del Castillo et al., 2006). EGFR activity is necessary for resistance to TGF-β-induced apoptosis in these cells through a PI3 kinase-mediated mechanism inhibiting cytochrome C release and caspase-3 activation (Fabregat et al., 2000). Although the involvement of EGFR in EMT remains elusive, EGFR signaling down regulates E-cadherin expression and cell adhesion in tumor cells. EGF or TGF-α treatment of breast cancer cells decreases E-cadherin expression and increases expression of Twist, a transcriptional repressor of E-cadherin (Lo et al., 2007). In human tumor cells, EGF treatment decreased E-cadherin expression and cell adhesion through a mechanism involving caveolin-1-mediated endocytosis of E-cadherin at early times after EGF treatment and through decreased E-cadherin transcription, correlating with increased expression of the transcriptional repressor Snail, at later time points (Lu et al., 2003). These studies suggest that EGFR contributes to EMT by increasing cell survival and disrupting cell-cell adhesion.

Although Ras is activated by EGFR signaling and is generally considered to be part of the downstream signaling, several studies have shown that TGF-α mRNA and protein expression increase in RasV12 transformed cells (Buick et al., 1987; Ciardiello et al., 1990). EGFR signaling is necessary for oncogenic RasV12-induced transformation, as an anti-TGF-α antibody partially inhibits Ras transformation, while specific inhibition of EGFR activity completely attenuated the Ras transformed phenotype in RIE cells (Gangarosa et al., 1997). However, another study in keratinocytes deficient in TGF-α expression showed that TGF-α is not necessary for Ras-mediated transformation and tumor formation in nude mice, suggesting that other EGFR ligands, such as HB-EGF, amphiregulin, and β-cellulin, may be able to substitute for TGF-α in Ras transformed

keratinocytes (Dlugosz et al., 1995). Together, these studies suggest a role for EGFR activation during Ras transformation, although the role of specific ErbB ligands remains unclear.

Results

Cooperative regulation of EGFR ligands and receptors

Analysis of the RIE:iRas microarray data reveals that oncogenic Ras and TGF-β regulate the expression of several EGFR family ligands and receptors (Table 2). Together Ras and TGF-β synergistically increase neuregulin and decrease β-cellulin and ErbB3 mRNA. While Ras and TGF-β together increased the expression of EGFR and HB-EGF mRNA more than either Ras or TGF-β alone, their effects were not more than additive. In addition, oncogenic Ras expression significantly increased amphiregulin and TGF-α mRNA expression, although TGF-β was able to block the Ras-mediated amphiregulin but not TGF-α expression.

Table 2: EGFR family genes differentially regulated by oncogenic Ras and TGF-β

Gene	TGF-β (Fold)	RasV12 (Fold)	RasV12 + TGF-β (Fold)
Amphiregulin	nc	3.75	nc
β-cellulin	nc	-8.23	-12.41
EGFR	nc	nc	2.13
ErbB3	nc	nc	-3.25
HB-EGF	5.68	5.61	6.03
Neuregulin	nc	3.72	5.08
TGF-α	nc	29.22	28.32

Microarray analysis of RIE:iRas cells treated for 72 hours with 5mM IPTG, 3ng/ml TGF-β, or IPTG and TGF-β together. Table lists EGF receptors and ligands affected by Ras expression or TGF-β treatment. Fold change represents expression in Ras expressing and/or TGF-β treated samples compared to untreated; numbers are the average of at least three biological replicates. nc = no change, defined as < 2-fold change in expression compared to untreated.

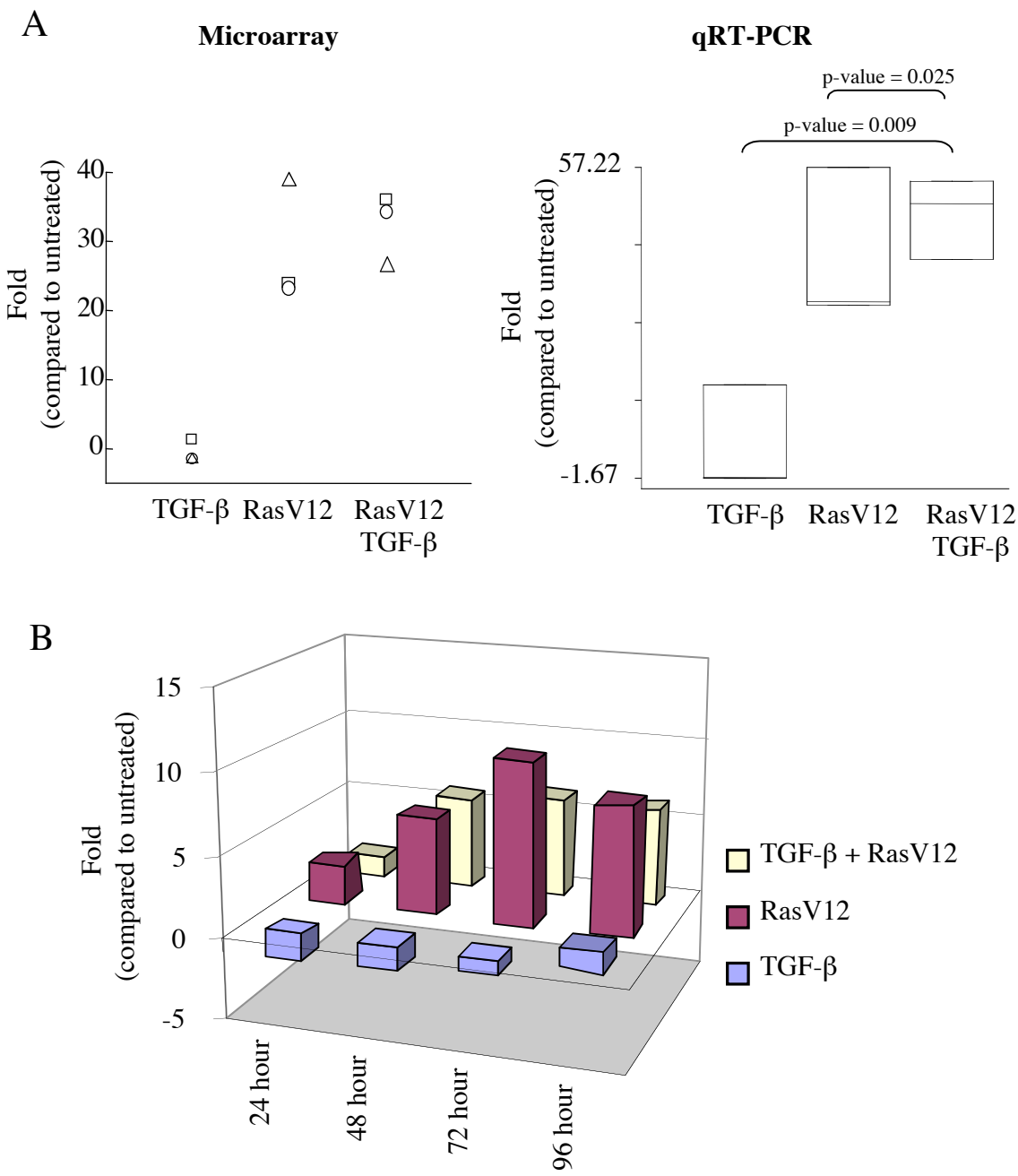


Figure 8: Oncogenic Ras and TGF-β cooperatively increase TGF-α mRNA expression. (**A**) TGF-α mRNA expression**.** Microarray intensity values. RNA was isolated from RIE:iRas cells treated for 72 hours with 5mM IPTG, 3ng/ml TGF-β, or IPTG and TGF-β together and expression of TGF-α quantified by real time RT-PCR. Changes in gene expression under treated conditions were calculated relative to untreated samples and all values were normalized to the housekeeping gene Pmm1. Dotted lines show mean expression for each treatment. Significance was determined by ANOVA with Bonferroni correction. Box plot shows data from three independent experiments. (**B**) Quantitation of TGF-α mRNA induction by real time RT-PCR, as above. Bar graph shows data from one representative experiment.

Validation of this increase in TGF-α expression after 72 hours of Ras induction or TGF-β treatment was confirmed by qRT-PCR (Figure 8A). Although the increase in TGF-α expression occurs primarily in response to oncogenic Ras expression, treatment of Ras expressing cells with TGF-β modestly increases TGF-α mRNA levels. The kinetics of TGF-α induction were measured by qRT-PCR after 24 to 96 hours of treatment with TGF-β and/or induction of RasV12 (Figure 8B). TGF-β did not induce TGF-α at any time point, as noted after 72 hour of treatment. Oncogenic Ras induction of TGF-α peaks within 48 hours and remains elevated for more than four days. In this representative experiment TGF-β treatment did not augment Ras induction of TGF-α

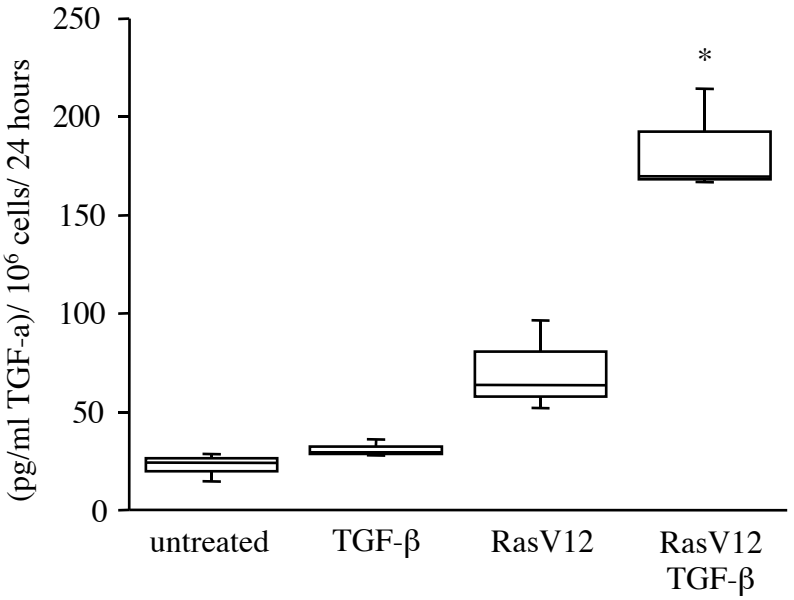
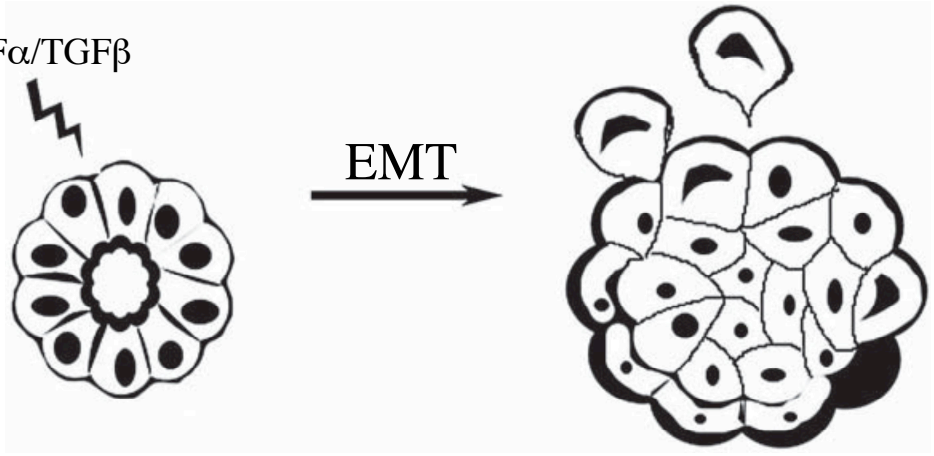


Figure 9: Oncogenic Ras and TGF-β cooperatively increase TGF-α protein expression. TGF-α protein expression in RIE:iRas cell lysates treated with 5 mM IPTG and/or 3 ng/ml TGF-β for 24 hours was measured by RIA. Box plot shows data from 3 independent experiments. *p-value<0.004 compared to all other treatments (ANOVA).

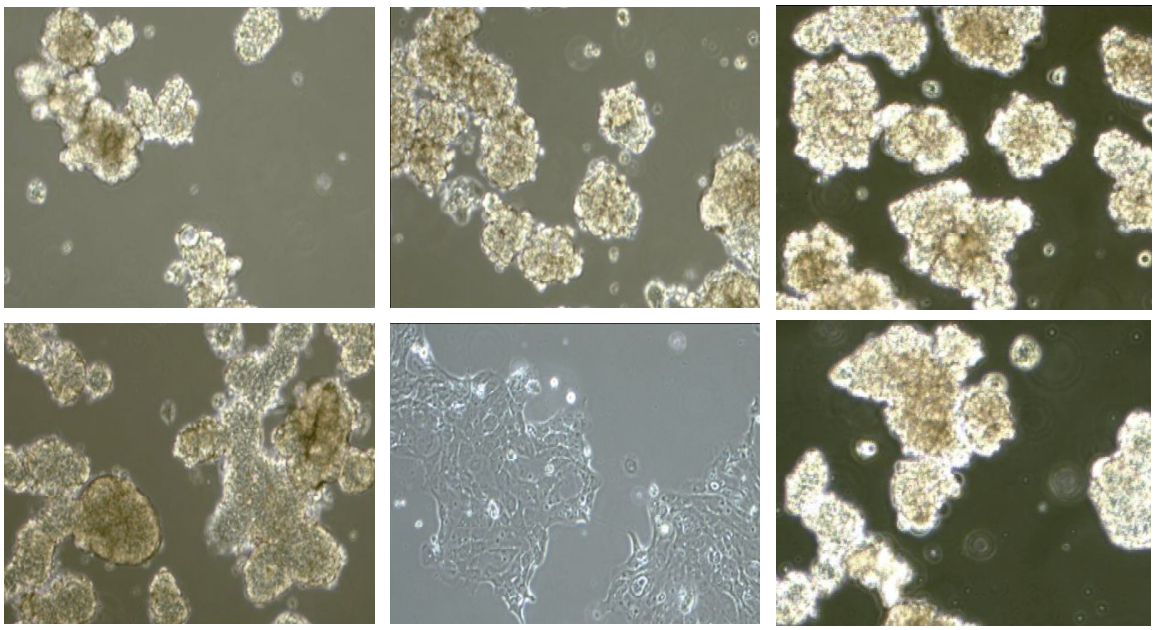
mRNA. Regulation of TGF-α expression was also examined at the protein level (Figure 9), as detectable in cell lysates using a highly sensitive ELISA developed by Dr. Robert Coffey (Vanderbilt University). Oncogenic Ras expression induced a 3-fold increase in TGF-α protein compared to untreated cells while TGF-β alone did not affect TGF-α expression, similar to the mRNA expression pattern. TGF-β treatment and RasV12 expression together synergistically increased TGF-α expression more than 8-fold.

Based on the observation that Ras and TGF-β synergistically upregulate TGF-α expression in RIE:iRas cells, a potential role for TGF-α in the EMT response to TGF-β was extended to the human colon cancer cell line, LIM1863, developed Dr. Robert Whitehead (Whitehead et al., 1987). LIM1863 cells are well-differentiated colon carcinoma cells that grow in suspension as organoids around a central lumen. TGF-β induces nearly 100% of the floating organoids to attach to the plastic substrate and begin to spread into a monolayer exhibiting phenotypic characteristics of EMT (Figure 10A) (Bates and Mercurio, 2003). While TGF-β-induced attachment occurs within 24 hours of treatment, migration into a monolayer and loss of E-cadherin occur over a period of 3-7 days, a process which is dramatically accelerated by treatment with tumor necrosis factor α (TNFα) (Bates and Mercurio, 2003). TGF-β-induced attachment is specific, as demonstrated by the ability of a TβRI tyrosine kinase inhibitor, LY364947 (TRKi), to block attachment of LIM1863 cells (Figure 10B). Furthermore, TGF-β treatment of LIM1863 cells induced a significant increase in TGF-α protein expression (Figure 10C), consistent with our data from RIE:iRas cells.

A


TNFα/TGFβ
EMT
Suspension,
3-D morphology
Adhesion,
migration

B


untreated
TNF-α
TRKI
TGF-β
TGF-β+TNF-α
TRKI + TGF-β

C

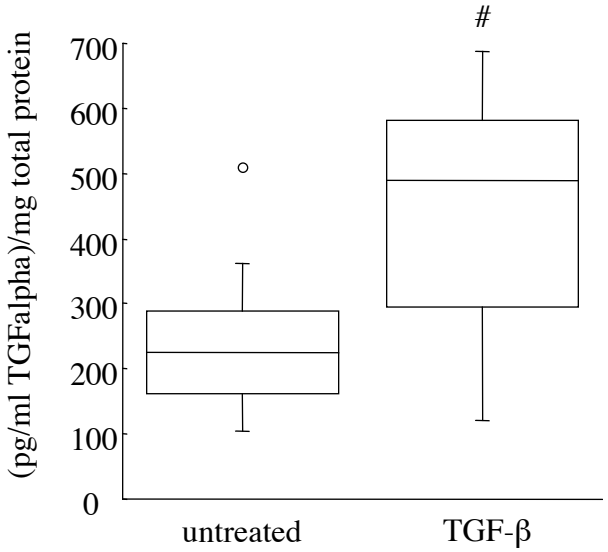


Figure 10: TGF-β induces EMT and TGF-α expression in LIM1863 cells. (A) Diagram of TGF-β-induced attachment and EMT from Bates et al., 2007 Cells Tissues Organs 185:29. (**B**) LIM1863 cells were treated with or without 2 ng/ml TGF-β and/or 10 ng/ml TNF-α for 48 hours. Phase-contrast photomicrographs show floating and attached cells (100x). (**C**) TGF-α protein expression in LIM1863 cell lysates treated with or without 2 ng/ml TGF-β for 48 hours was measured by RIA. Box plot shows data from 5 independent experiments. # p-value = 0.003 (ANOVA).

EGFR activity during EMT

Activation of EGFR during TGF-β-induced EMT. In order to determine whether TGF-α, or other EGFR ligands, activate EGFR during TGF-β-induced EMT, phosphorylation of EGFR was examined. RIE:iRas cells were pretreated with or without EKI-785 and then treated for 24 hours with IPTG and/or TGF-β. Western blotting for EGFR phosphorylation at tyrosine 1068 revealed that TGF-β and Ras expression, alone or together, induce transient phosphorylation of EGFR (Figure 11A).

EGFR phosphorylation was also examined in LIM1863 cells. Immunoprecipitation of EGFR and western blotting for phospho-EGFR showed that EGFR is phosphorylated and activated within 4 days of TGF-β treatment, at a time when

the cells are attached and spreading into a monolayer (Figure 11B). Phosphorylated and total EGFR levels decrease over the following two days.

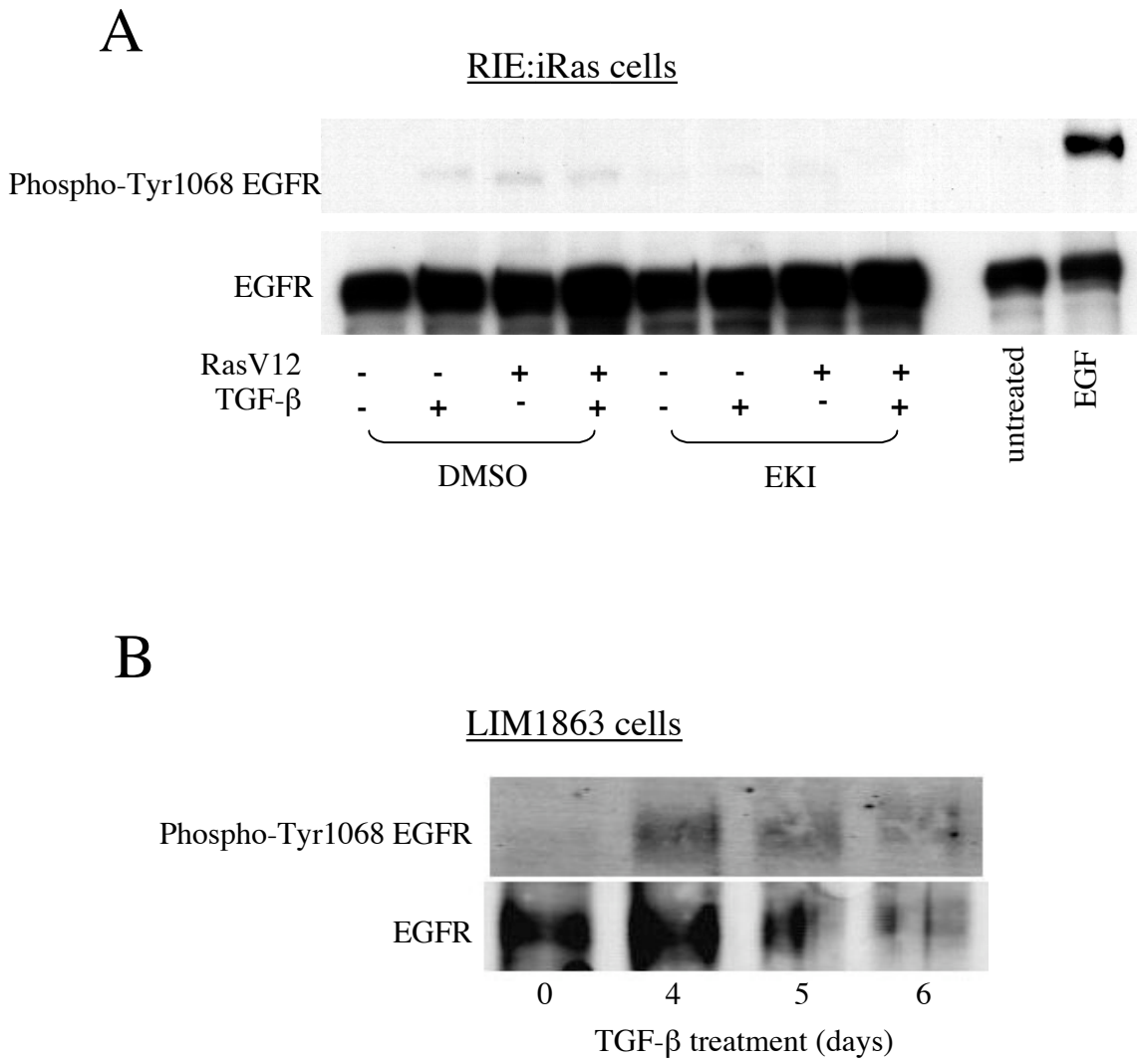


Figure 11: EGFR is phosphorylated during TGF-β-induced EMT. (**A**) RIE:iRas cells were pretreated for 15 minutes with DMSO or 1 μM EKI, then treated for 24 hours with or without 5 mM IPTG and/or 3 ng/ml TGF-β. Western blotting was performed on cell lysates for phospho and total EGFR. (**B**) LIM1863 cells were treated 0 to 6 days with 2ng/ml TGF-β. EGFR was immunoprecipitated from cell lysates and Western blotting performed for total EGFR and phospho-Tyr1068 EGFR.

Blocking EGFR activity prevents TGF-β-induced EMT. To determine whether TGF-α expression and subsequent activation of EGFR is necessary for TGF-β-induced EMT, cells were treated with an EGF receptor tyrosine kinase inhibitor (EKI-785). RIE:iRas cells were pretreated with EKI-785 then treated with TGF-β or IPTG to induce RasV12 expression. After 72 hours, combined Ras expression and TGF-β treatment induced a spindle-shaped phenotype and EMT in RIE:iRas cells (Figure 12). Although the EKI-785, IPTG, and TGF-β treated cells were less organized and cuboidal than untreated cells, treatment with EKI-785 blocked the Ras and TGF-β-induced transition to a fibroblastoid phenotype and prevented the cells from piling up on each other to form foci.

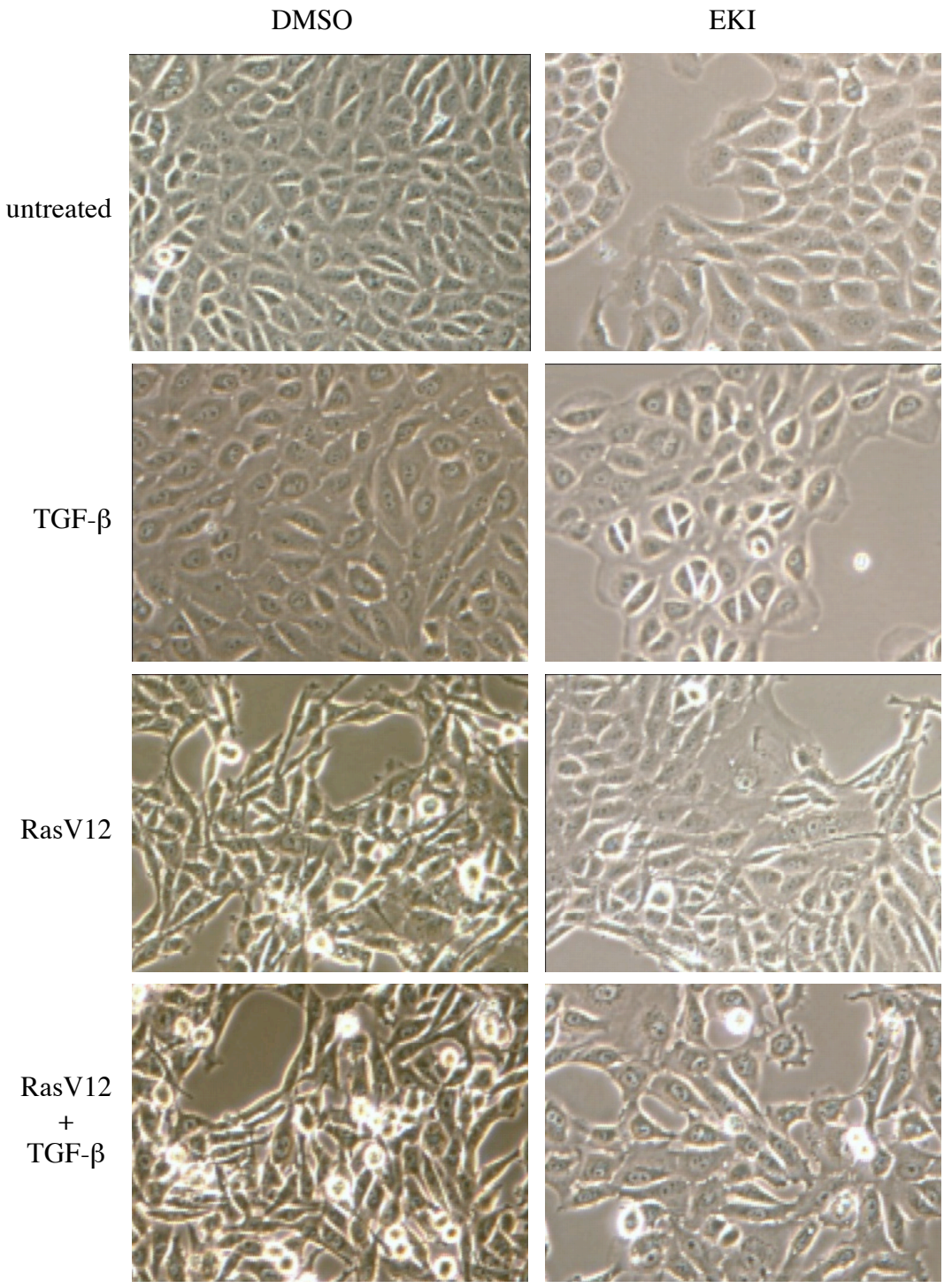


Figure 12: EGFR activity required for TGF-β-induced EMT in RIE:iRas cells. Cells were pretreated for 15 minutes with DMSO or 1 μM EKI, then treated for 72 hours with or without 5 mM IPTG and/or 3 ng/ml TGF-β. Phase-contrast photomicrographs show cell morphology (100x).

Similar results were observed in LIM1863 cells. The organoids were pretreated with or without EKI-785, treated with or without TGF-β for 24hrs, and then the cells were photographed before and after through washing with PBS to remove any floating cells. Treatment with EKI-785 prevented TGF-β-induced attachment of LIM1963 cells to the plastic substrate (Figure 13). The Ras-MEK-ERK pathway is activated downstream of EGFR signaling. LIM1863 cells were pretreated with the MEK inhibitor U0126 and then treated with TGF-β. Blockade of MEK-ERK signaling prevented TGF-β-induced attachment of LIM1863 cells (Figure 14). Interestingly, while activation of EGFR signaling is required for TGF-β-induced EMT in these cells signaling, addition of exogenous EGFR ligands, amphiregulin, TGF-α, or EGF, is not able to accelerate TGF-β-induced attachment of LIM1863 cells (Figure 15), suggesting that EGFR ligand endogenous ligand production is sufficient to saturate receptor signaling.

EGFR activity is necessary for TGF-β-induced invasion. The role of EGFR signaling plays in malignant cells behaviors, such as invasion and migration, was examined. RIE:iRas cells were treated with or without EKI-785 and in the presence or absence of IPTG and TGF-β. Cell invasion through matrigel coated transwells was quantified after 72 hours of treatment. Untreated or EKI-785 treated cells were not invasive, while oncogenic Ras expression and TGF-β treated cells were highly invasive (Figure 16A). Inhibition of EGFR signaling with EKI-785 treatment significantly decreased cell invasion. Similarly, LIM1863 cells are normally not invasive (Figure 16B). While TGF-β and TNFα transformed cells were highly invasive, EKI-785 treatment blocked cell invasion.

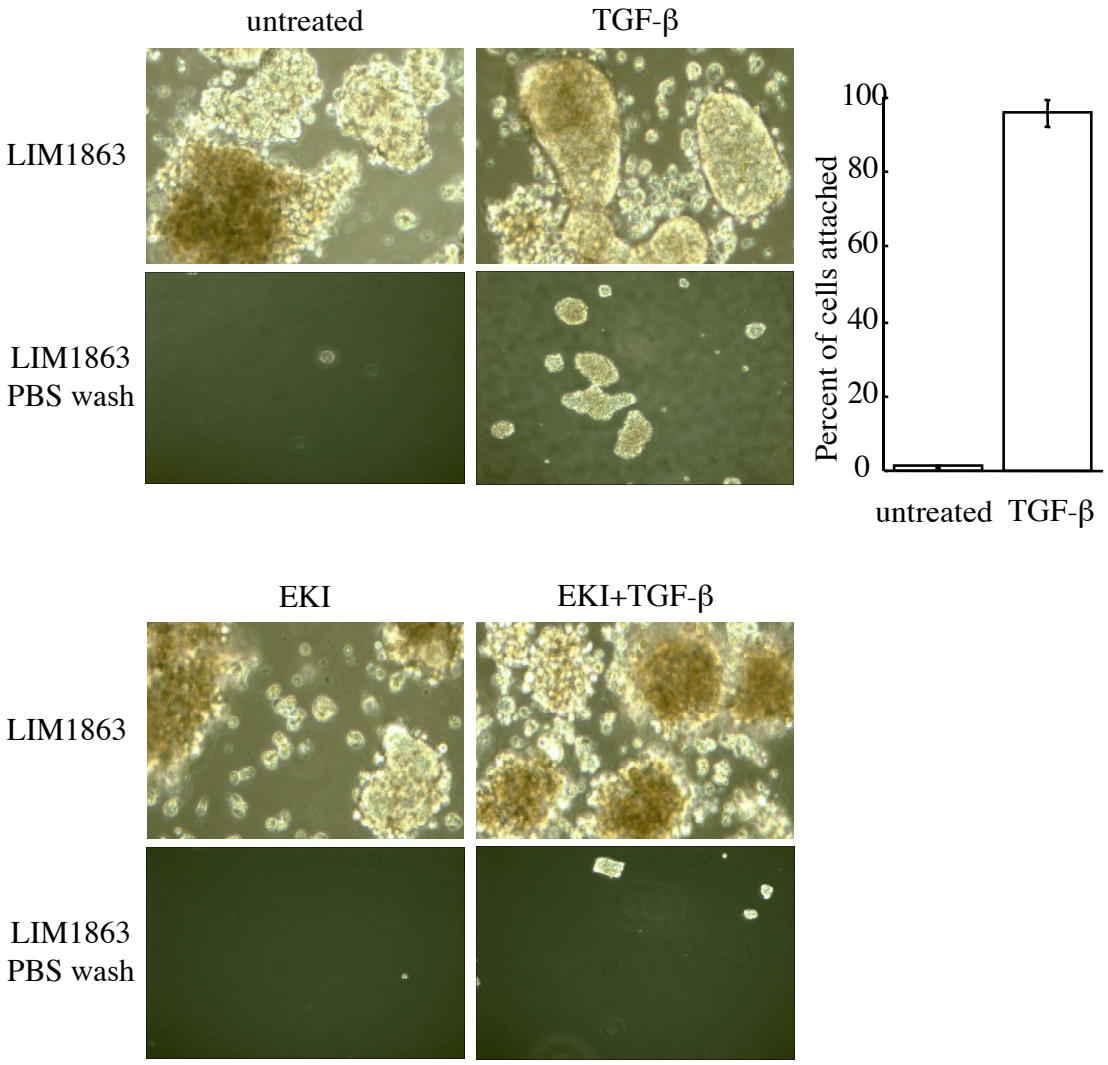


Figure 13: EGFR activity required for TGF-β-induced EMT in LIM1863 cells. LIM1863 cells were treated with or without 2 ng/ml TGF-β and 10 ng/ml TNF-α for 48 hours. Floating were collected, including two PBS washes, and attached cells were detached with trypsin. Phase-contrast photomicrographs show floating and attached cells before (400x) and after PBS washing (100x). Both floating and attached cells from untreated and TGF-β treated samples were disrupted into single cell suspensions and counted on a hemocytometer. Bar graph represents the average of three independent experiments +/- SD. p-value<0.0001 (ANOVA).

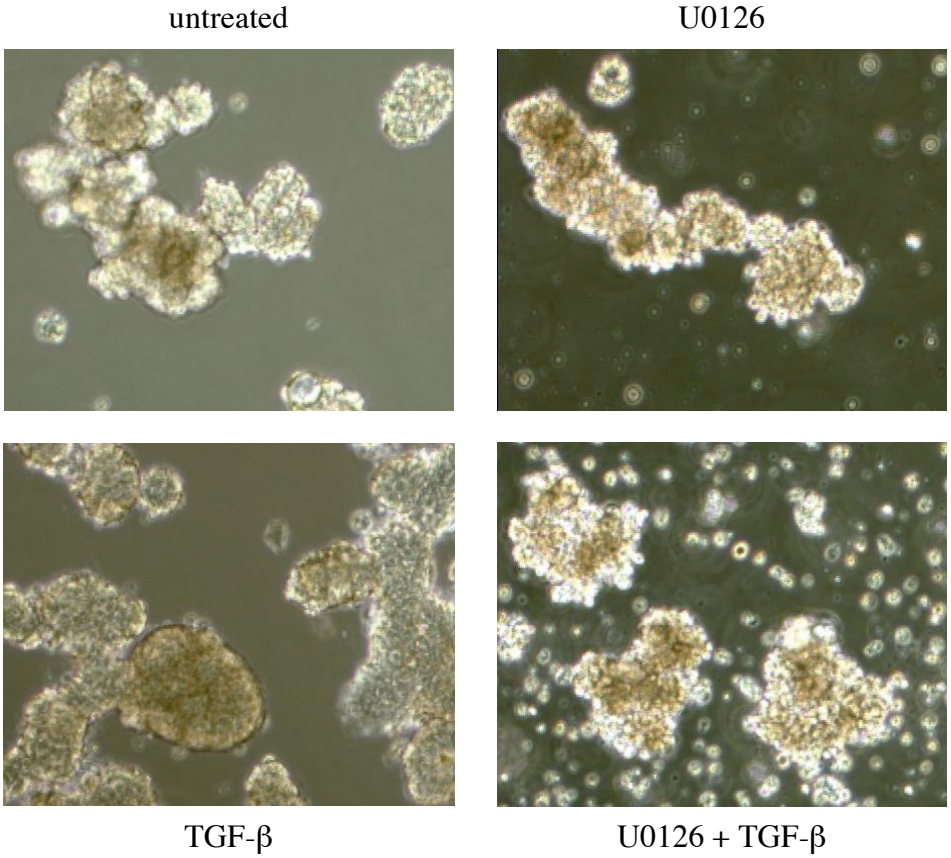


Figure 14: MEK-ERK activity required for TGF-β-induced EMT in LIM1863 cells. Cells were pretreated with 10 μM U0126 for 30 minutes, then treated with or without 2 ng/ml TGF-β for 48 hours. Phase-contrast photomicrographs show floating and attached cells (200x).

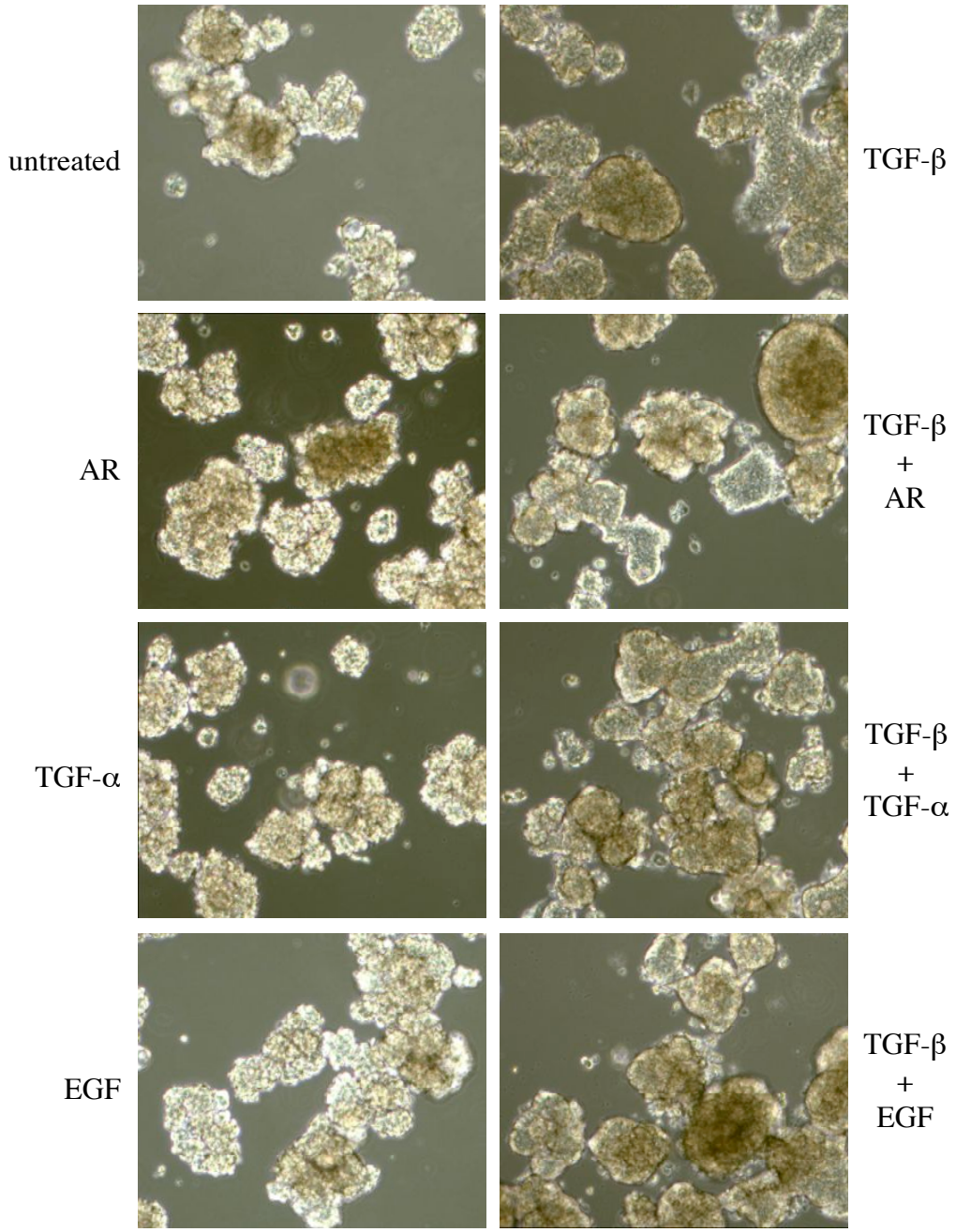


Figure 15: Exogenous EGFR ligands do not augment TGF-β-induced EMT in LIM1863 cells. Cells were left untreated or treated with EGFR ligand (AR, TGF-α, or EGF) and simultaneously treated with or without 2 ng/ml TGF-β for 48 hours. Phase-contrast photomicrographs show floating and attached cells (200x).

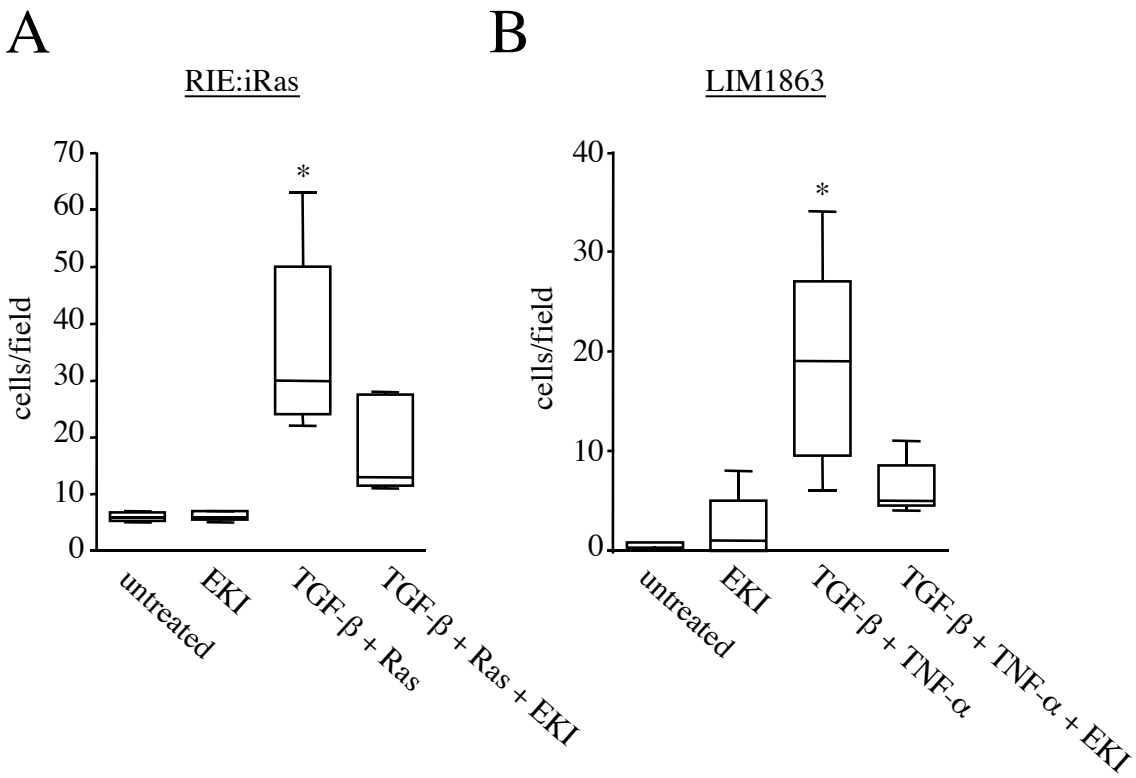


Figure 16: EGFR activity required for TGF-β-induced cell invasion. Cells were plated Matrigel coated Transwells and treated. After 72 hours, cells were removed from the top of the filter, then the bottom was fixed and mounted with DAPI mounting media. (**A**) RIE:iRas cells were grown in the presence of DMSO, 1 μM EKI, 5 mM IPTG and 3 ng/ml TGF-β, or IPTG, TGF-β, and EKI. (**B**) LIM 1863 cells were grown in DMSO, 1 μM EKI, 10 ng/ml TNF-α and 2 ng/ml TGF-β, or TNF-α, TGF-β, and EKI. Five high power fields (hpf) of cells were counted per filter. *p-value < 0.05 for TGF-β + Ras/TNF-α vs. all other treatments (ANOVA).

Summary

Microarray analysis reveals that oncogenic Ras and TGF-β cooperate to alter the expression of the EGF receptor and several EGF-related ligands. In addition, an increase in EGFR phosphorylation and activation was observed in both RIE:iRas cells and the LIM1863 colon cancer cell line during TGF-β-induced EMT. TGF-α is one potential mediator of increased EGFR activity in these cells. While treatment with TGF-β

modestly augments the oncogenic Ras-induced increase in TGF-α mRNA expression, TGF-β show marked synergy with RasV12 in increasing TGF-α protein expression, suggesting that the cooperation between Ras and TGF-β signaling occurs through a post-transcriptional mechanism by increasing TGF-α mRNA stability, translation efficiency, or TGF-α protein stability. Similar increases in TGF-α expression were observed in LIM1863 cells after TGF-β treatment, demonstrating that increased TGF-α expression is associated with TGF-β-induced EMT in multiple model systems. Increased EGFR activity has profound effects on cell behavior since blocking EGFR signaling with the small molecule EKI-785 inhibited the phenotypic transformation of both RIE:iRas and LIM1863 cells. Furthermore, EGFR inhibition was able to block TGF-β-induced invasion of both cell lines. These results provide evidence of an important role for EGFR signaling during the malignant transformation of intestinal epithelial cells.

CHAPTER V

ONCOGENIC RAS AND TGF-β REGULATE VEGF AND OTHER GENES BY A POST-TRANSCRIPTIONAL MECHANISM

Introduction

mRNA stability

Gene expression is tightly regulated at multiple levels including transcription, mRNA stability, translation efficiency, and protein stability. There is growing evidence that mRNA turnover plays a central role in the regulation of gene expression. The rate at which RNA is degraded is a key point of regulation, particularly for immediate early genes, such as myc, which are tightly regulated and often have unstable RNAs. Many of these same factors are known mediators of oncogenic transformation, whose expression is significantly enhanced in cancer, partially through mRNA stabilization, including proto-oncogenes, growth factors and cytokines (Audic and Hartley, 2004).

The two main components responsible for regulating RNA stability and translation efficiency are *cis*-elements and trans-acting factors. One of the most important *cis*-acting elements in mRNA is the adenylate/uridylate-rich regions, or AU-rich elements (ARE), in the 3' untranslated region (3'UTR) of messenger RNA molecules (Caput et al., 1986; Shaw and Kamen, 1986). AREs contain multiple adenylate-uridylate AUUUA repeats and often have a high uridylate content (Figure 17). The ARE targets mRNA for rapid and selective degradation and can inhibit translation (Akashi et al., 1994; Chen et al., 1994; Shaw and Kamen, 1986). *Trans*-acting factors also play an important role in

regulating mRNA stability. There are many RNA-binding proteins that bind the AUUUA motif and are able to modulate mRNA decay (Bevilacqua et al., 2003). These RNA-binding proteins can function as positive or negative regulators of mRNA stability or translational efficiency, in part by regulating the subcellular localization of mRNA (Hollams et al., 2002; Perrotti and Calabretta, 2002).

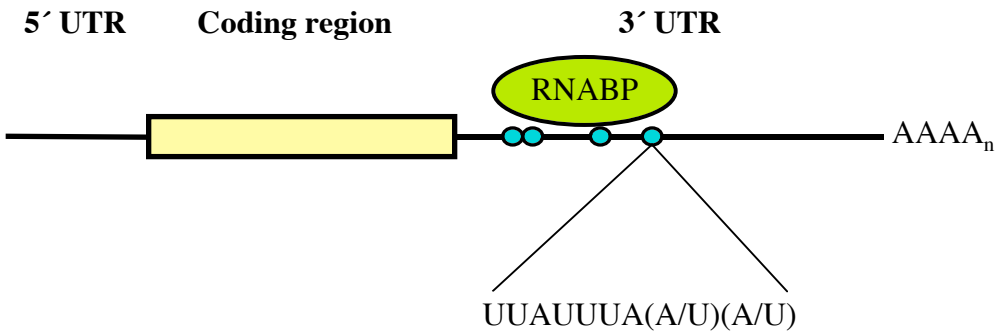


Figure 17: AU-rich element and mRNA structure. Illustration shows 5' untranslated region (5' UTR), coding region, and 3' untranslated region (3' UTR) of messenger RNA. Blue circles denote adenylate/uridylate rich elements (AREs) consisting of at least two ARE consensus sequences (AUUUA). *Trans*-acting factors, or RNA binding proteins (RNABPs) bind AREs and modulate mRNA stability and translation.

Altered mRNA stability is important in regulating tumor-associated genes that participate in cancer progression. For example, general mRNA turnover and RNase activity are higher in host livers than in transplanted hepatomas (Sidransky et al., 1978). Importantly, the mRNA of several normally unstable genes, such as cytokines and cell cycle regulators, are preferentially stabilized in tumor cells compared to normal cells (Lee et al., 1998; Ross et al., 1991). Rapid decay of c-myc mRNA is mediated by cis-elements in the 3'UTR, yet in certain cases of myeloma and leukemia, the c-myc 3'UTR is translocated or lost leading to increased c-myc mRNA stability (Hollams et al., 2002).

An mRNA binding protein, CRD-BP, thought to stabilize c-myc mRNA, is amplified in 35% of breast cancers (Doyle et al., 2000). We have recently shown that COX-2, an important factor in inflammation and carcinogenesis, is regulated on a post-transcriptional level through its relatively long AU-rich 3'UTR which contains numerous AREs sequences that are necessary for stabilization of COX-2 by oncogenic Ras and TGF-β (Sheng et al., 2000). Furthermore, activity of the RNA binding protein HuR has been associated with stabilization of COX-2 mRNA in ovarian cancer, brain tumors, and colon cancer (Denkert et al., 2004; Dixon et al., 2001; Nabors et al., 2001).

VEGF

Angiogenesis is an essential process of endothelial cell proliferation, migration and vessel formation that occurs during development and is necessary for tumor growth and progression, when the formation of nutrient supplying blood vessels is vital for tumors grow beyond 1-2mm in size (Folkman, 2002). Over-expression of VEGF has been observed in several different cancers including pancreatic and colon, where it is often highly expressed at an early stage and associated with tumor progression and metastasis (Fujimoto et al., 1998; Konno et al., 1998; Takahashi et al., 1995; Wong et al., 1999). Expression of VEGF is stimulated by a variety of conditions and factors, such as hypoxia, nitric oxide, several cytokines and growth factors and by the expression of oncogenes like *myc* (Gale and Yancopoulos, 1999; Hanahan and Folkman, 1996; Rak et al., 2000). In addition, oncogenic Ras and TGF-β have each independently been shown to increase steady state levels of VEGF mRNA and protein in a variety of cell types. TGF-β increases VEGF expression in lung, breast, and kidney cancer cell lines (Donovan et al.,

1997; Pertovaara et al., 1994; Wang et al., 2004) and oncogenic Ras increases VEGF expression in intestinal epithelial cells, endothelial cells, and keratinocytes (Arbiser et al., 1998; Rak et al., 2000; Segrelles et al., 2004).

VEGF regulation and mRNA stability

The transcriptional regulation of VEGF by oncogenes and growth factors has been well characterized, however recent evidence suggests that changes in VEGF mRNA stability contribute significantly to the regulation of VEGF expression. Stabilization of VEGF mRNA was first observed in response to low oxygen conditions, or hypoxia (Finkenzeller et al., 1995; Ikeda et al., 1995; Levy et al., 1995; Shima et al., 1995; Stein et al., 1995). This stabilization of VEGF mRNA requires the binding of RNA binding proteins, such as HuR and hnRNP L, to the ARE region of the VEGF mRNA 3'UTR (Dibbens et al., 1999; Levy et al., 1998; Shih and Claffey, 1999). In addition to hypoxic conditions, VEGF mRNA is stabilized by RasV12 expression in fibroblasts (White et al., 1997). VEGF mRNA was not stabilized by TGF-β in renal proximal tubular epithelial cells (Kitamura et al., 2003). However, these results should be viewed with some caution since the kinetics examined were not optimized for elucidating the stabilizing effects of TGF-β. Although it is clear that oncogenic Ras expression and TGF-β treatment are each able to induced VEGF expression, the mechanisms by which they regulate VEGF expression and their combined effects on VEGF are not well understood.

Results

Oncogenic Ras and TGF-β synergistically increase VEGF expression

One of the genes synergistically regulated by oncogenic Ras and TGF-β in the microarray experiment was VEGF, showing no change after TGF-β treatment, a 5.9-fold increase with RasV12 expression, and combined RasV12 and TGF-β further increased VEGF mRNA expression to 7.5-fold. Since the angiogenic factor VEGF has a well-established role in promoting cancer progression and metastasis (Fujimoto et al., 1998; Konno et al., 1998; Takahashi et al., 1995; Wong et al., 1999), we conducted a thorough and in depth analysis of VEGF mRNA and protein levels induced by activated Ras in combination with TGF-β exposure. First, VEGF mRNA expression patterns were validated by Northern blotting mRNA from RIE:iRas cells treated for 24 hours with IPTG to induce oncogenic Ras expression, TGF-β, or both IPTG and TGF-β. Steady state VEGF mRNA levels are very low in untreated RIE:iRas cells and increase 6- and 4-fold after IPTG induction of RasV12 or TGF-β treatment, respectively, but increase more than 13-fold with RasV12 expression and TGF-β together (Figure 18A). TGF-β maximally induces VEGF mRNA within 24 hours, after which VEGF is still expressed, albeit at lower levels (Figure 18B). Oncogenic Ras induces VEGF expression more slowly, delayed in part due to the lag between IPTG treatment and RasV12 expression, and steadily increases between 12 and 72 hours. The profound cooperative effect of Ras and TGF-β together on VEGF mRNA expression can be seen within 12 hours and is sustained for more than 72 hours.

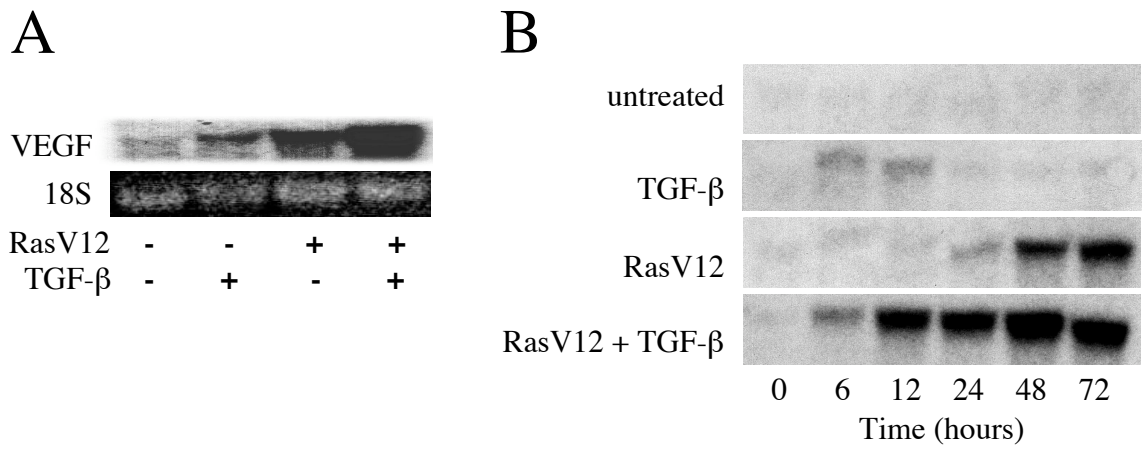


Figure 18: Oncogenic Ras and TGF-β synergistically increase VEGF mRNA expression in RIE:iRas. RIE:iRas cells were treated with either vehicle, 5mM IPTG, 3ng/ml TGF-β, or both IPTG and TGF-β. VEGF mRNA levels in RIE:iRas cells were visualized by Northern blot using a mouse $VEGF_{165}$ cDNA probe and 18S rRNA visualized with ethidium bromide. Northern blot is representative of at least three separate experiments. (**A**) VEGF mRNA expression after 24 hours of treatment. (**B**) Time course of VEGF mRNA expression.

Likewise, VEGF levels were determined by ELISA in conditioned media from RIE:iRas cells treated for 24 hours with IPTG, TGF-β, or both IPTG and TGF-β. Untreated RIE:iRas cells produce low levels of VEGF protein. Treatment of RIE:iRas cells with TGF-β or expression of oncogenic Ras alone increased VEGF protein levels in the media by 3-4 fold, whereas oncogenic Ras and TGF-β together synergistically increased VEGF protein levels more than 15-fold (Figure 19A). Similar results were observed after 72 hours of treatment (data not shown). The cooperative effects of RasV12 and TGF-β on VEGF protein expression were confirmed independently in young adult mouse colonocyte (YAMC) cells and YAMC cells stably transfected with RasV12 (YAMC-Ras). Oncogenic Ras expression increased VEGF expression in YAMC-Ras cells compared to parental cells and TGF-β treatment cooperated with RasV12 to further

increase VEGF expression (Figure 19B). These data demonstrate that oncogenic Ras and TGF-β cooperatively regulate VEGF expression in multiple gastrointestinal epithelial cell lines.

Induction of VEGF by TGF-β treatment is dose-dependent, leveling out at 0.2ng/ml TGF-β (Figure 19C). IPTG-induced oncogenic Ras expression also stimulated VEGF in a dose dependent manner (Figure 19D), however the nature of the lac operon controlled inducible system relies more on a threshold IPTG dose to induce Ras expression than on a gradient response. However, TGF-β treatment and RasV12 induction together have a synergistic interaction on the production of VEGF (all $p<0.0001$, test for interaction in regression). These results demonstrate the dose-dependent and synergistic induction of VEGF expression stimulated by Ras and TGF-β together compared to each alone.

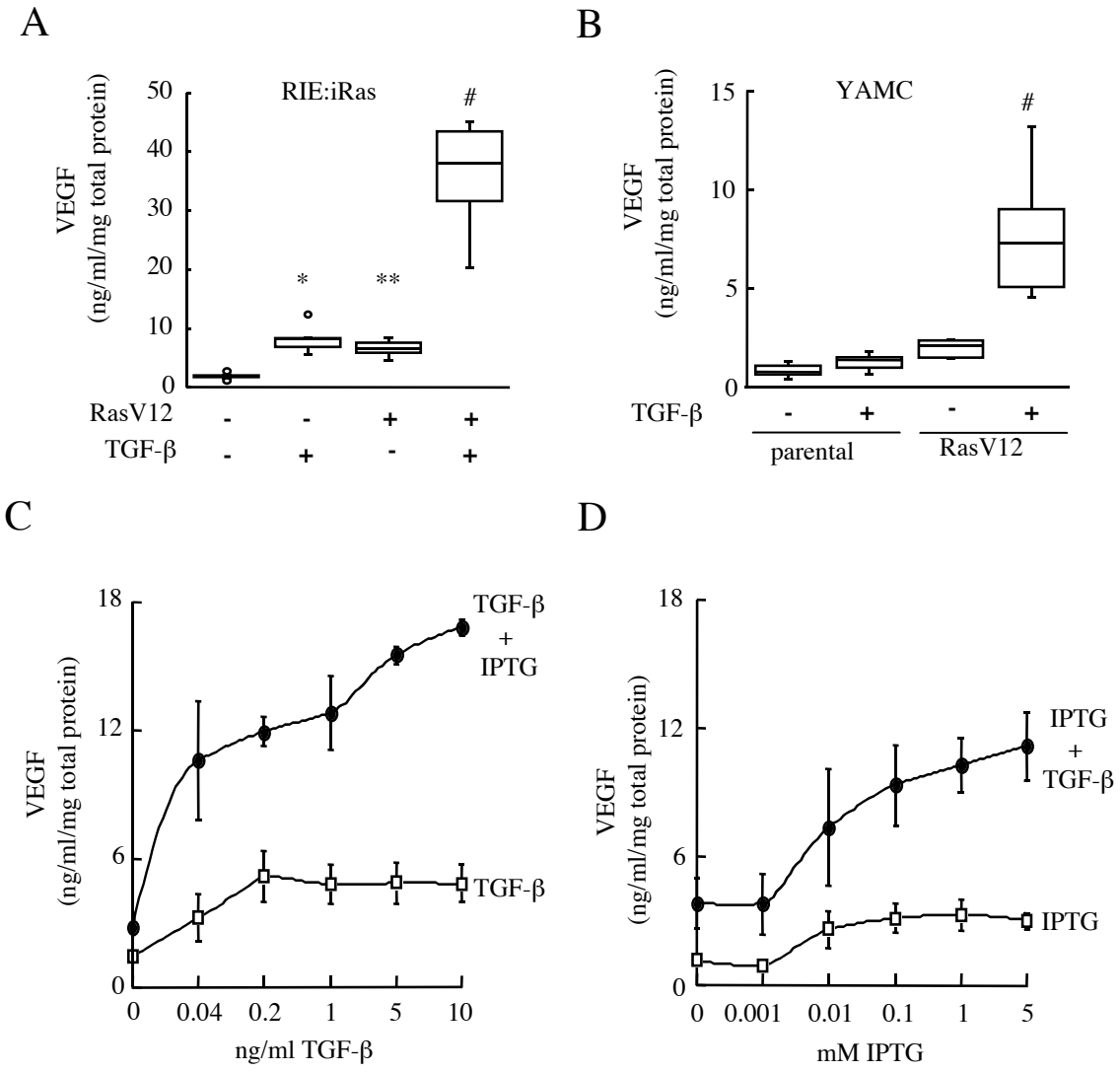


Figure 19: Oncogenic Ras and TGF-β synergistically increase VEGF protein expression in RIE:iRas and YAMC cells. (A) RIE:iRas cells were treated with either vehicle, 5mM IPTG, 3ng/ml TGF-β, or both IPTG and TGF-β for 24 hours. VEGF protein levels were measured in conditioned media by ELISA and normalized to total protein concentrations. Box plot shows data summarized from seven independently replicated experiments. *p-value <0.004 compared to untreated. **p-value <0.03 compared to untreated. #p-value <0.002 compared to all treatments. **(B)** YAMC and YAMC-Ras cells were treated with or without 5ng/ml TGF-β for 24 hours then VEGF levels were measured in conditioned media by ELISA and normalized to total protein concentrations. Box plot shows data from eight independently replicated experiments. #p-value <0.0001 compared to all samples. **(C-D)** RIE:iRas cells were treated with IPTG and/or TGF-β at varying doses for 24 hours and VEGF levels were measured in conditioned media by ELISA and normalized to total protein concentrations. Cells were treated with or without 5mM IPTG and 0, 0.04, 0.2, 1, 5, or 10 ng/ml TGF-β or cells were treated with or without 3ng/ml TGF-β and 0, 0.001, 0.01, 0.1, 1, or 5 mM IPTG. Graphs show average of three independent experiments +/- SE. Dose-dependence and synergistic interaction was confirmed via multiple linear regression (all p<0.0001).

Signaling pathways involved in VEGF regulation

TGF-β and MEK-ERK signaling. An inhibitor of the kinase activity of TGF-β type I and type II receptors, which blocks their ability to activate down stream effectors, was used for two purposes: first, to confirm the specificity of TGF-β treatment and second, to determine whether oncogenic Ras expression induces expression of TGF-β in the RIE:iRas cells and whether this TGF-β then acts alone or in concert with RasV12 to increase VEGF expression. TGF-β treatment, alone or with Ras induction, phosphorylates Smad2, and this phosphorylation is blocked by TRKi pretreatment. Inhibition of the kinase activity of TβRI and TβRII with LY364947 (TRKi) blocks the TGF-β-mediated increase in VEGF protein and mRNA expression (Figure 20A). However, TβRI and TβRII activity is not necessary for the oncogenic Ras-mediated increase in the expression of VEGF protein, but TRKi does decrease VEGF mRNA levels slightly. The synergistic increase in VEGF protein and mRNA expression by simultaneous induction of oncogenic Ras and TGF-β treatment is blocked by inhibition of TβRI/II kinase activity, lowering it to the same level as oncogenic Ras induction alone (Figure 20A). These data demonstrate that the TGF-β-receptors type I and type II (TβRI and TβRII) are required for TGF-β but not RasV12 to increase VEGF expression.

The Raf-MEK-ERK signaling cascade is activated by oncogenic Ras and required for Ras and TGF-β-induced EMT in keratinocytes (Janda et al., 2002). The MEK inhibitor, U0126, was used to determine whether oncogenic Ras and TGF-β utilize MEK/ERK signaling to increase VEGF expression. U0126 pretreatment prevents ERK phosphorylation in response to oncogenic Ras expression or TGF-β treatment (Figure 20B). Inhibition of MEK with U0126 completely blocks VEGF protein and mRNA

expression induced by oncogenic Ras and TGF-β, alone or in combination (Figure 20B). Although ERK activity is required for either oncogenic Ras or TGF-β to induce VEGF, there was no additional activation of ERK by RasV12 and TGF-β together, so ERK activity may not be sufficient to account for the cooperative increase in VEGF expression by oncogenic Ras and TGF-β.

PI3 kinase activity contributes to oncogenic Ras and TGF-β induction of VEGF. The PI3 kinase-Akt signal transduction pathway is another of the prominent pathways activated by oncogenic Ras and TGF-β (Rommel and Hafen, 1998; Zavadil and Bottinger, 2005). To address whether oncogenic Ras and TGF-β increase VEGF expression by signaling through PI3 kinase and its downstream effector, Akt, activation status of PI3K/Akt was assessed in RIE:iRas cells. Oncogenic Ras and TGF-β induce phosphorylation and activation of Akt, either alone or together (Figure 21). Inhibition of PI3K with LY294002 blocks most of the oncogenic Ras-induced increase in VEGF mRNA and protein but has no effect on the TGF-β-induced increase in VEGF expression (Figure 21A). In the presence of the PI3K inhibitor, oncogenic Ras and TGF-β still cooperatively increased VEGF expression, albeit not to the same level as in the absence of the inhibitor. Similar results were obtained with YAMC and YAMC-Ras cells treated with and without TGF-β and LY294002 (Figure 21B). This suggests that while the PI3K/Akt pathway has a role in the cooperative increase in VEGF by oncogenic Ras and TGF-β, there appears to be a PI3K-independent component.

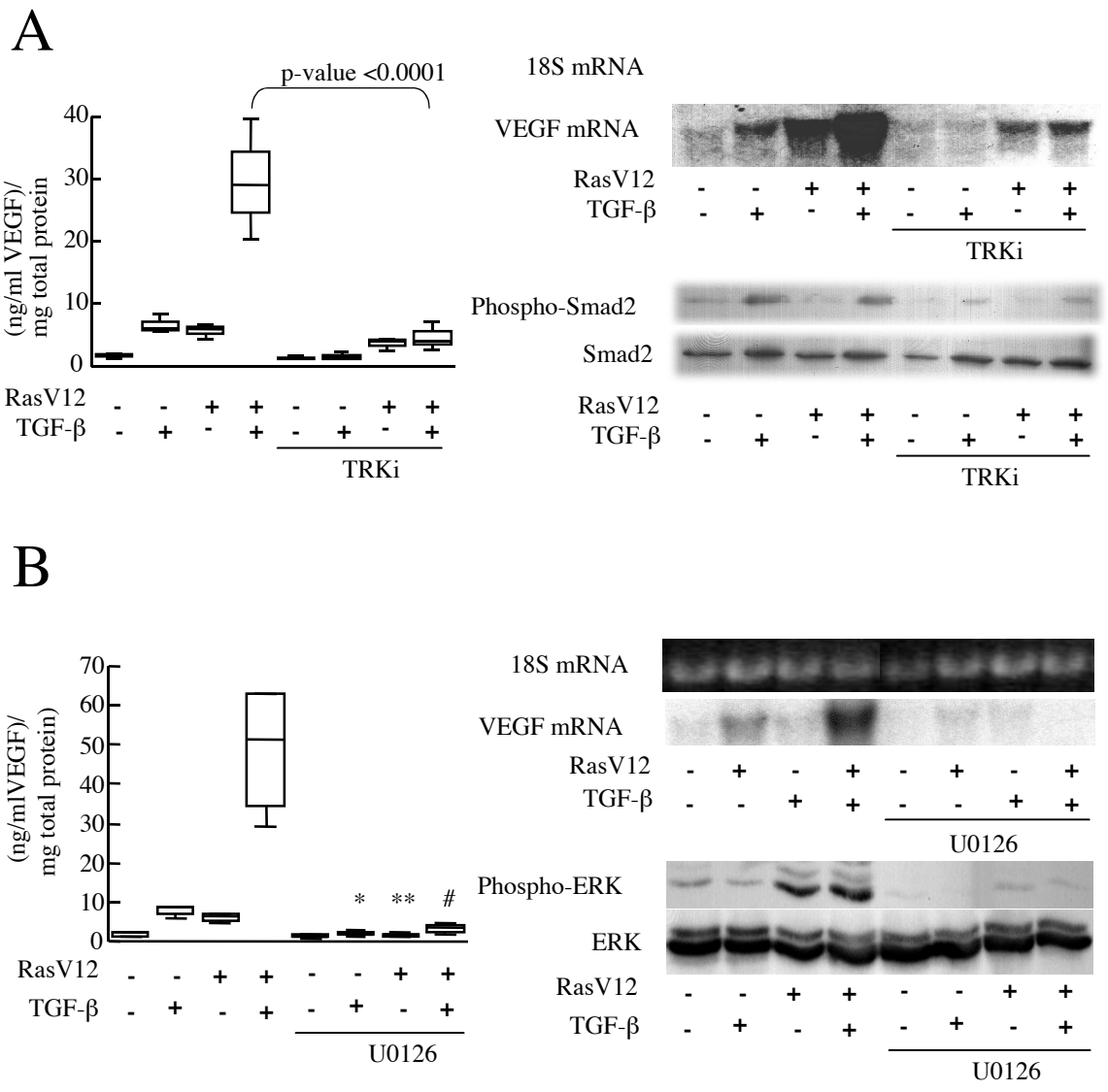
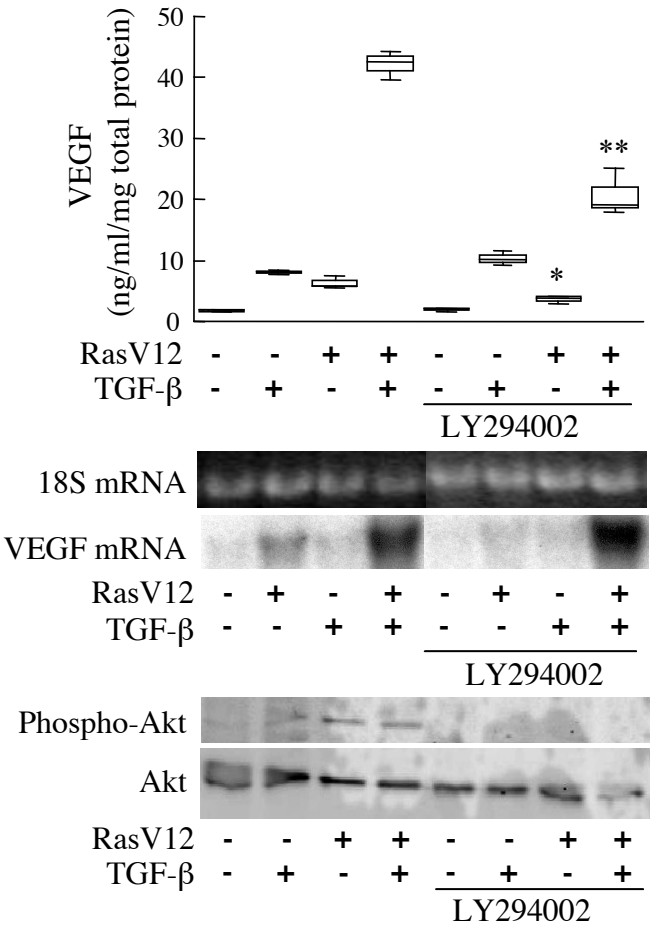


Figure 20: TβRII and ERK activity are necessary for oncogenic Ras and TGF-β to increase VEGF expression in RIE:iRas cells. RIE:iRas cells were pretreated for 15 minutes with a **(A)** TβRII inhibitor (2μM TRKi) or **(B)** MEK inhibitor (10μM U0126) then treated for 24 hours with 5mM IPTG and/or 3ng/ml TGF-β. VEGF protein levels were assayed by ELISA and normalized to total protein levels. VEGF Northern blot (with18S rRNA loading control band) is shown along with corresponding Western blot of phosphorylated and total Smad2 or ERK. (**A**) Box plot shows data from three independently replicated experiments. (**B**) Box plot shows data from four independently replicated experiments. *p-value = 0.17 compared to TGF-β. **p-value = 0.31 compared to Ras. #p-value <0.0001 compared to Ras + TGF-β.

A


VEGF
(ng/ml/mg total protein)
50
40
30
20
10
0
**
*
RasV12 - - + + - - + +
TGF-β - + - + - + - +
LY294002
18S mRNA
VEGF mRNA
RasV12 - + - + - + - +
TGF-β - - + + - - + +
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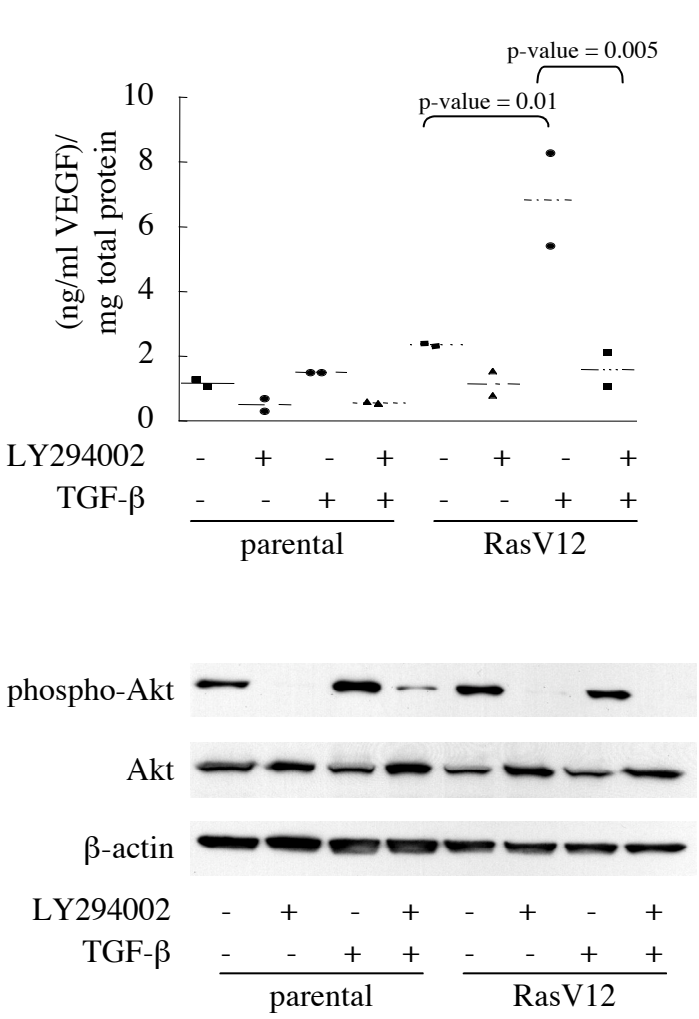


Figure 21: Synergistic induction of VEGF by oncogenic Ras and TGF-β is PI3 kinase and Akt dependent. (**A**) RIE:iRas cells were pretreated for 15 minutes with a PI3K inhibitor (20μM LY294002) then treated for 24 hours with 5mM IPTG and/or 3ng/ml TGF-β. VEGF protein levels were assayed by ELISA and normalized to total protein levels. Box plot shows data from four independently replicated experiments. *p-value<0.08 compared to Ras. **p-value<0.0001 compared to Ras + TGF-β (ANOVA). VEGF Northern blot (with18S rRNA loading control band) is shown along with corresponding Western blot of phosphorylated Akt and total Akt. (**B**) YAMC and YAMC-Ras cells were pretreated for 15 minutes with a PI3K inhibitor (20μM LY294002) then treated for 24 hours with or without 5ng/ml TGF-β. VEGF protein levels were assayed by ELISA and normalized to total protein levels. Dot plot shows data from two independently replicated experiments. Western blot showing phosphorylated Akt and total Akt.

p38-independent induction of VEGF expression. Another candidate signaling cascade that may contribute to the regulation of VEGF is the p38 MAPK signaling pathway, which is activated during TGF-β-induced EMT in keratinocytes (Davies et al., 2005). A recent study demonstrated that p38 signaling down regulates VEGF expression in H-RasV12 transformed liver epithelial cells (Okajima and Thorgeirsson, 2000). Expression of oncogenic Ras and treatment with TGF-β does not phosphorylate and activate p38 MAPK in RIE:iRas cells (Figure 22). Inhibition of p38 activity with SB203580 increases Ras and TGF-β-induced expression of VEGF mRNA and protein (Figure 22).

EGFR signaling is required for increased VEGF expression. TGF-α treatment of keratinocytes induces bioactive VEGF expression (Detmar et al., 1994). My previous data demonstrate that RasV12 expression and TGF-β treatment activate EGFR signaling in RIE:iRas cells (Chapter IV). To examine whether EGFR activation affects VEGF expression, RIE:iRas cells were pretreated with EKI-785, treated with IPTG and/or TGF-β, then conditioned media collected and levels of secreted VEGF were measured. EKI-785 blockade of EGFR signaling inhibited TGF-β or Ras-induced VEGF expression (Figure 23A). In addition, EKI-785 significantly reduced VEGF expression induced by oncogenic Ras and TGF-β together. Similar results were observed in another colon cancer cell line, YAMC cells (Figure 23B).

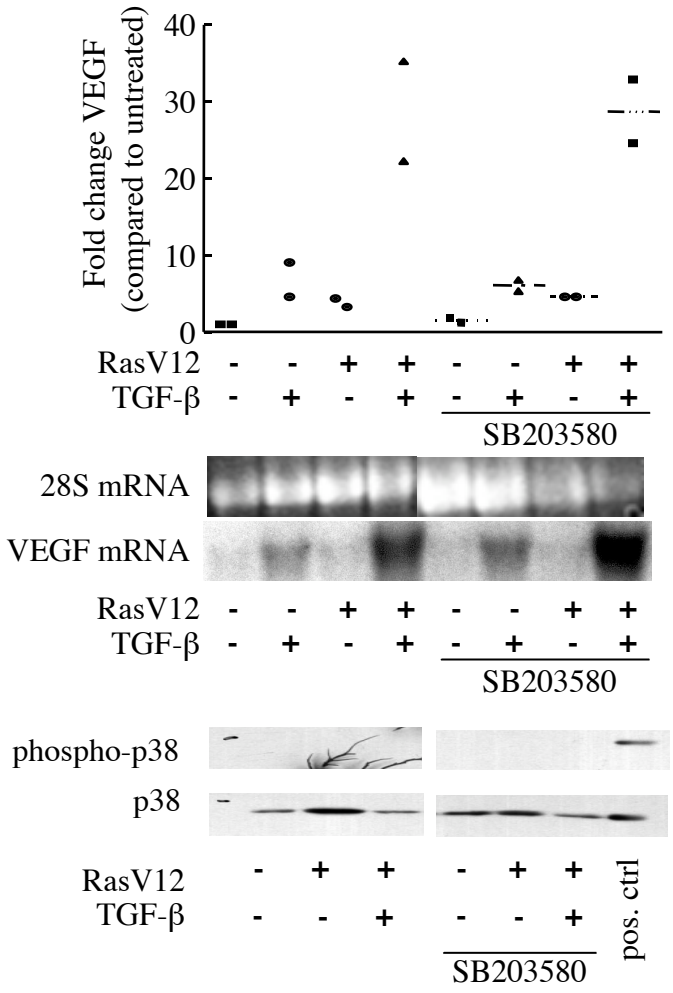


Figure 22: Synergistic induction of VEGF by oncogenic Ras and TGF-β does not require p38 activity. RIE:iRas cells were pretreated for 15 minutes with a p38 inhibitor (2μM SB203580) then treated for 24 hours with 5mM IPTG and/or 3ng/ml TGF-β. VEGF protein levels were assayed by ELISA and normalized to total protein levels. Dot plot shows data from two independently replicated experiments. VEGF Northern blot (with 28S rRNA loading control band) is shown along with corresponding Western blot of phosphorylated and total p38.

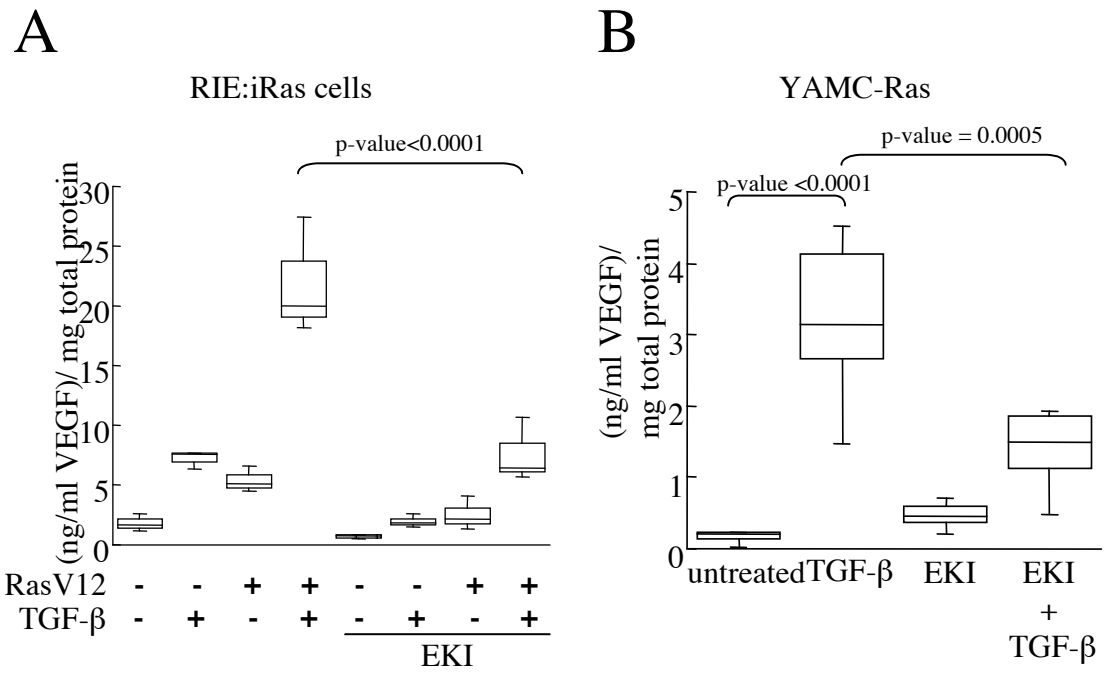


Figure 23: EGFR signaling contributes to the synergistic induction of VEGF by oncogenic Ras and TGF-β. (**A**) RIE:iRas cells were pretreated for 15 minutes with an EGFR inhibitor (1μM EKI) then treated for 24 hours with 5mM IPTG and/or 3ng/ml TGF-β. VEGF protein levels were assayed by ELISA and normalized to total protein levels. Box plot shows data from three independently replicated experiments. (**B**) YAMC-Ras cells were pretreated for 15 minutes with an EGFR inhibitor (1μM EKI) then treated for 24 hours with or without 5ng/ml TGF-β. VEGF protein levels were assayed by ELISA and normalized to total protein levels. Box plot shows data from six independently replicated experiments.

VEGF induction by oncogenic Ras and TGF-β is independent of COX-2 activity.

Previous studies indicated a role for COX-2 in the induction of VEGF in tumor cells (Abdelrahim and Safe, 2005; Kim et al., 2005b; Masunaga et al., 2000; Nishikawa et al., 2004; Tsujii et al., 1998), therefore the secondary effects of COX-2 activity in RIE:iRas cells were examined. A COX-2 specific inhibitor, NS398, was used at a concentration (10μM) that completely inhibits COX-2-dependent prostaglandin production (Tsujii et al., 1998). Inhibition of COX-2 activity and prostaglandin production at this dose had no

effect on the synergistic induction of VEGF by oncogenic Ras and TGF-β (Figure 24A), indicating that Ras and TGF-β signaling regulate VEGF expression independently of COX-2. Prostaglandin levels, and thus COX-2 activity, were assessed by 6-keto-PGF1α, a stable metabolite of PGI_2 and one of the major prostaglandins produced by COX-2 activity in these cells (Figure 24B). Similar results were obtained with the COX-2 inhibitor celecoxib and a similar response was observed for PGE_2 levels (data not shown).

These results were futher confirmed in another colon cancer model cell line, YAMC, by activating Ras signaling with EGF treatment. Parental YAMC cells or COX-2 null YAMC cells (Figure 24C) were treated with or without TGF-β and EGF, then VEGF protein levels in conditioned media were measured. TGF-β and EGF treatment together cooperatively increased VEGF expression compared to either treatment alone, although EGF cooperated to a lesser degree than oncogenic Ras expression (Figure 24D). COX-2 null YAMC cells showed the same VEGF expression pattern as the parental cells. These data from two separate colon cancer model cell lines demonstrate that activation of Ras and TGF-β signaling regulates VEGF gene expression through a COX-2-independent mechanism.

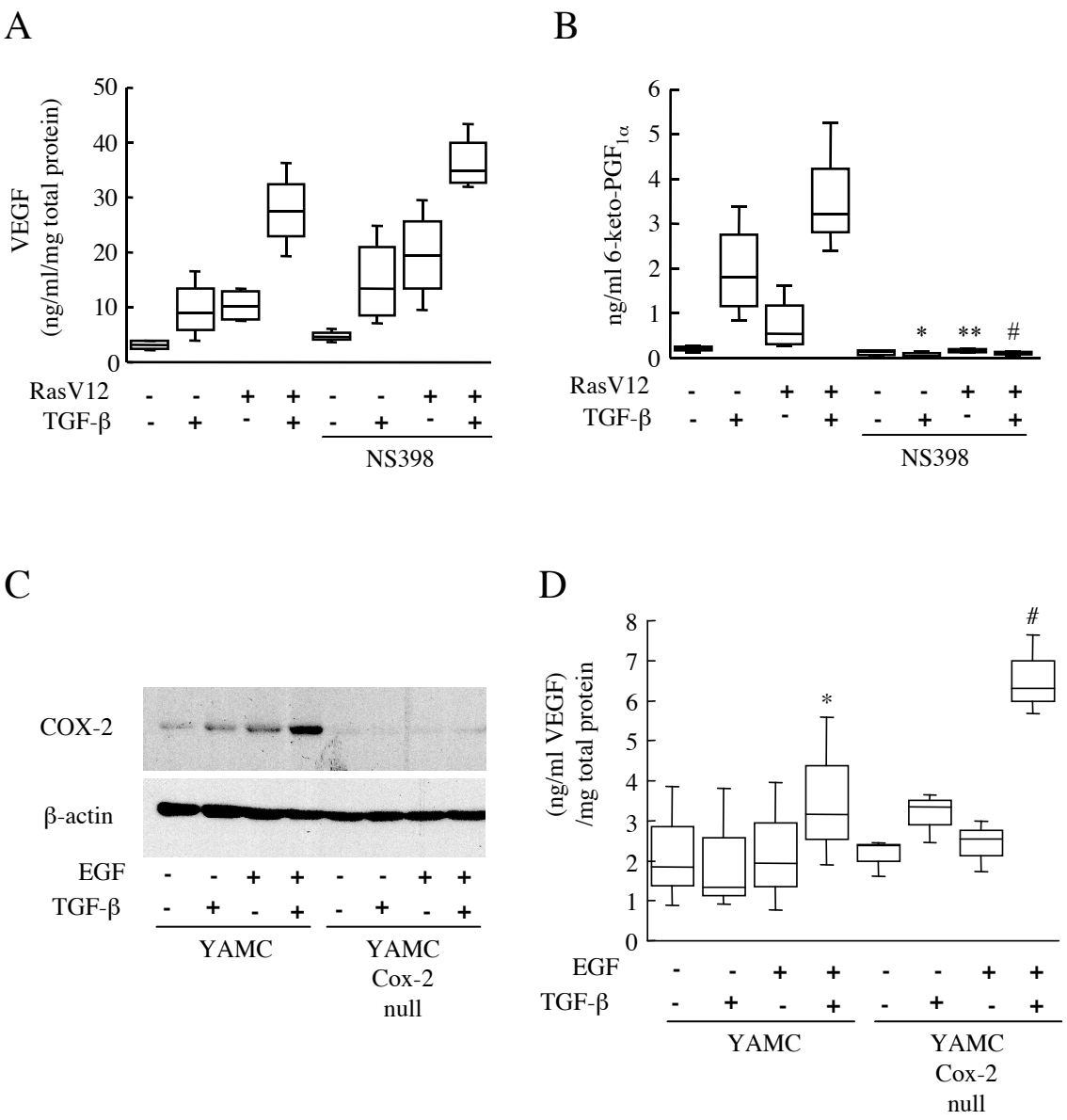


Figure 24: Ras and TGF-β -mediated increase in VEGF expression is COX-2 independent. RIE:iRas cells were pretreated with DMSO or 10μM NS398 for 15 minutes then treated with vehicle, 5mM IPTG, 3ng/ml TGF-β, or both IPTG and TGF-β for 24hrs. **(A)** VEGF protein in conditioned media was measured by ELISA and normalized to total protein concentration. Box plots show data from four independently replicated experiments. **(B)** Prostacyclin (6-keto-PGF1α) levels in conditioned media were measured by gas chromatography-mass spectrometry. Box plots show data from four independently replicated experiments. Significance between no inhibitor and NS398 treatment was determined by ANOVA with Bonferroni correction. *p-value <0.0001 compared to TGF-β. **p-value = 0.09 compared to Ras. #p-value <0.0001 compared to Ras + TGF-β. (**C**) YAMC and COX-2 null YAMC cells were treated for 24 hours with EGF and/or TGF-β. Western blotting for COX-2 and β-actin. (**D**) YAMC and COX-2 null YAMC cells were treated for 24 hours with EGF and/or TGF-β. VEGF protein in conditioned media was measured by ELISA and normalized to total protein concentration. Box plots show data from three independently replicated experiments. ***** p-value = 0.02 compared to all other treatments of YAMC cells, # p-value<0.03 compared to untreated and EGF in COX-2 null cells (ANOVA)

Oncogenic Ras and TGF-β cooperate to increase VEGF mRNA stability

Our previous work demonstrates that oncogenic Ras and TGF-β synergistically increase COX-2 expression and mRNA stability through a mechanism involving AU-rich elements (AREs) in the 3'untranslated region (UTR) of its mRNA transcript (Sheng et al., 2000). VEGF mRNAs also contain AREs in their 3'UTR that target VEGF mRNA for rapid degradation (Claffey et al., 1998; Levy et al., 1995; Levy et al., 1998; Shima et al., 1995). The half-life of VEGF mRNA in the context of oncogenic Ras expression and TGF-β treatment was examined and in untreated cells rapid decay was observed yielding a half-life of 25 minutes for VEGF mRNA (Figure 25). Either Ras induction or TGF-β treatment alone increased the stability of VEGF mRNA significantly (both $p<0.0001$), extending the half-life to 50-55 minutes. Furthermore, combined Ras activation and TGF-β treatment together also significantly increased VEGF mRNA stability ($p<0.0001$), extending the half-life to 120 minutes. Although no interaction above the additive effects of TGF-β and RasV12 were seen in mRNA stability (all $p > 0.1788$), combined oncogenic Ras expression and TGF-β markedly potentiated VEGF mRNA stability compared to each alone (Figure 25).

Increased VEGF mRNA stabilization may not be the sole mechanism whereby VEGF mRNA is increased. To determine whether oncogenic Ras and TGF-β increased the transcription of VEGF, RIE-H-RasV12 cells were transiently transfected with a VEGF promoter/luciferase reporter construct. TGF-β treatment did not enhance the luciferase reporter activity of Ras expressing cells (Figure 26). These results demonstrate that oncogenic Ras and TGF-β synergistically increase VEGF expression through increased VEGF mRNA stability.

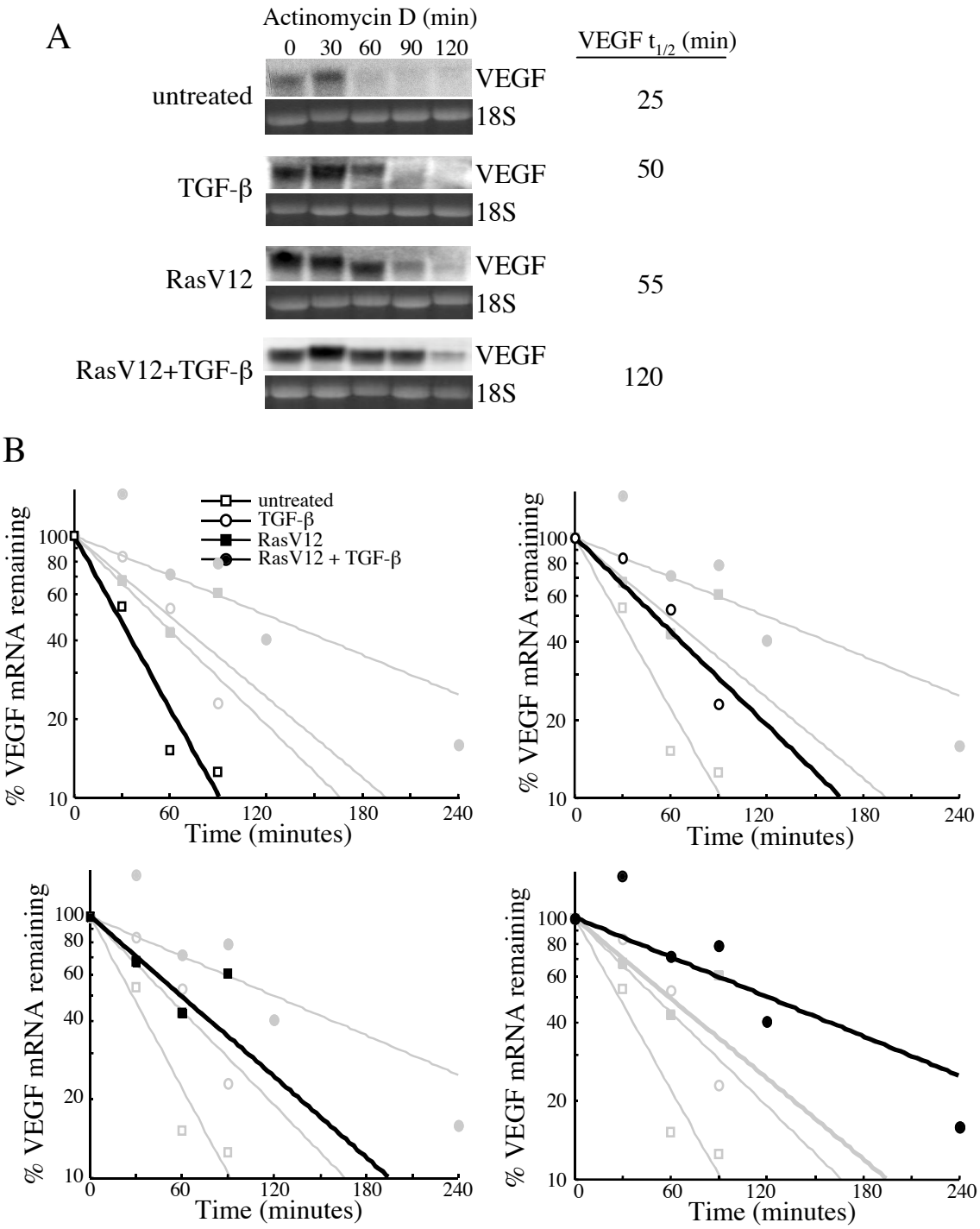


Figure 25: Oncogenic Ras and TGF-β cooperate to synergistically stabilize VEGF mRNA. Northern blot analysis of RIE:iRas cells after treatment for 24 hours with vehicle, 5mM IPTG, 3ng/ml TGF-β, or both IPTG and TGF-β and then treatment with 10μg/ml Actinomycin D for up to 4 hours. **(A)** VEGF Northern blot and 18S rRNA, as detected by ethidium bromide staining. **(B)** Percentage of VEGF mRNA, normalized to 18S rRNA levels, remaining after actinomycin D treatment. Each treatment group is highlighted in a separate graph and data are representative of three separate experiments. □ untreated, ○ TGF-β, ■ IPTG, ● IPTG + TGF-β.

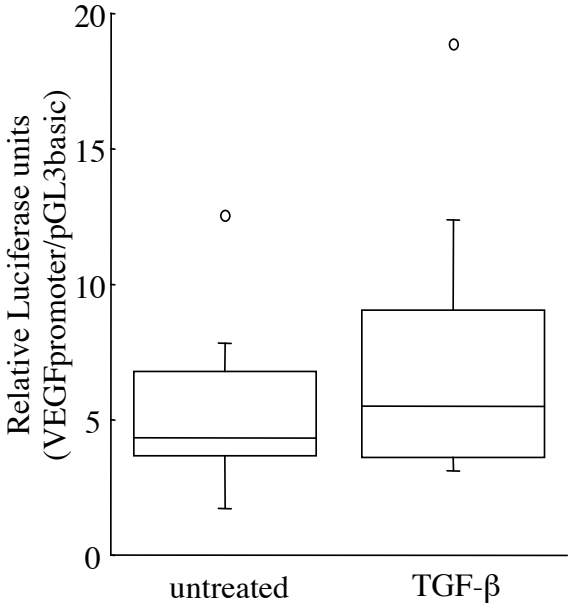


Figure 26: Ras activation of VEGF promoter-reporter is not affected by TGF-β. RIE-H-RasV12 cells were transiently transfected with a VEGF promoter-luciferase reporter or pGL3-basic control plasmid. Cells were treated with or without 3 ng/ml TGF-β for 48 hours and luciferase activity measured. VEGF promoter activity was normalized to pGL3 control plasmid. Box plot shows data from seven independent experiments. p-value = 0.3 (t-test).

A global mechanism of post-transcriptional gene regulation

Oncogenic Ras and TGF-β regulate ARE-containing genes. Based on the observed regulation of both COX-2 and VEGF by a post-transcriptional mechanism, the hypothesis that Ras and TGF-β cooperatively regulate global gene expression profiles through a post-transcriptional mechanism involving AREs was formed. To test this hypothesis, the Ras and TGF-β signature was examined for AU-rich element (ARE) motifs that mediate post-transcriptional regulation of gene expression (Bakheet et al., 2006) and function as binding sites for specific RNA binding proteins that regulate the rate of RNA degradation and translation (Lee et al., 1998; Ross et al., 1991). ARE analysis using a multiple search approach revealed that 39% of the 379 synergistically

regulated transcripts contained AREs in their 3'UTRs. Among the 191 unique genes in this set with annotations, 105 (56%) contain AU-rich elements (Appendix Table 6). Since only 5-8% of the transcriptome is estimated to contain such elements (Bakheet et al., 2006), the Ras and TGF-β signature was found to be enriched more than 4-fold with ARE-containing genes. In order to assess the statistical significance of ARE gene representation, we employed the recently developed and stringent ARED-Organism database (Halees et al., 2008). We observed a statistically significant 4-fold increase in ARE-gene representation in the set of synergistically regulated genes ($p=0.001$). Interestingly, we also observed a 4-fold enrichment of ARE-containing genes among those genes differentially regulated in response to Ras expression ($p=0.0001$) or TGF-β treatment ($p=0.0002$) alone or together ($p=0.0001$). It should be noted that the ARED-Organism method is very stringent and non-comprehensive due to the high number of rat genes with incomplete or missing 3'UTRs. Gene ontology and KEGG analysis of the list of synergistic ARE-containing genes using IPA revealed that this list is significantly enriched for genes associated with cell migration and invasion ($p<0.002$), cell growth and proliferation ($p<0.001$), and cancer ($p<0.004$). The biological and molecular functions of some of these genes are listed in Table 3. The over-representation of ARE-containing genes in the Ras and TGF-β signature suggests that neoplastic transformation results in profound changes in gene expression through post-transcriptional mechanisms.

Table 3: Functions of ARE-containing genes synergistically regulated by oncogenic Ras and TGF-β

Gene Symbol	Gene Name	Rat Ensembl GID	Fold change		
			TGF-β	RasV12	RasV12 + TGF-β
Adhesion & Motility					
Nrg1	neuregulin 1	ENSRNOG00000010392	-0.02	3.15	4.38
Podxl	podocalyxin-like	ENSRNOG00000012495	-1.34	-1.65	-2.90
Marcks	myristoylated alanine rich protein kinase C substrate	ENSRNOG00000000579	0.01	1.38	3.20
Vnn1	vanin 1	ENSRNOG00000016219	-2.09	-5.77	-7.94
Gne	glucosamine	ENSRNOG00000014365	1.77	4.11	6.52
Olr1	oxidized low density lipoprotein (lectin-like) receptor 1	ENSRNOG00000008375	-1.84	-4.26	-21.26
Col14a1	procollagen, type XIV, alpha 1 (predicted)	ENSRNOG00000026415	-1.67	-8.14	-13.31
Igsf4a	immunoglobulin superfamily, member 4A	ENSRNOG00000018778	-1.33	-2.11	-4.23
Lamc2	lamimin, gamma 2	ENSRNOG00000002667	1.78	7.49	9.63
Cxadr	coxsackie virus and adenovirus receptor	ENSRNOG00000001557	-1.65	-7.32	-10.53
Ctnnal1	catenin (cadherin associated protein), alpha-like 1 (predicted)	ENSRNOG00000010593	-2.57	-2.31	-7.18
Angiogenesis					
Vegfa	vascular endothelial growth factor	ENSRNOG00000019598	1.45	5.90	7.52
Fgf13	fibroblast growth factor 13	ENSRNOG00000003523	-1.29	-7.46	-13.92
Growth & proliferation					
Gas6	growth arrest specific 6	ENSRNOG00000018233	-2.08	-10.17	-27.00
Nppb	natriuretic peptide precursor type B	ENSRNOG00000008141	2.73	9.17	14.57
Fhl1	four and a half LIM domains 1	ENSRNOG00000000875	1.30	-11.85	-18.74
Dusp1	dual specificity phosphatase 1	ENSRNOG00000003977	1.85	-1.03	-3.36
Mycn	v-myc myelocytomatosis viral related oncogene, neuroblastoma derived (avian)	ENSRNOG00000006308	-1.58	-18.99	-67.66
Transcription					
Klf4	kruppel-like factor 4 (gut)	ENSRNOG00000016299	-1.29	1.04	-2.54
Id2	inhibitor of DNA binding 2	ENSRNOG00000007237	-3.40	-2.15	-6.66
Tcf7	transcription factor 7, T-cell specific (predicted)	ENSRNOG00000005872	1.85	-1.10	20.51
Stat1	signal transducer and activator of transcription 2	ENSRNOG00000014079	-1.80	-1.28	-3.16
Nrg1	neuregulin 1	ENSRNOG00000010392	-0.02	3.15	4.38
ECM related					
Adamts1	a disintegrin-like and metallopeptidse with thrombospondin type 1 motif, 1	ENSRNOG00000001607	-2.32	-12.63	-19.39
Col14a1	procollagen, type XIV, alpha 1 (predicted)	ENSRNOG00000026415	-1.67	-8.14	-13.31
Mmp13	matrix metallopeptidase 13	ENSRNOG00000008478	2.18	3.23	23.10
Mmp10	matrix metallopeptidase 10	ENSRNOG00000032832	1.24	2.71	4.10
Cytoskeleton					
Marcks	myristoylated alanine rich protein kinase C substrate	ENSRNOG00000000579	0.01	1.38	3.20
Map1b	microtubule-associated protein 1b	ENSRNOG00000017428	4.01	5.37	9.94
Ctnnal1	catenin (cadherin associated protein), alpha-like 1 (predicted)	ENSRNOG00000010593	-2.57	-2.31	-7.18
Cav	caveolin	ENSRNOG00000006694	-1.68	-2.13	-5.01
RNA binding proteins					
Zfp36l1	zinc finger protein 36, C3H type-like 1	ENSRNOG00000030024	-2.35	-2.13	-6.25
Cugbp2	CUG triplet repeat, RNA binding protein 2	ENSRNOG00000023661	-2.01	-2.58	-5.64
A2bp1	ataxin 2 binding protein	ENSRNOG00000002827	-1.42	3.09	4.71
Growth factors & cytokines					
Nrg1	neuregulin 1	ENSRNOG00000010392	-0.02	3.15	4.38
Bmp4	bone morphogenetic protein 4	ENSRNOG00000009694	-1.90	-10.34	-18.82
Bmp2	bone morphogenetic protein 2	ENSRNOG00000021276	1.81	20.10	25.40
Wnt5a	wingless-type MMTV integration site 5A	ENSRNOG00000015618	1.09	2.98	7.41
Wnt2	wingless-related MMTV integration site 2	ENSRNOG00000007843	2.49	1.16	7.79
Pdgfa	platelet derived growth factor, alpha	ENSRNOG00000001312	1.30	2.24	6.74
Vegfa	vascular endothelial growth factor	ENSRNOG00000019598	1.45	5.90	7.52
Il24	interleukin 24	ENSRNOG00000004470	1.30	2.95	23.63
Il1a	interleukin 1 alpha	ENSRNOG00000004575	1.08	1.46	16.17
Ccl2	chemokine (C-C motif) ligand 2	ENSRNOG00000007159	5.47	17.28	37.90
Btc	betacellulin	ENSRNOG00000002728	-1.58	-7.33	-11.47
Wnt signaling					
Wnt5a	wingless-type MMTV integration site 5A	ENSRNOG00000015618	1.09	2.98	7.41
Wnt2	wingless-related MMTV integration site 2	ENSRNOG00000007843	2.49	1.16	7.79
Fzd1	frizzled homolog 1 (Drosophila)	ENSRNOG00000016242	1.20	1.24	2.70
Axin2	axin2	ENSRNOG00000003612	1.42	2.26	4.26
TGF-β signaling					
Bmp4	bone morphogenetic protein 4	ENSRNOG00000009694	-1.90	-10.34	-18.82
Bmp2	bone morphogenetic protein 2	ENSRNOG00000021276	1.81	20.10	25.40
Serpine1	serine (or cysteine) peptidase inhibitor, clade E, member 1 (PAI-1)	ENSRNOG00000001414	33.57	4.60	57.69

All genes listed in the table are significantly and synergistically regulated by a combination of Ras induction and TGF-β treatment and contain an ARE (AU-rich element) motif in the 3'UTR. Fold change represents expression in Ras expressing and/or TGF-β treated samples compared to untreated. Gene expression increases and decreases are defined as greater than 2-fold. Presence of an ARE was determined by analysis using the ARE database, ARED3.0 (Bakheet et al., 2006).

Cooperative regulation of RNA binding proteins. Analysis of the RIE:iRas microarray showed a synergistic change in the expression of two known mRNA binding proteins in response to Ras and TGF-β. A 3.2-fold increase in heterogeneous nuclear

ribonucleoprotein A/B (hnRNP A/B), a member of the hnRNP family of RNA binding proteins involved in RNA trafficking and splicing, was induced by oncogenic Ras and TGF-β. The cooperative increase in hnRNP A/B by oncogenic Ras and TGF-β at various time points was confirmed by qRT-PCR, although synergy was only observed at the 48 hour time point (Figure 27B).

Another RNA binding protein, CUGBP2, has been shown to bind the COX-2 3'UTR and inhibit its translation in response to ionizing radiation (Mukhopadhyay et al., 2003), consistent with our observed 4.3-fold decrease in CUGBP2 expression and increase in COX-2 expression in response to oncogenic Ras expression and TGF-β treatment. The synergistic decrease in CUGBP2 expression was confirmed at the mRNA level by qRT-PCR (Figure 27A). Validation of endogenous CUGBP2 and hnRNP A/B expression could not be confirmed at the protein level due to a lack of commercially available antibodies.

Oncogenic Ras and TGF-β cooperate to alter HuR localization and expression. Data indicating the synergistic effects of oncogenic Ras and TGF-β directly effect the expression of ARE-containing genes and influence the stabilization of VEGF and COX-2 mRNA suggested that the ability of AREs to promote rapid decay was compromised. Based on its ability to bind AREs and stabilize the mRNA of genes such as COX-2 and VEGF (Dixon et al., 2001), involvement of the mRNA stability factor HuR was examined. Under normal conditions HuR is primarily localized in the nucleus. However, in response to cellular signaling, it can shuttle to the cytoplasm where it can influence mRNA stabilization and translational efficiency (Brennan and Steitz, 2001). Based on this, changes in cytoplasmic trafficking of HuR could account for the observed VEGF

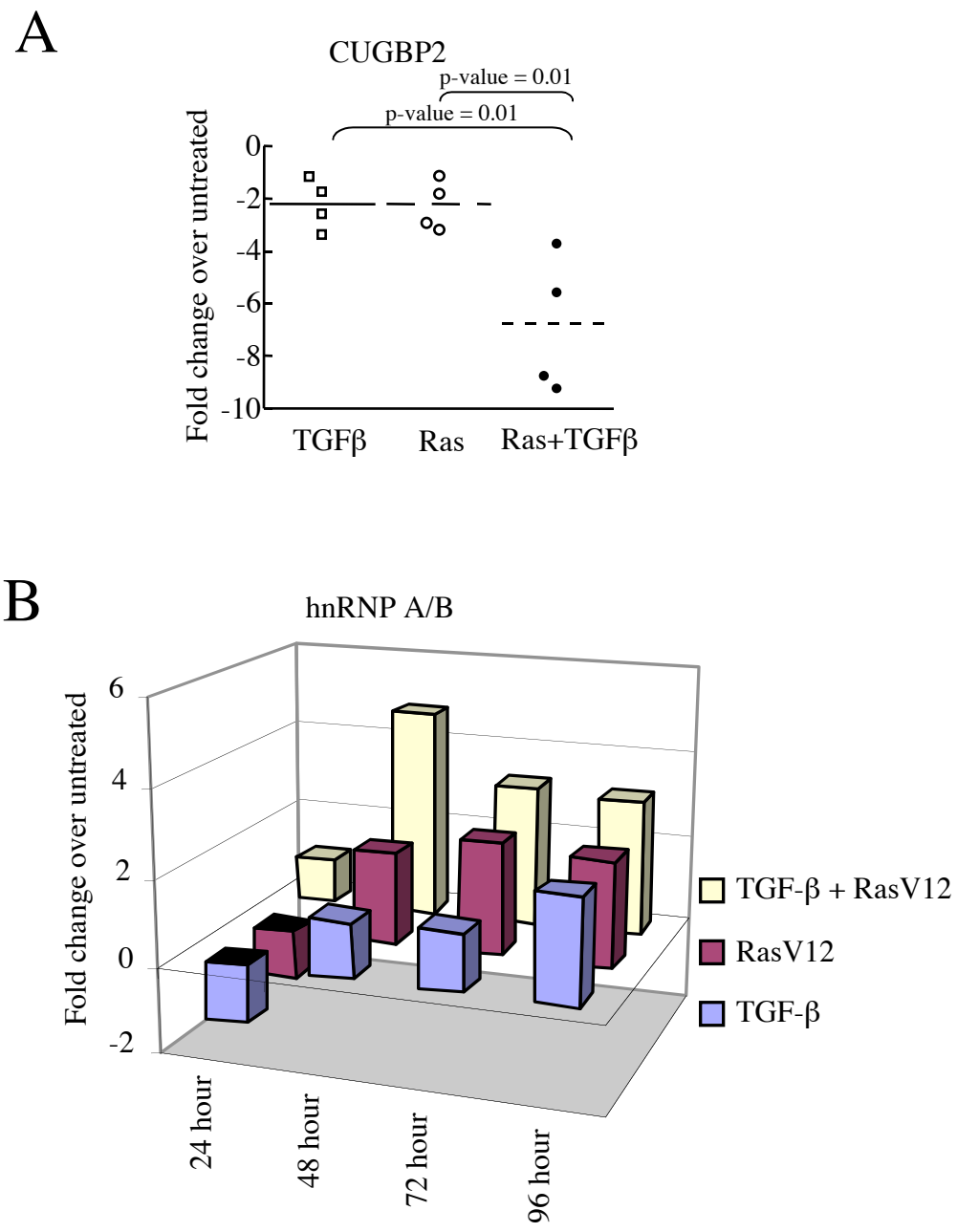
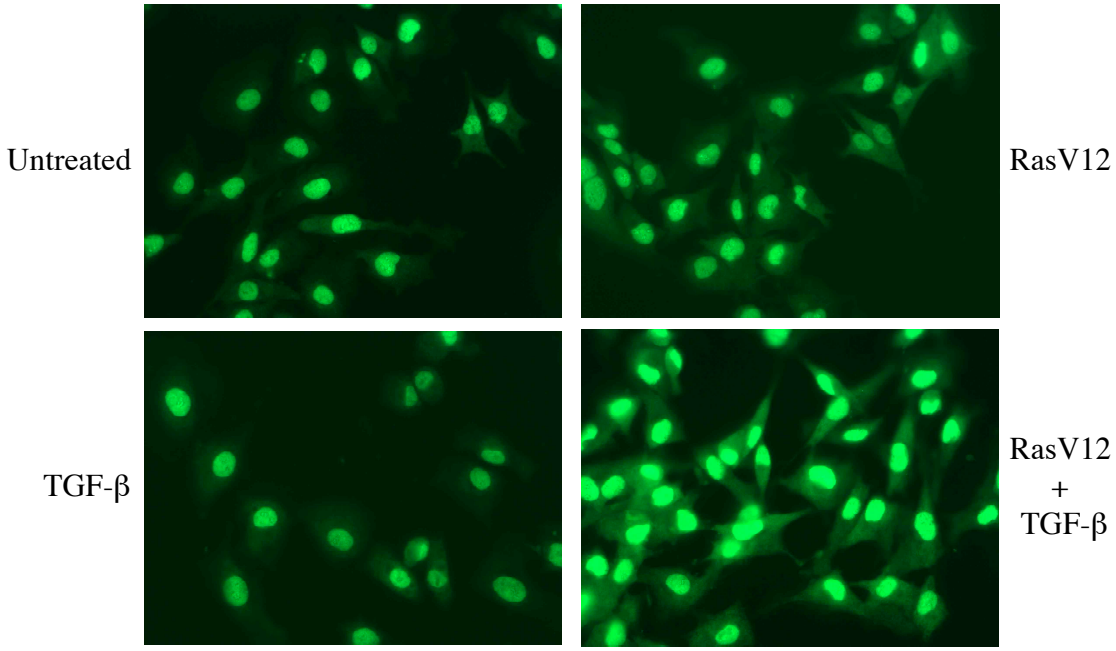


Figure 27: Ras and TGF-β regulate RNA binding protein expression. RNA was isolated from RIE:iRas cells treated for 72 hours with 5mM IPTG, 3ng/ml TGF-β, or IPTG and TGF-β together and expression of CUGBP2 and hnRNP A/B quantified by real time RT-PCR. Changes in gene expression under treated conditions were calculated relative to untreated samples and all values were normalized to the housekeeping gene Pmm1. Dotted lines show mean expression for each treatment. Significance was determined by ANOVA with Bonferroni correction. (**A**) RNA was isolated from cells treated for 72 hours with 5mM IPTG, 3ng/ml TGF-β, or IPTG and TGF-β together. Dotted lines show mean expression for each treatment. Significance determined by ANOVA with Bonferroni correction. (**B**) RNA was isolated from cells treated 24, 48, 72, or 96 hours with 5mM IPTG, 3ng/ml TGF-β, or IPTG and TGF-β together. Bar graph shows data from one representative experiment.

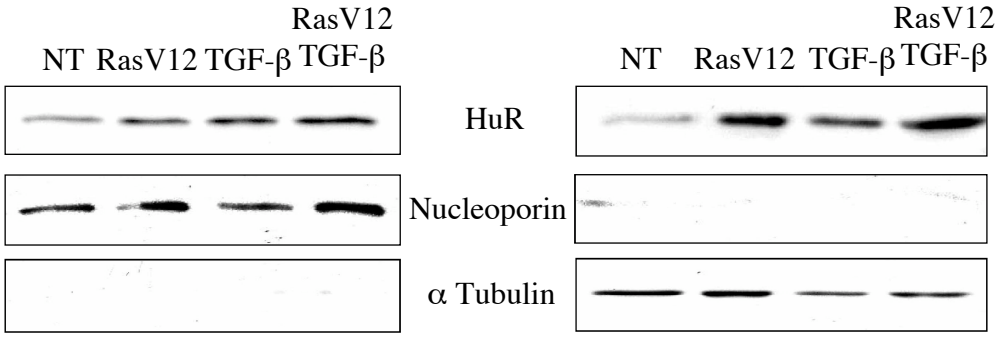
mRNA stabilization and protein overexpression promoted by oncogenic Ras and TGF-β. RIE:iRas cells were incubated with IPTG to induce Ras expression, TGF-β, or a combination of both, and localization of HuR was examined by immunofluorescence (Figure 28A). HuR was primarily detected in the nuclei of untreated RIE:iRas cells. Expression of oncogenic Ras or treatment with TGF-β for 24 hours increased HuR levels in the cytoplasm and cytoplasmic HuR was robustly detected by immunostaining after oncogenic Ras and TGF-β treatment together. Similar results were obtained upon examination of HuR subcellular localization by SDS-PAGE (Figure 28B). In addition to the nuclear to cytoplasmic translocation of HuR, total levels of HuR protein, but not mRNA, were cooperatively increased by oncogenic Ras and TGF-β (Figure 28C-D).

A role for HuR in the regulation of VEGF. To examine the role of HuR in the regulation of VEGF expression, siRNA against HuR was transiently expressed in RIE cells. A dose-dependent decrease in HuR expression was observed after HuR siRNA transfection (Figure 29). A concomitant decrease in VEGF protein expression was also seen (Figure 29). These results indicate that HuR is translocated from the nucleus to the cytoplasm under conditions of oncogenic Ras and TGF-β signaling in intestinal epithelial cells and suggest that this regulated translocation of HuR contributes to mRNA stabilization and protein synthesis in these cells.

A


Untreated
RasV12
TGF-β
RasV12
+
TGF-β

B


Nuclear
Cytoplasmic
NT
RasV12
TGF-β
RasV12
TGF-β
NT
RasV12
TGF-β
RasV12
TGF-β
HuR
Nucleoporin
α Tubulin

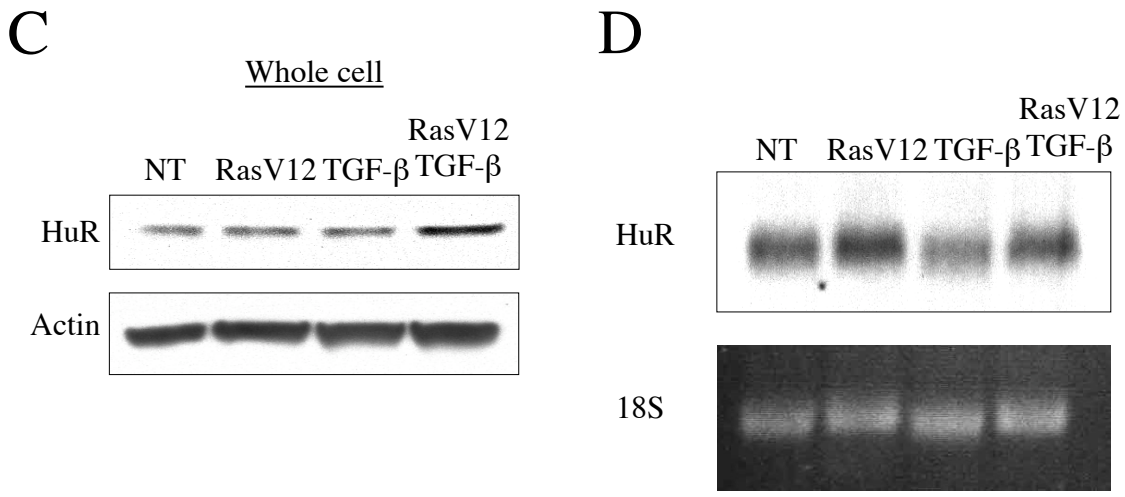


Figure 28: Oncogenic Ras and TGF-β influence subcellular localization and expression of HuR in RIE:iRas cells. (**A**) RIE:iRas cells (1 x 10^5) were plated on cover slips and were subsequently left untreated or incubated for 24 hours with 5mM IPTG, 5 ng/ml TGF-β, or both IPTG and TGF-β followed by examination of HuR localization by immunofluorescence. (**B**) Western blot analysis of nuclear (20 μg) and cytoplasmic (40 μg) levels of HuR in RIE:iRas cells that were either untreated (labeled NT) or treated as described above. Nucleoporin and α-tubulin served as loading controls for nuclear and cytoplasmic preparations, respectively. (**C**) Western blot analysis of total (30 μg) HuR levels that were either left untreated or treated as stated above. β-actin served as a loading control. (**D**) Northern blot analysis of HuR mRNA levels. 18S ribosomal RNA served as a loading control.

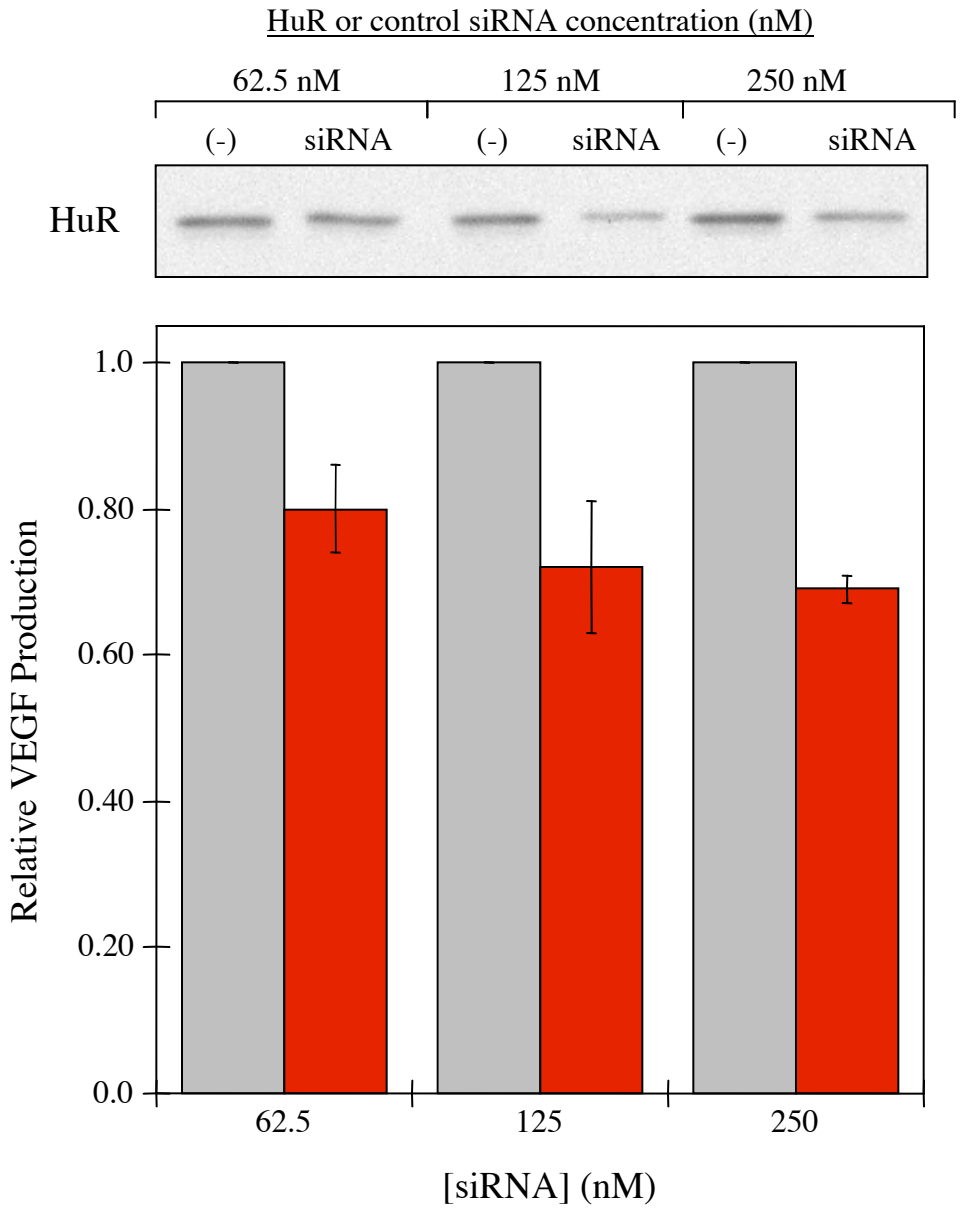


Figure 29: Inhibition of HuR expression attenuates VEGF expression in RIE cells. RIE-1 cells were transfected with increasing concentrations of a control siRNA (-) or a siRNA against HuR (siRNA). After 24 hr of transfection, media was changed and cells were grown for another 24 hr. Cultured media was then collected for VEGF expression analysis by ELISA (bottom panel) and cell lysates were analyzed for HuR expression by SDS-PAGE (top panel). Relative VEGF production was assessed as VEGF secretion in cultured media normalized to total cell protein. All values shown are normalized to VEGF expression in cells transfected with the respective amount of control siRNA and indicate the averages triplicate experiments.

Summary

Oncogenic Ras and TGF-β cooperate in a dose-dependent manner to synergistically increase VEGF expression in multiple gastrointestinal epithelial cell lines. Several signaling pathways known to be activated by both Ras and TGF-β participate in this synergistic increase in VEGF, as MEK/ERK and EGFR activity are necessary and PI3K/Akt signaling also contributes. However, the cooperative interactions of Ras and TGF-β increase VEGF expression independently of COX-2 activity and p38 signaling. The synergistic increase in VEGF expression by oncogenic Ras and TGF-β occurs through a mechanism of increased VEGF mRNA stability, while a cooperative increase in VEGF promoter activity is not observed. Furthermore, this study suggests a global mechanism of post-transcriptional regulation is involved in altering gene expression during EMT. The set of synergistically regulated genes is enriched with gene containing AU-rich elements and Ras and TGF-β cooperatively affect RNA binding proteins, decreasing CUGBP2 (an inhibitor of translation) and increasing cytoplasmic HuR (a mRNA stabilizing protein).

CHAPTER VI

COLORECTAL CANCER MICROARRAY

Introduction

Recently, advances in gene array technology have made it possible to evaluate gene expression patterns across much of the known genome. Microarray analysis of colorectal tumors facilitates identification of genes that differentiate biological behavior or response to therapeutic intervention. Due to the multitude of variables affecting *in vivo* tumorigenesis, such as genetic background, patient history and diet, it is useful to inform gene expression analyses of malignant transformation with those occurring in well-controlled experiments through cell culture model systems. Such model systems have demonstrated important mechanistic principles such as the loss of a growth inhibitory response to TGF-β in the process of carcinogenesis and that TGF-β can promote tumor progression, increase cell motility and invasiveness, and promote metastasis (Cui et al., 1996; Friedman et al., 1995; Janji et al., 1999; Robson et al., 1996). Oncogenic Ras can switch TGF-β from a growth suppressor to a growth promoter and TGF-β enhances the transforming effects of oncogenic Ras (Filmus et al., 1992; Fujimoto et al., 2001; Oft et al., 1996).

One objective of this study is to determine whether the our cell model of EMT is applicable to human cancers. The approach described here is to perform combined analysis of microarray data sets derived from cell culture model systems of colorectal

cancer and clinical colorectal data sets. The Ras inducible rat intestinal epithelial (RIE:iRas) cell culture model is used to examine the biological relevance of genes involved in malignant transformation, such as is seen in the cooperation of TGF-β and oncogenic Ras. The Ras and TGF-β signature was compared with expression patterns seen in human colorectal tumors. The hypothesis of this study states that interrogation of clinical microarray data sets using parallel data sets from cell culture model systems leads to the identification of biomarkers and novel therapeutic targets for colon cancer.

Results

In order to determine whether the Ras and TGF-β signature derived from RIE:iRas cells provides mechanistic insights into the human disease, global gene expression profiles from human colorectal cancers were examined. Gene expression profiles from colorectal cancer (CRC) samples were obtained using the Affymetrix U133 Plus 2.0 array. These profiles were examined for genes significantly (Q-value< 0.05) changed in human stage 4 metastatic colorectal adenocarcinomas as compared with adenomas and 7721 transcripts, corresponding to 3694 unique Ensembl gene IDs, were differentially regulated.

Overlap between CRC and RIE:iRas microarray

The 379 transcripts synergistically up- or down-regulated by Ras and TGF-β in RIE:iRas cells were mapped to human orthologs based on Ensembl annotation, resulting in 189 genes. These synergistic genes were then intersected with the 3694 genes

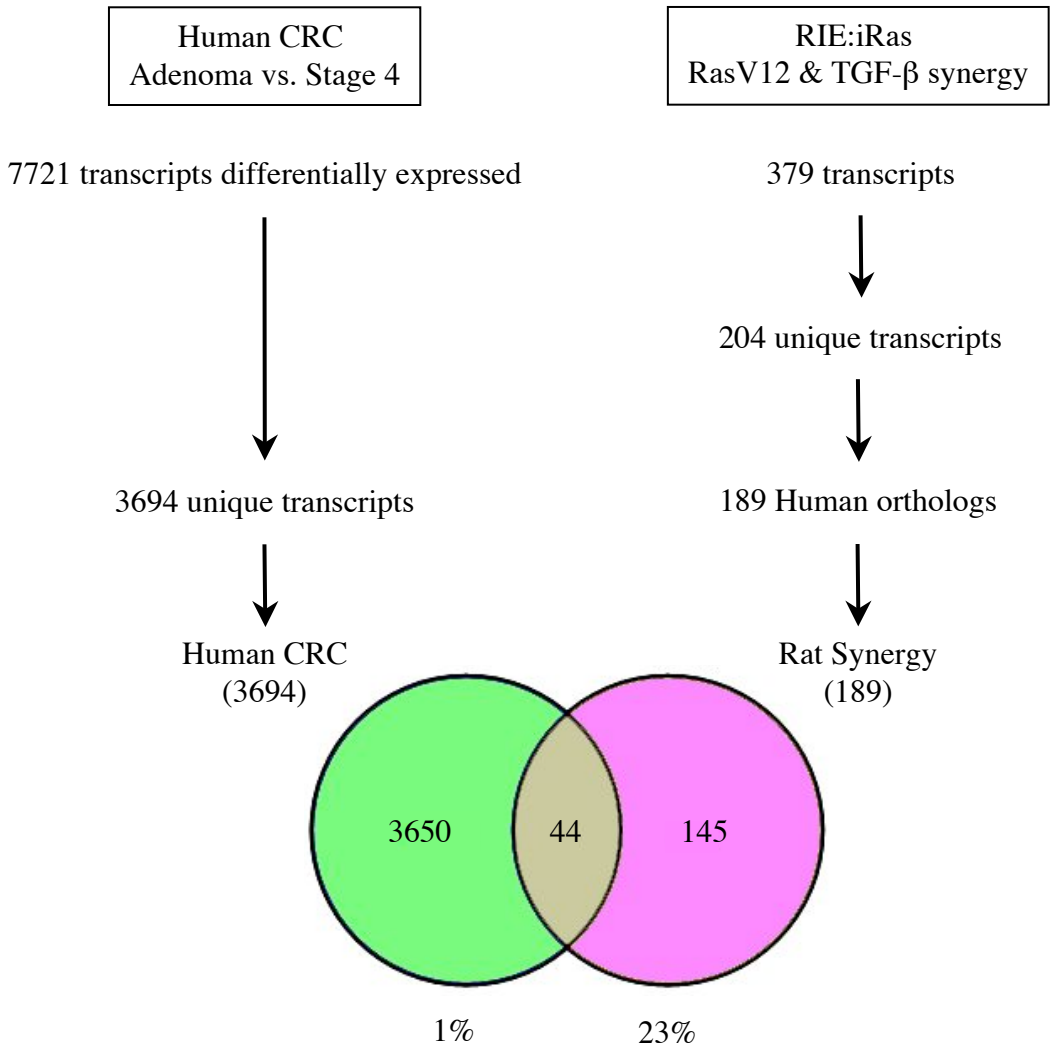


Figure 30: Molecular events in transformed RIE:iRas cells reflect gene expression changes in human colorectal tumors. Flow chart and Venn diagram show the intersection of genes differentially expressed in stage 4 human colorectal cancer samples compared to adenomas (Q-value<0.05) with human homologs of the RIE:iRas Ras-TGF-β signature genes using Webgestalt.

differentially expressed in stage 4 adenocarcinoma compared to adenomas (Figure 30). Among the 44 genes in common between human CRCs and RIE:iRas cells are several genes involved in TGF-β signaling (*Bmp1, Bmp4, Follistatin, Inhibinba, Pai1, Tgfb1)*, EGFR signaling (*Erbb3, Epiregulin)*, and adhesion and migration (*Col14a1, Col5a3,*

Integrina5, Timp2) (Appendix Table 6). Annotation of the rat genome is less complete than that of the human genome, thus gene overlap between the RIE:iRas and CRC lists is likely incomplete.

Several ARE-containing genes that are differentially expressed between human adenomas and stage 4 CRC overlap with genes that are also present in the Ras and TGF-β gene signature derived from RIE:iRas cells. There is a high correlation (8/11) in the direction of expression change in ARE-containing genes between the oncogenic Ras and TGF-β signature and stage 4 CRC (Table 4). Several of the genes in common between the rat cellular model and human CRC are involved in cell proliferation and adhesion, such as *Col14a1, Gne, Mbp, Pai-1, Tfp1*, and *Vegfa*. Furthermore, three of these genes, *Vegfa, Pai-1*, and *Tfp1*, have well established roles in cancer.

Table 4: Human and rat differentially expressed ARE-containing genes

Gene Symbol	Gene Name	Rat RasV12 + TGF-β vs. untreated	Human Stage 4 vs. adenoma
AKAP7	A-kinase anchor protein 7 isoform gamma	down	down
ANKH	Progressive ankylosis protein homolog	up	down
ARL4C	ADP-ribosylation factor-like protein 4C	up	up
CHSY1	Chondroitin sulfate synthase 1	up	up
COL14A1	Collagen alpha-1(XIV) chain	down	up
GNE	Bifunctional UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase	up	down
MBP	Myelin basic protein	down	down
RAB27A	Ras-related protein Rab-27A	down	down
SERPINE1	Plasminogen activator inhibitor 1 (PAI-1)	up	up
TFPI	Tissue factor pathway inhibitor	up	up
VEGFA	Vascular endothelial growth factor A	up	up

All genes listed contain AU-rich elements, are synergistically regulated by oncogenic Ras and TGF-β in RIE:iRas cells, and are significantly changed in human stage 4 adenocarcinomas compared to adenomas. Presence of an ARE (AU-rich element) was determined by analysis using the ARE database, ARED3.0 (Bakheet et al., 2006).

In addition to changes in specific genes that were synergistically regulated by Ras and TGF-β in RIE:iRas cells, numerous genes involved in the signaling pathways investigated here are differentially regulated in CRC. KEGG based pathway analysis of human CRC microarray revealed that 11 genes associated with the EGF pathway are differentially regulated in stage 4 adenocarcinomas compared to adenomas. Among these are several signaling mediators, such as *Grb2, HRas, Jak1* and *Shc1*, which have increased expression in stage 4 adenocarcinomas and activate downstream signaling. In addition, two separate oligo probe sets for the EGF receptor, *Erbb1*, are down regulated in stage 4 adenocarcinomas compared to adenomas (Figure 31). Profound misregulation of genes involved in TGF-β signaling was also observed in the human CRC microarray, KEGG based pathway analysis revealed that 27 genes associated with the TGF-β

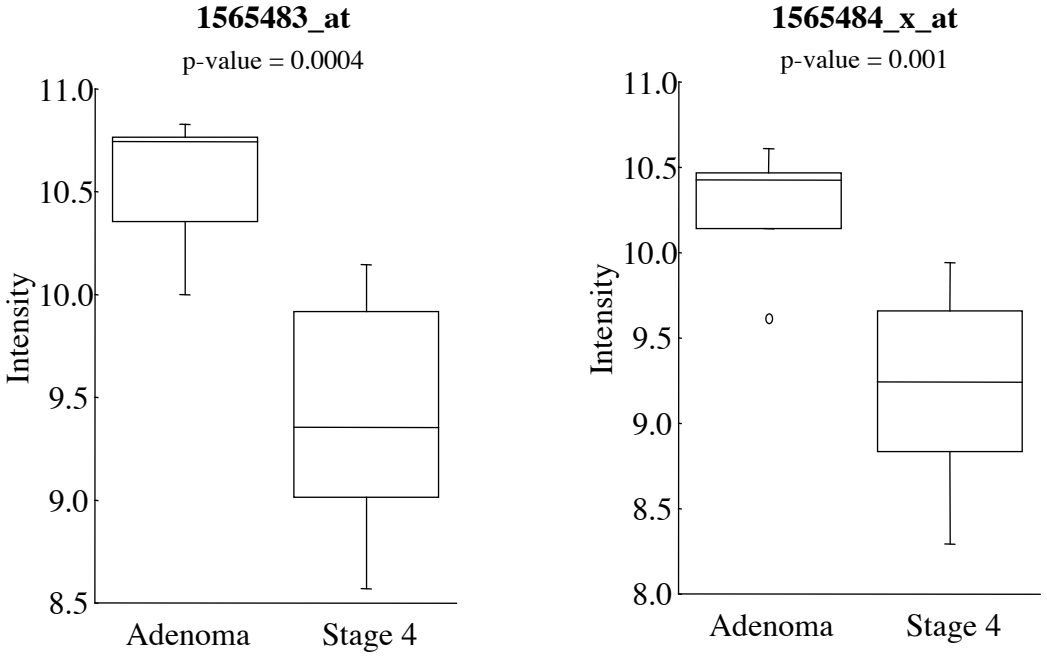


Figure 31: EGFR expression is significantly decreased in stage 4 CRC. Box plots show microarray expression values for two oligonucleotide probe sets in adenomas (n=5) and stage 4 adenocarcinomas (n=19).

pathway are differentially regulated in stage 4 adenocarcinomas compared to adenomas. Among these are several ligands *(Bmp1, Bmp4, Bmp7, Tgfb1, Tgfb2, Nodal*), inhibitors (*Inhibinba, Inhibinbb, Follistatin*), receptors (*Bmpr2, Tgfbr1, Acvr1, Acvr1c*), and signaling mediators (*Smurf1, Smad7, Smad5*). All of these genes, except *Smad7* and *Acvr1c*, show increased expression in stage 4 adenocarcinomas. Overall, these changes suggest increased TGF-β signaling, through increased expression of ligands and type I receptor and decreased expression of inhibitory Smad7, and decreased BMP signaling due to increased expression of the BMP inhibitor Follistatin and increased expression of Smurf1, which targets BMP specific Smad1/5 for degradation, despite increases in BMP ligands.

Clinical significance of post-transcriptional gene regulation

ARE-containing genes in CRC progression. Previous studies have demonstrated that dysregulation of mRNA stability may occur during the malignant transformation of cancer cells through mutations in the cis-regulatory elements or by dysregulation of the trans-acting proteins that bind these elements (Denkert et al., 2004; Dixon et al., 2001; Hollams et al., 2002). These studies, together with the observation that ARE-containing genes are enriched in the Ras and TGF-β signature, led to the hypothesis that a similar ARE-mRNA enrichment occurs in human CRC samples. The set of 3694 genes differentially regulated between adenomas and stage 4 adenocarcinomas were examined for the presence of conserved ARE motifs (Bakheet et al., 2006). A 3- to 4-fold enrichment in ARE-containing genes was observed, showing an increase in ARE-mRNAs from 5-8% of the whole transcriptome to 20.2% within the differentially

regulated transcripts (Appendix Table 7). To facilitate statistical analysis, we used the stringent ARED database (Halees et al., 2008) and found that ARE representation in this gene list was statistically significant (p-value = 0.001). Furthermore, we observed AREs in 21-24% of the genes differentially expressed between adenomas and any stage of adenocarcinoma, including stage 1 (p=0.001), 2 (p=0.001), or 3 (p=0.002), and all adenocarcinomas combined (p=0.0002), representing a statistically significant enrichment. A notable enrichment of ARE-containing genes (20.3%) was also observed in the list of genes differentially regulated in late stage CRC samples that were analyzed with the ABI microarray platform (data not shown). Taken together, these results indicate that molecular events in transformed RIE:iRas cells reflect the gene expression changes in human tumors and could provide mechanistic insights into the oncogenic process.

RNABPs in CRC. Among the genes differentially expressed in stage 4 CRC

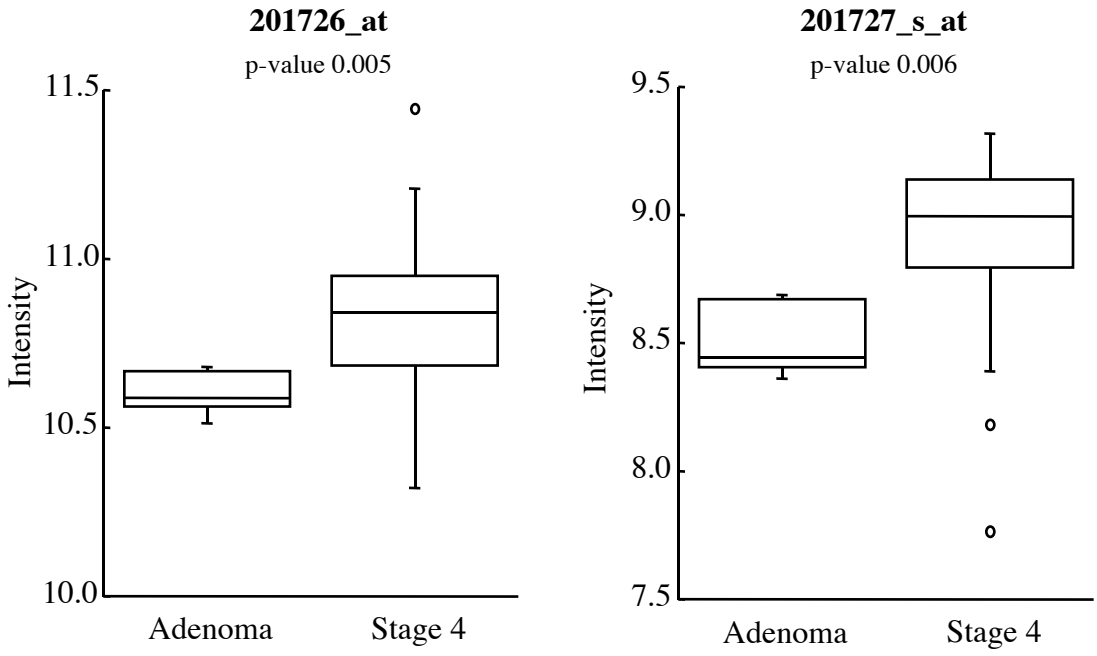


Figure 32: HuR expression is significantly increased in stage 4 CRC. Box plots show microarray expression values for two oligonucleotide probe sets in adenomas (n=5) and stage 4 adenocarcinomas (n=19).

compared to adenomas are 151 RNA binding protein encoding genes, representing a significant enrichment ($p<0.0006$) of genes with this molecular function. Among these RNABPs, HuR is significantly increased in stage 4 adenocarcinoma as compared with adenomas (Figure 32). Furthermore, 17 genes involved in translation initiation, elongation, and repression are differentially regulated in late stage CRC.

Summary

Analysis of microarray gene expression patterns in human colorectal cancer samples suggest that stage 4 adenocarcinomas have increased signaling through Ras and TGF-β and decreased BMP signaling, consistent with known roles of these pathways in EMT and MET, respectively. In addition, several genes that are regulated by oncogenic Ras and TGF-β in the RIE:iRas cells are also differentially regulated in stage 4 adenocarcinomas compared to adenomas, including increases in VEGF, an important angiogenic factor, and PAI-1, a regulator of adhesion and migration. Furthermore, the list of genes differentially expressed in stage 4 adenocarcinomas compared to adenomas is enriched for genes with AU-rich elements and contains many RNA binding proteins, such as HuR, suggesting a role for post-transcriptional gene regulation during colorectal cancer progression. Together these results demonstrate that gene expression patterns in transformed RIE:iRas cells grown under highly controlled conditions are reflective of some of the gene expression changes that occur during tumor development and progression, suggesting that this cell culture system could yield a better understanding of the molecular mechanisms underlying carcinogenesis.

CHAPTER VII

DISCUSSION AND FUTURE DIRECTIONS

The molecular mechanisms leading to the metastatic spread of cancer cells are incompletely understood but critically important to the prevention of cancer-related fatalities. Given the heterogeneity and complexity of human tumors, experimental models of tumor cell behavior are invaluable for the elucidation of key regulators and mechanisms contributing to malignancy. In this study, we used a rat intestinal epithelial cell line under non-transforming and highly transforming conditions to characterize pathway-specific gene expression signatures in well-controlled conditions, finding that a global mechanism of post-transcriptional regulation of gene expression is important during Ras and TGF-β-mediated EMT *in vitro* and represents a clinically relevant target.

Cooperation between Ras and TGF-β

TGF-β treatment enhances oncogenic Ras-induced transformation and invasion in intestinal epithelial cells. One explanation for this is that Ras and TGF-β each moderately activate the same pathways and that threshold effects are achieved when the two are activated in combination. In this study, TGF-β-mediated activation of Smad2 was not effected by Ras expression and Ras activation of ERK was not effected by TGF-β treatment, though Ras and TGF-β together cooperate to induce COX-2 expression. These results show that synergy between Ras and TGF-β occurs downstream or in parallel with the primary effectors of Ras and TGF-β signaling. Thus, crosstalk between Ras and TGF-

β may result in threshold activation of a downstream target that in turn uniquely regulates a distinct set of genes important for EMT.

The purpose of this study is to examine the mechanism and effect of the interaction of oncogenic Ras expression and TGF-β treatment. We used microarray analysis to define a Ras and TGF-β expression signature consisting of genes that are regulated in a more than additive, or synergistic, way in response to this unique interaction. Quantitative validation of several genes in the Ras and TGF-β signature at the mRNA and protein levels demonstrated that Ras and TGF-β interact to synergistically regulate gene expression, which is of particular importance since microarray technology is not purely quantitative.

The Ras and TGF-β signature revealed numerous cooperatively regulated genes that are known to have relevance for EMT. For example, a synergistic increase in expression of the transcriptional repressor Snail is consistent with the increase in Slug we recently observed and reported during Ras-induced transformation and EMT in the RIE:iRas cells (Schmidt et al., 2005). We have previously shown that oncogenic Ras and TGF-β cooperate to synergistically increase COX-2, integrin β1 and PAI-1 expression (Fujimoto et al., 2001; Saha et al., 2001; Sheng et al., 2000), validating our expression profiling experiment. TGF-β also cooperates with Ras-mediated transformation to decrease E-cadherin and TGF-β type II receptor expression (Fujimoto et al., 2001). These gene expression changes were also noted in our present gene expression array experiment.

A model of the differing roles that members of the TGF-β superfamily play in the regulation of epithelial to mesenchymal transition is emerging. As we and others have

shown, TGF-β signaling through Smad 2/3 can promote epithelial to mesenchymal transition. BMP-7 signaling through Smad 1/5/8 blocks TGF-β-induced EMT and promotes a mesenchymal to epithelial transition (Lee et al.), particularly during the process of renal fibrosis in response to injury (Zeisberg et al., 2003). Changes in the expression of two genes, follistatin and Id2, from the microarray stood out for their potential roles in regulating or mediating this balance between TGF-β-induced EMT and BMP-induced MET. Follistatin was originally identified for its role in preventing Activin from binding its receptor and has since been found to also bind BMP-2, BMP-4 and BMP-7, blocking signaling through the Activin and BMP receptors. Upregulation of Follistatin, as seen in these experiments, would be predicted to block BMP signaling, promoting the transforming effects of TGF-β signaling.

The Id family of proteins play a role in EMT and inhibit differentiation by antagonizing basic helix-loop-helix (bHLH) transcription factors (Ruzinova and Benezra, 2003). A recent study demonstrated that Id2 and Id3 are differentially expressed in response to TGF-β and BMP-7. TGF-β treatment causes sustained inhibition of Id2 and Id3, whereas BMP-7 treatment results in sustained upregulation of these two proteins (Kowanetz et al., 2004). Forced overexpression of Id2 or Id3 by adenoviral infection in this study was sufficient to block the downregulation of E-cadherin and α-smooth muscle actin by TGF-β treatment of a clone of NMuMG cells (NMe cells), whereas knockdown of Id2 enhanced EMT induced by TGF-β and enabled cells to undergo EMT in response to BMP-7 (Kowanetz et al., 2004). The observed synergistic decrease in Id2 expression observed in this study suggests that this is one of the mechanisms by which activated Ras and TGF-β can collaboratively induce EMT.

One important question raised by these studies is whether the observed cooperation between oncogenic Ras and TGF-β on malignant behaviors *in vitro* also occurs *in vivo*. To address this question, it would be of interest to determine whether cells expressing both oncogenic RasV12 and active TGF-β signaling, through overexpression of TGF-β ligand or constitutively active TGF-β type II receptor, are able to form more aggressive tumors in mice. We would expect to see an increase in tumor size, vascularization, and perhaps local invasion in tumors expressing Ras and TGF-β together compared to either alone. It would be particularly interesting to determine whether injection of these cells into the tail vain, spleen, or cecum led to increased metastases.

Role of EGFR signaling

Microarray analysis revealed that several EGF family ligands and receptors show altered expression patterns in response to oncogenic Ras and TGF-β. Additional studies validating these gene expression changes, beyond TGF-α, and exploring the kinetics of EGFR activation will provide important mechanistic insights into the role of this pathway during TGF-β-induced EMT. Since EGF receptors homo- and heterodimerize, we cannot exclude the possibility that other ErbB receptors are involved in EGFR mediated EMT. It will also be important to determine whether expression of these receptors and ligands requires additional transcription or translation.

Oncogenic Ras and TGF-β cooperate to increase TGF-α mRNA expression and synergistically increase TGF-α protein, suggesting that Ras and TGF-β cooperate to regulate TGF-α by a post-transcriptional mechanism. Furthermore, increased TGF-α expression during TGF-β-induced EMT was confirmed in LIM1863 cells. ErbB ligands

are synthesized as transmembrane precursors that are proteolytically cleaved to release biologically active soluble growth factors. TACE (TNF-α converting enzyme)/ADAM17 (A disintegrin and metalloprotease 17) is the major convertase mediating cleavage and release of ErbB ligands, such as TGF-α, AR, and HB-EGF (Sunnarborg et al., 2002). After secretion, EGFR ligands rapidly bind their receptors and are internalized into the cells (Dempsey and Coffey, 1994), consistent with our observation of increased TGF-α in cell lysates but not in conditioned media. It remains to be clarified whether EGF ligands are acting in an autocrine, paracrine, or juxtacrine manner to contribute to TGF-β-induced EMT.

Stimulation of EGFR ligand binding and activation can occur through several different mechanisms, including changes in ligand production, ligand secretion, or receptor expression. Upregulation of transcription, translation, and secretion of ErbB ligands is one way to increase ligand availability to bind and activate receptors. In addition, activation of TACE or ADAMs increases proteolytic cleavage of existing transmembrane precursor ligands to release active growth factors. Transactivation of the EGF receptor by G-protein coupled receptors has been well characterized and is mediated by activation of ADAMs (Ohtsu et al., 2006). Recently, TGF-β was reported to stimulate transactivation of the EGFR independent of ligand release, similar to G-protein coupled receptors, (Joo et al., 2007). Another mechanism to increase ErbB activity involves increased receptor expression, which can help overcome ligand-induced receptor internalization and ubiquitin-mediated degradation (Sweeney et al., 2006). The data presented here suggest that Ras and TGF-β activation of EGFR signaling could occur through several of these mechanisms. Ras and TGF-β increase EGFR expression and

increased TGF-α mRNA levels suggests that Ras and TGF-β stimulate transcription of TGF-α, but the synergy observed at the TGF-α protein level suggests a combination of transcription and transactivation are responsible for increasing TGF-α expression.

These studies have shown that EGFR activity is required for TGF-β-induced EMT and invasion of RIE:iRas and LIM1863 cells. Nearly 100% of LIM1863 cells attach to the plastic substrate upon TGF-β-treatment and EGFR signaling seems to be required for this process, however further quantitation of the effects of blocking EGFR signaling on TGF-β-induced attachment need to be performed. An EGFR tyrosine kinase inhibitor blocks the TGF-β-induced phenotypic transformation of RIE:iRas and LIM1863 cells, but these studies would benefit from an examination of molecular markers of EMT, such as E-cadherin, N-cadherin, α-smooth muscle actin, and vimentin expression and actin re-arrangement to confirm EMT. Although the invasion assay results are preliminary and further confirmation is needed, they suggest that EGFR signaling is necessary for TGF-β to enhance cellular invasiveness.

As outlined in the introduction of chapter 4, the exact role of EGFR signaling in cancer is unclear. EGFR expression is low in normal colon but increases in aberrant crypt foci and colorectal cancer (Borlinghaus et al., 1993; Ciardiello et al., 1991; Cohen et al., 2006). However, the prognostic value of EGFR expression remains controversial (Overman and Hoff, 2007). A comparison of EGFR mRNA expression in adenomas and stage 4 adenocarcinomas in this study shows that EGFR expression is decreased in later stage colorectal cancers. Further examination of EGFR expression in normal colon and a larger number of patients with early stage colorectal adenomas and adenocarcinomas would clarify the pattern of EGFR expression during colorectal cancer progression.

Based on our current knowledge of EGFR actions, we propose that EGFR plays a role in early cancer development, increasing proliferation and acting in concert with other oncogenic events to increase malignant behaviors, such as loss of cell-cell adhesion and increased migration, but that EGFR activity becomes less important as tumors acquire other mechanisms to grow independently of growth factors and invade.

Regulation of VEGF expression

The collaboration of Ras and TGF-β in regulating VEGF was of particular interest to us since VEGF is known to play an important role in the angiogenesis necessary for tumor growth and metastasis (Fujimoto et al., 1998; Konno et al., 1998; Takahashi et al., 1995; Wong et al., 1999). Our results show that Ras and TGF-β synergistically regulate VEGF mRNA and protein expression in RIE:iRas cells and in an independent colon cancer cell line, demonstrating the universality of this response in intestinal cells. VEGF is induced in a dose-dependent manner by both TGF-β and Ras induction and the combination of Ras and TGF-β together induces VEGF expression more than predicted from the effect of each alone.

COX-2 expression is rapidly induced by a variety of mitogens and tumor promoters (DuBois et al., 1994). A key enzyme in the metabolism of arachadonic acid, COX-2 increases the production of prostaglandins, which can promote survival and growth of colorectal cancer cells. Increased expression of COX-2 is frequently observed in colon cancer (Eberhart et al., 1994) and treatment with non-steroidal anti-inflammatory drugs (NSAIDs) that inhibit COX-2 is associated with a decreased incidence of colon cancer (Smalley and DuBois, 1997; Thun et al., 1991). High COX-2 expression in colon

cancer has been correlated with increased VEGF expression and microvessel density (Masunaga et al., 2000). Although COX-2 inhibitors clearly decrease tumor formation, the direct effects of COX-2 activity on VEGF expression are less clear. In one study, HCA-7 cells and Caco-2 colon cancer cells over-expressing COX-2 produced high levels of VEGF that were decreased by COX-2 inhibitors (Tsujii et al., 1998). In another study, colon cancer cells treated with a high dose of COX-2 inhibitors (60 μM), were shown to exhibit COX-2-independent effects on cell growth and VEGF expression (Abdelrahim and Safe, 2005). Other studies have demonstrated either no change or a slight increase in VEGF expression following treatment with COX-2 inhibitors, consistent with the observations presented here (Kim et al., 2005b; Nishikawa et al., 2004). These studies demonstrate the complex regulation of VEGF expression in colon cancer and suggest that there are COX-2-dependent and independent mechanisms, determined in part by the microenvironment and genetic factors.

The synergistic increase in VEGF expression during oncogenic Ras and TGF-β-induced EMT may not be accounted for due to increased transcriptional activity of the VEGF gene. The post-transcriptional regulation of COX-2 by Ras and TGF-β (Sheng et al., 2000) and recent literature demonstrating that under certain conditions VEGF mRNA stability may be altered (Claffey et al., 1998; Levy et al., 1995; Levy et al., 1998; Shima et al., 1995) suggest that changes in post-transcriptional regulation are an important determinant of VEGF expression in cancer cells. This study demonstrates that cooperation between TGF-β and oncogenic Ras increased VEGF mRNA and protein expression by stabilizing VEGF mRNA. However, we cannot eliminate the possibility of a transcriptional contribution to the Ras and TGF-β cooperative increase in VEGF

expression, as the VEGF promoter activity shown here is from a single representative experiment and should be verified. VEGF mRNA stabilization under hypoxic conditions is mediated by conserved ARE sequences in the 3'UTR (Claffey et al., 1998). One study has also shown that destabilizing elements in the 5'UTR and coding region contribute to the regulation of VEGF mRNA stability (Dibbens et al., 1999). It remains to be determined which VEGF mRNA regions are necessary for regulating Ras and TGF-β-mediated stabilization.

Global post-transcriptional mechanism

One goal of this study was to determine the precise molecular mechanism by which oncogenic Ras and TGF-β synergistically regulate gene expression. The synergistic regulation of both VEGF and COX-2 (Sheng et al., 2000) mRNA stability suggested to us that post-transcriptional gene regulation is an important component of the collaborative effects of oncogenic Ras and TGF-β signaling. The primary mechanism for post-transcriptional regulation is through AU-rich elements that are located in the 3'UTR of mRNA (Caput et al., 1986; Shaw and Kamen, 1986) and significantly, 39% of genes synergistically regulated in this study contain AU-rich elements representing a greater than 4-fold enrichment compared to the genome as a whole (Bakheet et al., 2006). Among the genes increased or decreased by oncogenic Ras expression and TGF-β treatment, alone or together, we also observed a 4-fold enrichment of ARE-containing genes, suggesting that in addition to COX-2 and VEGF, a number of other genes known to be involved in tumor progression may also be co-regulated by a similar post-

transcriptional mechanism. This enrichment of ARE-containing genes indicates that the regulation of mRNA stability is an important mechanism of gene regulation during EMT.

There is a growing body of evidence suggesting that defects in mRNA turnover play a central role in cellular transformation. Specific immediate-early genes (proto-oncogenes among them), growth factors, cytokines and genes encoding other inflammatory mediators, such as COX-2 and VEGF, have been shown to be regulated though mRNA stabilization (Audic and Hartley, 2004). For example, the rapid decay of c-myc mRNA is mediated by *cis*-elements in the 3'UTR, yet in certain cases of myeloma and leukemia, the c-myc 3'UTR is translocated or lost, leading to increased c-myc mRNA stability (Hollams et al., 2002). Through specific interaction with AREs, RNA-binding proteins can be either positive or negative regulators of stability and translation (Bevilacqua et al., 2003). The Ras and TGF-β signature contains two known RNA binding proteins. Heterogeneous nuclear ribonucleoprotein A/B (hnRNP A/B), a member of the hnRNP family of RNA binding proteins involved in RNA trafficking and splicing, increased more than 3-fold with combined Ras expression and TGF-β treatment. In addition, CUGBP2, which has been shown to bind the COX-2 3'UTR and promote its stability while inhibiting its translation in response to ionizing radiation (Mukhopadhyay et al., 2003), decreased more than 4-fold in response to Ras expression and TGF-β treatment. The altered expression of hnRNP A/B and CUGBP2 mediated by Ras and TGF-β remains to be confirmed at the protein level. The role of these proteins in the regulation of VEGF mRNA stability can be assessed through knockdown or overexpression, respectively.

Another important RNA binding protein that acts in *trans* with the 3'UTR AU rich elements is HuR, a member of the ELAV family of RNA binding proteins. HuR is a nuclear-cytoplasmic shuttling protein (Fan and Steitz, 1998a) and it is generally believed that the ability of HuR to promote mRNA stabilization requires its translocation to the cytoplasm (Fan and Steitz, 1998b; Peng et al., 1998) where it is able to stabilize ARE-containing mRNA transcripts such as COX-2 (Dixon et al., 2001; Fan and Steitz, 1998b; Peng et al., 1998). Furthermore, the increased expression and cytoplasmic localization of HuR is correlated with elevated COX-2 expression and poor outcome in ovarian, breast, and colon cancers (Denkert et al., 2004; Dixon et al., 2001; Erkinheimo et al., 2003; Heinonen et al., 2005). In the present studies, oncogenic Ras and TGF-β cooperate to increase both the total steady-state level of HuR and to increase the relative amount of HuR in the cytoplasm. The increase in HuR protein expression after Ras induction and TGF-β treatment was not associated with an increase in HuR mRNA, suggesting that oncogenic Ras and TGF-β signaling may influence HuR expression through translation or protein stabilization. It remains to be determined whether HuR directly interacts with VEGF or COX-2 mRNA and if HuR expression is necessary for Ras and TGF-β to stabilize mRNA.

These data suggest a possible mechanism by which oncogenic Ras and TGF-β can cooperatively regulate ARE-containing genes, such as COX-2 and VEGF. Modulating the expression, activity, and binding specificity of these *trans*-acting regulatory proteins could change the RNA-protein complexes formed, profoundly affecting RNA decay and protein translation. While beyond the scope of this study, resolving the role of RNA

binding proteins during oncogenic Ras and TGF-β-mediated EMT could contribute important insights into the mechanisms of malignant transformation.

Oncogenic Ras and TGF-β each activate numerous downstream signaling pathways, including MEK/ERK, PI3K/Akt, JNK, and p38, that contribute to EMT. Many of these signaling pathways regulate mRNA decay during inflammation and transformation, such as p38, JNK, ERK, and PKC (Eberhardt, 2007 #187). Further defining the signaling pathways that regulate RNA binding proteins and ARE-mediated turnover is important for understanding the mechanisms underlying malignant cell behavior and cancer progression. Although no drugs have yet been identified that target ARE-binding proteins, the development of novel therapeutics effecting mRNA decay is a promising approach to treating inflammatory disease and cancer.

Clinical relevance

During Ras and TGF-β-induced EMT and invasiveness, RIE:iRas cells model aspects of human tumor cell behavior (Fujimoto et al., 2001; Sheng et al., 2000). We found that several of the ARE-containing transcripts in the Ras and TGF-β gene signature derived from RIE:iRas cells are also differentially regulated in metastatic tumors compared to early adenomas, including VEGFA and PAI-1. To further determine whether genes in the Ras and TGF-β signature are significant for human malignancy, we analyzed human CRC gene expression profiles for the presence of RNA regulatory elements. The genes that are differentially regulated in late stage carcinomas compared to adenomas show a 3-fold enrichment in ARE-containing genes compared to the genome as a whole (Bakheet et al., 2006) and a similar enrichment is seen as early as Stage 1,

suggesting that post-transcriptional gene regulation could be an important regulatory mechanism involved in early tumor progression from adenoma to invasive carcinoma.

Cross-platform comparison of large microarray data sets, such as the human colorectal cancer and rat cell culture model system described here, will help to establish standards and tools for selecting biologically relevant gene lists for further validation studies. Such procedures will allow us to closely examine different genetic models and molecular mechanisms of gene regulation that may be important in altering the expression of genes necessary for tumor cell survival, growth, and metastasis. Future experiments could include the analysis of the effects of other oncogenes and purified growth factors, of pharmacological inhibitors of COX-2 activity and other biologically relevant targets, and of the effects of the impact of co-cultured fibroblast and matrix determinants. This type analysis will facilitate the discovery of novel intracellular regulatory mechanisms, improve molecular classification of human cancers, and identification of relevant cancer biomarkers and novel therapeutic targets.

The application of microarray technology to cancer research has the potential to assist in the classification of different types of cancer and to predict clinical outcome and survival. Microarray based gene expression profiling has already contributed to the molecular classification of hepatocellular carcinomas (Lee and Thorgeirsson, 2004). Genome-wide gene expression analysis of human colorectal cancers, transgenic mouse models of colon cancer, and mouse embryonic development were combined in a large-scale comparison revealing extensive similarities between the gene expression patterns in mouse models and human cancers and early embryonic development (Kaiser et al., 2007). In addition, several recent studies from the Thorgeirsson lab have utilized gene

expression profiles generated from cell lines and mouse models to identify subsets of hepatocellular carcinoma patients with poor prognosis (Kaposi-Novak et al., 2006; Lee et al., 2004a; Lee et al., 2006). It would be interesting to perform similar analyses with the gene expression profiles generated from Ras and TGF-β transformed intestinal epithelial cells and the human CRC data from the GI SPORE.

Conclusion

These studies show that oncogenic Ras and TGF-β together regulate the expression of a set of genes that includes members of the EGFR family, the TGF-β and Wnt signaling pathways, and an important angiogenic factor, VEGF. Analysis of a Ras and TGF-β gene signature reveals that these conditions enrich for ARE-containing mRNA transcripts. Consistent with this effect, oncogenic Ras and TGF-β synergistically increase the expression of VEGF through stabilization of its ARE-containing mRNA. Furthermore, VEGF and other ARE-containing transcripts are differentially regulated in stage 4 colorectal adenocarcinomas. The reflection of the Ras and TGF-β gene expression profile and enrichment of AREs in human CRC cancers demonstrates that this experimental model system can provide novel molecular explanations of EMT and cancer progression, revealing important potential therapeutic targets. Thus, these studies demonstrate that oncogenic Ras and TGF-β cooperate to induce EMT and malignant cell behaviors by post-transcriptional regulation of a unique set of mRNA transcripts with clinical relevance to human CRC.

Table 5: Genes synergistically regulated by oncogenic Ras and TGF-β

probe_id	Gene Name	Gene Symbol	AU-rich element	Fold change		
				TGF-β	RasV12	RasV12 + TGF-β
1368519_at	serine (or cysteine) peptidase inhibitor, clade E, member 1	Serpine1	ARE	42.22	5.53	75.28
1373000_at	sushi-repeat-containing protein, X-linked 2 (predicted)	Srpx2		19.24	25.59	73.63
1383486_at	Transcribed locus	---	ARE	5.93	27.94	52.85
1367581_a_at	secreted phosphoprotein 1	Spp1		2.46	19.28	49.76
1368359_a_at	VGF nerve growth factor inducible	Vgf		3.11	4.61	48.10
1392618_at	Transcribed locus	---	ARE	3.48	24.30	45.76
1398302_at	prolactin-like protein F	Prlpf	ARE	1.39	3.29	45.23
1392264_s_at	serine (or cysteine) peptidase inhibitor, clade E, member 1	Serpine1	ARE	24.92	3.67	40.09
1391022_at	laminin, beta 3	Lamb3		2.13	3.31	38.15
1384605_at	Transcribed locus	---		2.94	14.57	37.91
1367973_at	chemokine (C-C motif) ligand 2	Ccl2	ARE	5.47	17.28	37.90
1369249_at	progressive ankylosis homolog (mouse)	Ank	ARE	3.12	8.33	33.58
1398479_at	ryanodine receptor 3	Ryr3	ARE	1.42	9.28	29.65
1371194_at	tumor necrosis factor alpha induced protein 6	Tnfaip6	ARE	2.95	7.90	29.24
1386344_at	Progressive ankylosis homolog (mouse)	Ank		3.59	9.42	28.55
1368945_at	bone morphogenetic protein 2	Bmp2	ARE	1.69	22.39	27.95
1398662_at	Transcribed locus	---		8.35	12.74	26.27
1386552_at	Transcribed locus	---		2.26	15.31	25.51
1368210_at	interleukin 24	Il24	ARE	1.30	2.95	23.63
1388204_at	matrix metallopeptidase 13	Mmp13	ARE	2.18	3.23	23.10
1398270_at	bone morphogenetic protein 2	Bmp2	ARE	1.93	17.82	22.84
1383987_at	Transcribed locus	---		2.63	10.44	22.11
1391083_at	Rho GTPase activating protein 22 (predicted)	Arhgap22		1.30	19.08	21.97
1393439_a_at	progressive ankylosis homolog (mouse)	Ank	ARE	2.58	5.32	21.63
1384027_a_at	---	---		2.17	1.00	21.38
1382378_at	cathepsin W	Ctsw		6.39	-1.02	20.55
1376197_at	transcription factor 7, T-cell specific (predicted)	Tcf7	ARE	1.85	-1.10	20.51
1385709_x_at	Progressive ankylosis homolog (mouse)	Ank		2.30	5.15	20.45
1374594_at	similar to RIKEN cDNA 1600029D21	LOC363060		1.40	16.93	19.30
1382603_at	Similar to PD-1-ligand precursor (predicted)	RGD1566211		1.13	1.95	19.09
1369081_at	neuraminidase 1	Neu1	ARE	4.75	1.61	17.73
1378867_at	Similar to CG32425-PA (predicted)	RGD1307366		2.99	12.57	16.65
1390835_at	similar to 1300013J15Rik protein	RGD1311123		4.20	11.04	16.47
1368592_at	interleukin 1 alpha	Il1a	ARE	1.08	1.46	16.17
1370047_at	ectonucleotide pyrophosphatase/phosphodiesterase 1	Enpp1		7.78	5.19	15.56
1368564_at	solute carrier family 17 (sodium-dependent inorganic phosphate cotransporter), member 6	Slc17a6	ARE	-1.02	5.74	15.25
1367616_at	natriuretic peptide precursor type B	Nppb	ARE	2.73	9.17	14.57
1380028_at	similar to ADP-ribosylation factor-like protein 4C (ADP-ribosylation factor-like 7)	LOC367311		1.58	2.77	13.95
1375684_at	neuraminidase 1	Neu1		3.74	1.40	13.81
1388972_at	reticulon 4 receptor	Rtn4r		3.90	1.16	13.72
1387548_at	hyaluronan synthase 2	Has2	ARE	1.18	1.88	13.06
1378133_at	Transcribed locus	---		1.80	13.18	13.01
1384028_at	---	---		2.26	1.08	12.94
1393314_at	Transcribed locus	---		3.18	1.28	12.81
1387562_at	peptidyl arginine deiminase, type III	Padi3		2.40	1.16	12.57
1373008_x_at	reticulon 4 receptor	Rtn4r		3.33	1.18	12.43
1378556_at	Transcribed locus	---		6.54	4.22	12.21
1393728_at	Transcribed locus	---	ARE	1.65	7.90	11.60
1375815_at	Progressive ankylosis homolog (mouse)	Ank		1.32	2.21	11.54
1386529_at	Transcribed locus	---		3.71	3.88	11.53
1393927_at	wingless-related MMTV integration site 2	Wnt2		3.02	1.08	11.51
1395357_at	microtubule-associated protein 1b	Map1b	ARE	4.30	5.96	11.10
1389467_at	similar to RIKEN cDNA 1810057C19	MGC108778	ARE	1.08	1.08	10.34
1382482_at	Transcribed locus	---		1.41	2.17	10.01
1379340_at	lamimin, gamma 2	Lamc2	ARE	1.78	7.49	9.63
1397729_x_at	similar to RIKEN cDNA 1600029D21	LOC363060		1.18	8.10	9.30
1379284_at	similar to RIKEN cDNA 2810457I06 (predicted)	RGD1310357		1.59	3.68	9.23
1375014_at	similar to ADP-ribosylation factor-like protein 4C (ADP-ribosylation factor-like 7)	LOC367311	ARE	1.45	2.14	9.05
1373363_at	microtubule-associated protein 1b	Map1b	ARE	3.72	4.77	8.78
1385738_at	Transcribed locus	---		1.26	1.75	8.53
1385925_at	Transcribed locus	---		4.65	2.63	8.46
1367723_a_at	linker of T-cell receptor pathways	Lnk	ARE	1.21	3.78	8.02
1369008_a_at	olfactomedin 1	Olfm1		1.14	2.08	7.91
1382375_at	Wingless-type MMTV integration site 5A	Wnt5a		1.17	3.04	7.85
1397769_at	Similar to RIKEN cDNA 6330512M04 gene (predicted)	RGD1563319		-1.16	6.18	7.59
1373807_at	vascular endothelial growth factor A	Vegfa	ARE	1.45	5.90	7.52
1393003_at	Transcribed locus	---	ARE	2.43	2.99	7.28
1370427_at	platelet derived growth factor, alpha	Pdgfa	ARE	1.31	2.28	7.17
1377365_at	similar to hypothetical protein DKFZp434H2010 (predicted)	RGD1311019		1.01	2.25	7.04
1370387_at	cytochrome P450, family 3, subfamily a, polypeptide	Cyp3a13	ARE	1.08	5.26	7.04
1368374_a_at	gamma-glutamyltransferase 1	Ggt1		1.27	1.07	7.01
1369263_at	wingless-type MMTV integration site 5A	Wnt5a	ARE	1.02	2.91	6.96
1373298_at	Transcribed locus	---		1.16	2.56	6.90
1370336_at	pregnancy-induced growth inhibitor	Okl38		1.94	2.48	6.86
1392791_at	Early growth response 3	Egr3	ARE	1.16	3.84	6.81
1388596_at	coactosin-like 1 (Dictyostelium) (predicted)	Cotl1		1.64	1.40	6.78
1373759_at	Transcribed locus	---		1.14	5.17	6.76
1369012_at	inhibin beta-A	Inhba		1.57	2.87	6.76
1399032_at	excision repair cross-complementing rodent repair deficiency, complementation group 1 (predicted)	Ercc1		1.32	4.16	6.70
1378522_at	Transcribed locus	---		2.72	2.89	6.64
1375986_at	Similar to BTEB3 protein (predicted)	RGD1565099		2.29	2.95	6.59

1368804_at	leukemia inhibitory factor	Lif		1.16	4.56	6.53
1368009_at	glucosamine	Gne	ARE	1.77	4.11	6.52
1389463_at	protein kinase, cAMP dependent regulatory, type I, beta	Prkar1b		1.59	1.12	6.47
1392820_at	Fibroblast growth factor 1	Fgf1		2.25	1.33	6.46
1370023_at	gap junction membrane channel protein alpha 4	Gja4		1.29	4.14	6.39
1379598_at	Transcribed locus	---		1.16	1.36	6.34
1379375_at	Platelet derived growth factor, alpha	Pdgfa		1.29	2.21	6.31
1374817_at	Transcribed locus	---	ARE	-1.28	2.09	6.25
1387843_at	follistatin	Fst		2.83	2.69	6.16
1368350_at	protein tyrosine phosphatase, receptor-type, Z polypeptide 1	Ptprz1	ARE	-1.18	1.78	6.05
1369625_at	aquaporin 1	Aqp1		1.08	1.34	5.95
1395715_at	Transcribed locus	---		1.48	2.00	5.89
1368259_at	prostaglandin-endoperoxide synthase 1	Ptgs1	ARE	1.15	4.07	5.81
1375320_at	Similar to hypothetical protein DKFZp434H2010 (predicted)	RGD1311019		1.30	2.12	5.81
1396208_at	gamma-glutamyltransferase-like activity 1	Ggtla1		1.61	1.09	5.68
1376498_at	similar to 2900002H16Rik protein (predicted)	RGD1307973	ARE	1.26	1.70	5.62
1380410_at	Similar to GLI pathogenesis-related 2	LOC679819		2.97	2.08	5.58
1385805_at	---	---		1.49	1.19	5.52
1393494_at	Transcribed locus	---		1.22	1.88	5.48
1381243_at	Transcribed locus	---	ARE	1.24	3.69	5.45
1383096_at	amyloid beta (A4) precursor-like protein 2	Aplp2	ARE	1.02	2.51	5.43
1388785_at	dynein, axonemal, light chain 4	Dnalc4	ARE	1.34	3.05	5.35
1373751_at	Transcribed locus	---	ARE	1.81	2.01	5.31
1391563_at	similar to melanoma associated antigen (mutated) 1-like 1 (predicted)	RGD1565148	ARE	1.37	1.62	5.23
1390983_at	Transcribed locus	---	ARE	1.06	3.02	5.19
1385181_at	Transcribed locus	---		1.05	2.39	5.16
1371211_a_at	neuregulin 1	Nrg1		1.10	3.72	5.08
1380895_at	Amyloid beta (A4) precursor-like protein 2	Aplp2		1.14	1.77	5.05
1368545_at	CASP8 and FADD-like apoptosis regulator	Cflar	ARE	1.49	3.16	5.00
1385926_at	Transcribed locus	---		2.76	1.79	5.00
1369683_at	BH3 interacting domain death agonist	Bid		1.23	1.62	4.93
1368254_a_at	sphingosine kinase 1	Sphk1		1.78	2.19	4.83
1383883_at	similar to Hypothetical protein KIAA0469 (predicted)	RGD1309644		1.70	2.48	4.78
1370082_at	transforming growth factor, beta 1	Tgfb1		1.73	2.35	4.78
1374920_at	Transcribed locus	---		1.55	2.61	4.73
1378241_at	---	---		1.97	1.00	4.71
1385235_at	bol, boule-like (Drosophila) (predicted)	Boll	ARE	-1.42	3.09	4.71
1374537_at	carbohydrate (chondroitin) synthase 1 (predicted)	Chsy1	ARE	1.65	2.23	4.67
1390429_at	Transcribed locus	---	ARE	1.55	2.66	4.61
1377759_at	Transcribed locus	---	ARE	1.21	1.59	4.57
1375377_at	Transcribed locus	---		1.43	2.25	4.56
1384238_at	similar to Tweety homolog 2 (predicted)	RGD1562969	ARE	1.10	2.40	4.45
1388121_at	amyloid beta (A4) precursor-like protein 2	Aplp2		1.01	2.36	4.44
1393344_at	Transcribed locus	---	ARE	1.70	2.15	4.31
1387206_at	UDP-Gal:betaGlcNAc beta 1,4-galactosyltransferase, polypeptide 6	B4galt6	ARE	1.80	1.96	4.31
1387184_at	axin2	Axin2	ARE	1.42	2.26	4.26
1378032_at	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, zeta (predicted)	Nfkbiz	ARE	1.35	2.46	4.23
1382442_at	similar to Septin-6	LOC682750	ARE	1.22	2.14	4.23
1391121_at	similar to DNA segment, Chr 8, ERATO Doi 82, expressed (predicted)	RGD1311793	ARE	1.35	1.94	4.19
1383257_at	Transcribed locus	---	ARE	-1.81	2.79	4.14
1368713_at	matrix metallopeptidase 10	Mmp10	ARE	1.24	2.71	4.10
1394361_a_at	wingless-related MMTV integration site 2	Wnt2	ARE	1.97	1.24	4.07
1385706_at	Transcribed locus	---		1.50	1.77	4.01
1368681_at	parathyroid hormone-like peptide	Pthlh	ARE	1.12	1.95	4.00
1387301_at	fibroblast growth factor 1	Fgf1		1.84	1.25	3.99
1372569_at	four and a half LIM domains 3 (predicted)	Fhl3		1.59	2.25	3.93
1369293_at	reticulon 4 receptor	Rtn4r		1.65	1.06	3.84
1387975_at	UDP-glucose ceramide glucosyltransferase	Ugcg	ARE	1.27	2.47	3.81
1390249_at	similar to DKFZP434H132 protein	RGD1305464		1.06	1.48	3.80
1373970_at	similar to RIKEN cDNA 9230117N10	RGD1311155		-1.12	3.64	3.72
1369783_a_at	neuregulin 1	Nrg1	ARE	-1.15	2.58	3.69
1393252_at	Transcribed locus	---	ARE	1.44	1.04	3.65
1370948_a_at	myristoylated alanine rich protein kinase C substrate	Marcks	ARE	1.09	1.48	3.65
1378480_at	Transcribed locus	---		1.01	1.86	3.64
1370817_at	Sec11-like 3 (S. cerevisiae)	Sec11l3		1.44	1.59	3.55
1368323_at	tissue factor pathway inhibitor	Tfpi	ARE	1.36	1.06	3.55
1383286_at	pleckstrin 2 (predicted)	Plek2		1.34	1.49	3.54
1372277_at	Taste receptor, type 1, member 2	Tas1r2		-1.33	1.74	3.51
1373226_at	Transcribed locus	---		1.37	2.03	3.43
1383130_at	Transcribed locus	---		-1.24	2.38	3.42
1378009_at	Transcribed locus	---	ARE	1.06	2.00	3.35
1371696_at	similar to G protein-coupled receptor 56	LOC682401		1.22	1.26	3.34
1384878_at	N-myristoyltransferase 2	Nmt2		1.48	1.77	3.34
1399065_at	Ring finger protein 167	Rnf167		1.42	1.32	3.33
1381121_at	Basic helix-loop-helix domain containing, class B2	Bhlhb2		1.75	1.23	3.28
1373258_at	cathepsin F	Ctsf		1.89	-1.09	3.25
1371193_at	tumor necrosis factor alpha induced protein 6	Tnfaip6		1.20	1.30	3.22
1376362_at	neuronal pentraxin receptor	Nptxr	ARE	1.50	1.58	3.22
1379614_at	Transcribed locus	---	ARE	1.10	1.69	3.20
1393140_at	zinc finger CCCH type containing 12A (predicted)	Zc3h12a		1.05	1.57	3.20
1390386_at	caspase 3, apoptosis related cysteine protease	Casp3	ARE	1.20	1.94	3.16
1367905_at	ectonucleotide pyrophosphatase/phosphodiesterase 3	Enpp3		1.02	2.02	3.10
1368347_at	procollagen, type V, alpha 3	Col5a3		1.16	1.72	3.10
1371014_at	phospholipase C, beta 1	Plcb1	ARE	-1.02	1.30	3.09
1376799_a_at	cytokine receptor-like factor 1 (predicted)	Crlf1		1.95	1.10	3.07
1378262_at	Transcribed locus	---		1.10	1.41	3.05
1372935_at	Transcribed locus	---		1.95	-1.24	3.04
1368531_at	prolactin-like protein C 1	Prlpc1	ARE	1.09	1.14	3.03
1369686_at	double cortin and calcium/calmodulin-dependent protein kinase-like 1	Dcamkl1	ARE	1.19	1.38	2.99
1385649_at	integrin alpha 5	Itga5		1.22	1.71	2.99
1393191_at	similar to RIKEN cDNA 2610200G18 (predicted)	RGD1561205	ARE	1.28	1.19	2.93
1390925_a_at	Transcribed locus	---		1.17	1.56	2.91
1387121_a_at	N-myc downstream regulated gene 2	Ndrg2		1.46	-1.19	2.86

1373432_at	similar to Myristoylated alanine-rich C-kinase substrate	Marcks	ARE	-1.05	1.24	2.78
1377975_at	Transcribed locus	---	ARE	-1.03	1.13	2.76
1370949_at	myristoylated alanine rich protein kinase C substrate	Marcks	ARE	-1.07	1.28	2.76
1367754_s_at	heterogeneous nuclear ribonucleoprotein A/B	Hnrpab		1.14	1.20	2.72
1370256_at	frizzled homolog 1 (Drosophila)	Fzd1	ARE	1.20	1.24	2.70
1386645_at	similar to 2900002H16Rik protein (predicted)	RGD1307973		1.06	1.22	2.67
1373786_at	Transcribed locus	---		1.81	-1.52	2.64
1371127_at	bone morphogenetic protein 1	Bmp1		1.64	-1.09	2.61
1374779_at	Coagulation factor XIII, A1 subunit	F13a1		1.06	1.00	2.61
1375957_at	Transcribed locus	---		1.35	1.06	2.58
1382907_at	similar to 4930431B09Rik protein	LOC310721		1.03	1.05	2.57
1368916_at	argininosuccinate lyase	Asl	ARE	1.12	1.15	2.52
1387651_at	aquaporin 1	Aqp1		1.02	1.15	2.51
1389048_at	bone morphogenetic protein 1	Bmp1		1.28	-1.25	2.48
1391399_at	Transcribed locus	---		1.35	-1.13	2.46
1387729_at	gamma-glutamyltransferase-like activity 1	Ggtla1		1.19	-1.00	2.45
1378947_at	tensin 4	Tns4		-1.29	1.28	2.36
1374816_at	similar to hypothetical protein FLJ30973	LOC363091	ARE	1.24	-1.06	2.31
1396445_at	RIB43A domain with coiled-coils 2	Ribc2		1.08	-1.11	2.28
1389229_at	acid phosphatase-like 2	Acpl2	ARE	-1.45	1.07	2.07
1373657_at	solute carrier family 31, member 2	Slc31a2	ARE	1.26	-1.34	-2.17
1375028_at	Transcribed locus	---		1.05	-1.23	-2.22
1371131_a_at	thioredoxin interacting protein	Txnip	ARE	-1.12	-1.05	-2.27
1391458_at	N-myc downstream regulated gene 1	Ndrg1		-1.03	-1.10	-2.38
1389142_at	similar to Sulfide:quinone oxidoreductase, mitochondrial precursor	LOC691966		-1.42	1.18	-2.42
1398582_at	ribosomal protein S6 kinase, polypeptide 5 (predicted	Rps6ka5	ARE	-1.19	-1.20	-2.54
1387260_at	Kruppel-like factor 4	Klf4	ARE	-1.29	1.04	-2.54
1373079_at	Transcribed locus	---		1.54	-1.84	-2.61
1398387_at	Unknown (protein for MGC:72614)	MGC72614		2.15	1.11	-2.61
1375716_at	interferon gamma receptor 2 (predicted)	Ifngr2		1.09	-1.05	-2.63
1376920_at	similar to sterile alpha motif domain containing 9-like	LOC500013	ARE	-1.18	-1.25	-2.65
1371360_at	N-myc downstream regulated gene 1	Ndrg1	ARE	-1.04	-1.19	-2.69
1374142_at	similar to RIKEN cDNA E130201N16 (predicted)	RGD1311589		-1.10	-1.09	-2.72
1388791_at	similar to 2810022L02Rik protein	RGD1309930	ARE	-1.37	-1.10	-2.72
1376319_at	Transcribed locus	---		-1.20	-1.50	-2.79
1384617_at	Unknown (protein for MGC:72614)	MGC72614	ARE	2.37	1.24	-2.80
1393653_at	similar to putative protein product of HMFN2073 (predicted)	RGD1560766	ARE	2.22	-1.31	-2.83
1385150_at	Transcribed locus	---		-1.50	1.59	-2.84
1369895_s_at	podocalyxin-like	Podxl	ARE	-1.34	-1.65	-2.90
1374544_at	hypothetical protein LOC679150	LOC679150		-1.63	1.25	-2.90
1395336_at	hypothetical LOC501207	LOC501207		-1.52	-1.20	-2.97
1383194_a_at	CDNA clone IMAGE:7379585	---		-1.47	-1.51	-2.97
1387354_at	signal transducer and activator of transcription 1	Stat1	ARE	-1.65	-1.26	-2.99
1379444_at	Transcribed locus	---		1.53	-1.17	-2.99
1377669_at	RAB27A, member RAS oncogene family	Rab27a	ARE	-1.27	-1.06	-3.02
1384852_at	RAB27A, member RAS oncogene family	Rab27a		-1.29	-1.05	-3.03
1372757_at	signal transducer and activator of transcription 1	Stat1		-1.72	-1.27	-3.05
1398640_at	Transcribed locus	---		-1.03	-1.67	-3.07
1379606_at	RAB30, member RAS oncogene family	Rab30	ARE	1.22	-1.30	-3.11
1377821_at	Transcribed locus	---		1.12	-1.78	-3.13
1395427_at	Transcribed locus	---		-1.47	-1.51	-3.13
1376878_at	similar to RIKEN cDNA 2310022B05 (predicted)	RGD1559896		1.06	-2.20	-3.19
1387625_at	insulin-like growth factor binding protein 6	Igfbp6		-1.25	-1.40	-3.23
1396090_at	similar to RIKEN cDNA A630007N06 gene (predicted)	RGD1304572		-1.28	-1.88	-3.25
1384132_at	immunoglobulin superfamily, member 4A	Igsf4a		-1.21	-1.87	-3.31
1368146_at	dual specificity phosphatase 1	Dusp1	ARE	1.74	-1.06	-3.31
1382044_at	hypothetical protein LOC498796	LOC498796	ARE	-1.42	-1.91	-3.34
1380133_at	odd-skipped related 2 (Drosophila)	Osr2	ARE	-1.44	-1.42	-3.35
1367974_at	annexin A3	Anxa3	ARE	-1.09	-2.27	-3.35
1368147_at	dual specificity phosphatase 1	Dusp1		1.95	-1.00	-3.42
1368835_at	signal transducer and activator of transcription 1	Stat1		-2.02	-1.29	-3.42
1373521_at	similar to RIKEN cDNA D430044G18	RGD1309038	ARE	-1.52	-1.82	-3.45
1376337_at	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 2	Smarca2	ARE	-1.53	-1.69	-3.45
1372133_at	related RAS viral (r-ras) oncogene homolog 2	Rras2		-1.11	-2.13	-3.45
1383064_at	Transcribed locus	---		-1.43	-1.57	-3.53
1385759_at	serine (or cysteine) peptidase inhibitor, clade B (ovalbumin), member 10	Serpinb10		-2.89	1.17	-3.62
1372111_at	---	---	ARE	-1.39	-1.82	-3.64
1385074_at	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 2	Smarca2		-1.65	-1.74	-3.66
1382060_at	Transcribed locus	---		-1.46	-1.98	-3.74
1368322_at	superoxide dismutase 3, extracellular	Sod3		-1.76	-1.40	-3.79
1383795_at	Transcribed locus	---		-1.46	-1.62	-3.80
1374131_at	Transcribed locus	---		-1.32	-1.88	-3.87
1393281_at	CDC42 effector protein (Rho GTPase binding) 5 (predicted)	Cdc42ep5		-1.51	-1.87	-3.87
1389146_at	hypothetical protein LOC498796	LOC498796		-1.40	-2.12	-3.91
1370061_at	RAB3B, member RAS oncogene family	Rab3b		-1.48	-1.13	-3.93
1388471_at	t-complex 11 (mouse) like 2	Tcp11l2	ARE	-1.92	-1.90	-3.94
1392301_at	SH3 domain and tetratricopeptide repeats 1 (predicted)	Sh3tc1		-1.70	-2.42	-4.07
1372457_at	mitochondrial tumor suppressor 1	Mtus1		1.20	-3.22	-4.12
1383842_at	Transcribed locus	---		-1.86	-2.42	-4.22
1383063_a_at	Transcribed locus	---		-1.54	-1.59	-4.28
1392246_at	Transcribed locus	---		-1.45	-2.29	-4.29
1395313_s_at	Annexin A3	Anxa3		1.11	-3.37	-4.34
1397837_at	Transcribed locus	---		-2.00	-1.65	-4.34
1369182_at	coagulation factor III	F3	ARE	-1.60	-2.74	-4.42
1378413_at	Transcribed locus	---		-2.27	-2.42	-4.42
1386907_at	Carboxypeptidase D	Cpd		-1.29	-1.71	-4.45
1374159_at	Transcribed locus	---		-1.40	-2.77	-4.45
1378753_at	occludin	Ocln		2.16	-3.53	-4.50
1376919_at	Similar to expressed sequence AW212394 (predicted)	RGD1562317		-1.44	-2.26	-4.59
1373315_at	Aryl hydrocarbon receptor nuclear translocator 2	Arnt2		-1.72	-2.40	-4.62
1369973_at	Programmed cell death 4	Pdcd4	ARE	-1.31	-3.42	-4.62

1377968_at	Transcribed locus	---		-2.10	-2.26	-4.68
1380017_at	Transcribed locus	---		-1.25	-3.29	-4.68
1379252_at	Transcribed locus	---		-1.41	-2.24	-4.75
1373881_at	Rho, GDP dissociation inhibitor (GDI) beta	Arhgdib		-1.76	-2.34	-4.77
1371922_at	Transcribed locus	---		-1.81	-2.66	-4.78
1385876_at	similar to Gpc6 protein (predicted)	RGD1563063		-2.20	-2.45	-4.80
1369972_at	serine (or cysteine) peptidase inhibitor, clade B, member 5	Serpinb5	ARE	-1.53	-3.25	-4.84
1396736_at	Similar to Gpc6 protein (predicted)	RGD1563063		-2.31	-2.22	-4.84
1384163_at	Transcribed locus	---	ARE	-1.92	-2.60	-4.87
1387581_at	peptidylglycine alpha-amidating monooxygenase COOH-terminal interactor	Pamci	ARE	1.09	-3.92	-4.90
1367940_at	chemokine orphan receptor 1	Cmkor1	ARE	-1.02	-2.86	-4.94
1394833_at	Transcribed locus	---		-1.25	-3.25	-4.98
1370131_at	caveolin	Cav		-1.68	-2.13	-5.01
1385687_at	hypothetical protein LOC682044	LOC682044		-2.51	1.04	-5.02
1389867_at	Transcribed locus	---		-1.91	-2.52	-5.07
1390403_at	similar to CG8312-PA	RGD1304790	ARE	-2.35	-1.90	-5.10
1382185_at	similar to C1q and tumor necrosis factor related protein 2 (predicted)	RGD1561041		-1.07	-4.11	-5.13
1376657_at	immunoglobulin superfamily, member 4A	Igsf4a	ARE	-1.45	-2.34	-5.15
1374458_at	Transcribed locus, strongly similar to XP_001060531.1	---	ARE	-1.87	-3.17	-5.20
1393719_at	Transcribed locus	---		-1.85	-2.13	-5.23
1368052_at	tetraspanin 8	Tspan8		-1.27	-2.48	-5.33
1387004_at	neuroblastoma, suppression of tumorigenicity 1	Nbl1		-2.52	-2.89	-5.34
1369519_at	endothelin 1	Edn1		2.10	-4.90	-5.51
1367975_at	annexin A3	Anxa3		1.10	-3.98	-5.52
1372466_at	Transcribed locus	---		-1.78	-3.25	-5.60
1388195_at	CUG triplet repeat, RNA binding protein 2	Cugbp2	ARE	-2.01	-2.58	-5.64
1372610_at	procollagen-proline, 2-oxoglutarate 4-dioxygenase, alpha II polypeptide (predicted)	P4ha2		-1.31	-4.14	-5.74
1388792_at	growth arrest and DNA-damage-inducible 45 gamma	Gadd45g		1.53	-3.29	-5.82
1398727_at	CDNA clone IMAGE:7374368	---		-2.48	-2.80	-5.96
1379108_at	Proteasome (prosome, macropain) 26S subunit, non-ATPase, 7 (predicted)	Psmd7	ARE	-1.76	-2.79	-6.02
1375144_at	Transcribed locus	---		-1.81	-2.65	-6.04
1372820_at	Transcribed locus	---	ARE	-2.36	-2.70	-6.20
1369959_at	zinc finger protein 36, C3H type-like 1	Zfp36l1	ARE	-2.35	-2.13	-6.25
1388312_at	Transcribed locus	---		-1.89	-2.71	-6.34
1376520_at	Transcribed locus	---		-2.27	-3.42	-6.38
1392971_at	Transcribed locus	---		-2.58	-3.62	-6.42
1383212_at	CDNA clone IMAGE:7462850	---		-1.61	-2.43	-6.51
1368870_at	inhibitor of DNA binding 2	Id2	ARE	-3.40	-2.15	-6.66
1391154_at	Transcribed locus	---		-1.18	-2.71	-7.01
1386827_at	similar to procollagen, type IV, alpha 6	LOC363458		-3.41	-3.69	-7.14
1372403_at	similar to Nuclear membrane binding protein NUCLING (predicted)	RGD1560011		-1.40	-2.07	-7.17
1398354_at	catenin (cadherin associated protein), alpha-like 1 (predicted)	Ctnnal1	ARE	-2.57	-2.31	-7.18
1383012_at	rhophilin, Rho GTPase binding protein 2 (predicted)	Rhpn2	ARE	-2.86	-1.43	-7.20
1376285_at	GULP, engulfment adaptor PTB domain containing 1	Gulp1	ARE	-1.27	-6.12	-7.47
1390159_at	Transcribed locus	---		1.49	-5.67	-7.47
1374176_at	similar to DNA segment, Chr 4, Brigham & Womens Genetics 0951 expressed	RGD1308059	ARE	1.16	-5.85	-7.49
1374038_at	Transcribed locus	---		-1.51	-6.12	-7.55
1375270_at	Transcribed locus	---		-1.19	-6.39	-7.65
1383565_at	Transcribed locus	---	ARE	-1.17	-2.97	-7.66
1379526_at	Myelin basic protein	Mbp		-2.82	-2.12	-7.73
1374746_at	Ab1-152	LOC500877	ARE	-3.85	-4.32	-7.82
1383479_at	Myelin basic protein	Mbp	ARE	-2.80	-2.04	-7.94
1389253_at	vanin 1	Vnn1	ARE	-2.09	-5.77	-7.94
1381557_at	guanine nucleotide binding protein, alpha 14	Gna14	ARE	-2.31	-4.59	-7.98
1372728_at	Sortilin 1	Sort1		-1.90	-6.93	-8.06
1371988_at	mannosidase 1, alpha (predicted)	Man1a	ARE	-2.96	-3.93	-8.09
1398406_at	Transcribed locus	---		-1.68	-3.99	-8.13
1384834_at	cordon-bleu (predicted)	Cobl	ARE	-1.46	-2.68	-8.15
1384709_at	---	---	ARE	1.50	-4.31	-8.20
1373108_at	protein phosphatase 1, regulatory (inhibitor) subunit 3C	Ppp1r3c		-2.35	-4.87	-8.48
1387669_a_at	epoxide hydrolase 1, microsomal	Ephx1		-2.51	-4.75	-8.85
1389496_at	A kinase (PRKA) anchor protein 7	Akap7	ARE	-1.63	-2.40	-8.87
1389779_at	SH2 domain containing 4A	Sh2d4a		-1.04	-3.25	-8.91
1385098_at	Transcribed locus	---		1.19	-7.26	-9.05
1372708_at	---	---		-1.22	-6.27	-9.06
1385008_at	Transcribed locus	---		-3.57	-2.85	-9.27
1389600_at	---	---	ARE	-2.67	-1.91	-9.31
1374649_at	RAS guanyl releasing protein 2 (calcium and DAG-regulated) (predicted)	Rasgrp2		-2.55	-5.39	-9.33
1379493_at	Transcribed locus	---		-1.37	-4.19	-9.48
1390566_a_at	creatine kinase, mitochondrial 1, ubiquitous	Ckmt1		2.69	-6.76	-9.53
1393000_at	Zinc finger, DHHC domain containing 6	Zdhhc6		-1.82	-4.32	-9.79
1367823_at	tissue inhibitor of metalloproteinase 2	Timp2		-2.44	-3.71	-9.90
1393825_at	guanine nucleotide binding protein, alpha 14	Gna14		-2.37	-4.90	-9.94
1393015_at	similar to hypothetical protein FLJ14146	RGD1310587		-1.55	-5.79	-10.07
1397335_at	sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3D	Sema3d	ARE	1.35	-7.95	-10.14
1392079_at	A kinase (PRKA) anchor protein 7	Akap7		-1.68	-2.69	-10.23
1377795_at	Transcribed locus	---		-3.57	-2.95	-10.31
1387493_at	A kinase (PRKA) anchor protein 5	Akap5		-1.26	-8.58	-10.39
1384816_at	coxsackie virus and adenovirus receptor	Cxadr	ARE	-1.65	-7.32	-10.53
1376165_at	solute carrier family 24 (sodium/potassium/calcium exchanger), member 3	Slc24a3	ARE	1.21	-10.72	-10.82
1380477_at	Transcribed locus	---		-1.25	-7.01	-11.28
1387644_at	betacellulin	Btc	ARE	-1.58	-7.33	-11.47
1375026_at	Hypothetical protein LOC688757	LOC688757		-3.99	-6.37	-11.69
1398227_at	Transcribed locus	---		-1.33	-7.88	-11.73
1388936_at	cadherin 11	Cdh11	ARE	-1.83	-5.47	-11.86
1385243_at	V-maf musculoaponeurotic fibrosarcoma oncogene homolog (avian)	Maf	ARE	-1.60	-6.86	-12.32
1389107_at	Similar to KIAA1749 protein (predicted)	RGD1304623		-1.10	-5.98	-12.92
1373312_at	Transcribed locus	---		-3.56	-8.97	-13.31
1376105_at	procollagen, type XIV, alpha 1 (predicted)	Col14a1	ARE	-1.67	-8.14	-13.31
1368114_at	fibroblast growth factor 13	Fgf13	ARE	-1.29	-7.46	-13.92
1383460_at	---	---		-1.35	-9.32	-14.51
1378327_at	doublesex and mab-3 related transcription factor 2 (predicted)	Dmrt2		-1.41	-7.08	-14.61
1388753_at	sulfatase 2	Sulf2		-2.90	-6.37	-14.97

1374942_at	carboxypeptidase X 2 (M14 family) (predicted)	Cpxm2		-3.45	-7.34	-15.05
1372107_at	four and a half LIM domains 1	Fhl1	ARE	1.23	-8.73	-15.11
1367673_at	selenium binding protein 2	Selenbp1		-3.19	-6.07	-15.49
1384707_at	Transcribed locus	---		-3.99	-7.95	-15.80
1373740_at	---	---	ARE	-1.17	-8.67	-17.10
1387232_at	bone morphogenetic protein 4	Bmp4	ARE	-1.90	-10.34	-18.82
1368223_at	a disintegrin-like and metallopeptidse (reprolysin type) with thrombospondin type 1 motif, 1	Adamts1	ARE	-2.32	-12.63	-19.39
1384276_at	similar to procollagen, type IV, alpha 6	LOC363458	ARE	-5.62	-5.37	-19.94
1374089_at	Transcribed locus	---		-1.11	-17.79	-20.43
1368683_at	oxidized low density lipoprotein (lectin-like) receptor 1	Oldlr1	ARE	-1.84	-4.26	-21.26
1390049_at	four and a half LIM domains 1	Fhl1		1.36	-14.97	-22.37
1368991_at	sphingomyelin phosphodiesterase 3, neutral	Smpd3		-1.13	-8.61	-22.46
1384487_at	doublesex and mab-3 related transcription factor 2 (predicted)	Dmrt2		-1.57	-8.62	-22.82
1378699_at	Sorting nexin 22 (predicted)	Snx22		-4.89	-16.29	-23.06
1373148_at	carboxypeptidase X 2 (M14 family) (predicted)	Cpxm2		-4.14	-8.90	-23.47
1383047_at	growth arrest specific 6	Gas6	ARE	-2.08	-10.17	-27.00
1376619_at	similar to protein tyrosine phosphatase, receptor type, D (predicted)	RGD1561090		-2.13	-16.24	-30.65
1374591_at	similar to protein tyrosine phosphatase, receptor type, D (predicted)	RGD1561090	ARE	-2.05	-15.10	-32.59
1375638_at	serum deprivation response protein	Sdpr		-2.79	-7.30	-55.92
1382452_at	serum deprivation response protein	Sdpr		-2.94	-9.40	-56.26
1376648_at	v-myc myelocytomatosis viral related oncogene, neuroblastoma derived (avian)	Mycn	ARE	-1.58	-18.99	-67.66

Affymetrix probe IDs that were synergistically regulated after 72 hours of treatment with 5mM IPTG and 3ng/ml TGF-β together, compared to either treatment alone and untreated cells. Fold change represents expression in Ras expressing and/or TGF-β treated samples compared to untreated. Gene expression increases and decreases are defined as greater than 2-fold. Presence of an ARE (AU-rich element) was determined by analysis using the ARE database, ARED3.0 (Bakheet et al., 2006).

Table 6: Genes differentially expressed in RIE:iRas and human CRC

Human Ensembl Gene ID	Gene Symbol	Gene Name
ENSG00000078328	A2BP1	Ataxin-2-binding protein 1
ENSG00000118507	AKAP7	A-kinase anchor protein 7 isoform gamma
ENSG00000154122	ANKH	Progressive ankylosis protein homolog
ENSG00000188042	ARL4C	ADP-ribosylation factor-like protein 4C
ENSG00000198363	ASPH	Aspartyl/asparaginyl beta-hydroxylase
ENSG00000168487	BMP1	Bone morphogenetic protein 1
ENSG00000125378	BMP4	Bone morphogenetic protein 4
ENSG00000131873	CHSY1	Chondroitin sulfate synthase 1
ENSG00000187955	COL14A1	Collagen alpha-1(XIV)
ENSG00000080573	COL5A3	Collagen alpha-3(V)
ENSG00000108515	ENO3	Beta-enolase
ENSG00000065361	ERBB3	Receptor tyrosine-protein kinase erbB-3
ENSG00000124882	EREG	Epiregulin precursor
ENSG00000183508	FAM46C	Protein FAM46C
ENSG00000113578	FGF1	Heparin-binding growth factor 1
ENSG00000134363	FST	Follistatin
ENSG00000159921	GNE	Bifunctional UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase
ENSG00000122641	INHBA	Inhibin beta A
ENSG00000161638	ITGA5	Integrin alpha-5
ENSG00000136826	KLF4	Krueppel-like factor 4
ENSG00000131711	MAP1B	Microtubule-associated protein 1B
ENSG00000197971	MBP	Myelin basic protein
ENSG00000129422	MTUS1	Mitochondrial tumor suppressor 1 isoform 3
ENSG00000165795	NDRG2	Protein NDRG2
ENSG00000164125	NP_001026870.1	AD021 protein (C4orf18)
ENSG00000151690	NP_060164.2	CDNA FLJ20160
ENSG00000153823	NP_060403.3	CDNA FLJ43369
ENSG00000173391	OLR1	Oxidized low-density lipoprotein receptor 1
ENSG00000127838	PNKD	Myofibrillogenesis regulator 1 isoform 1
ENSG00000153707	PTPRD	Receptor-type tyrosine-protein phosphatase delta
ENSG00000069974	RAB27A	Ras-related protein Rab-27A
ENSG00000100784	RPS6KA5	Ribosomal protein S6 kinase alpha-5
ENSG00000143416	SELENBP1	Selenium-binding protein 1
ENSG00000106366	SERPINE1	Plasminogen activator inhibitor 1 (PAI-1)
ENSG00000104611	SH2D4A	SH2 domain-containing protein 4A
ENSG00000080503	SMARCA2	SWI/SNF-related matrix- associated actin-dependent regulator of chromatin subfamily A member 2
ENSG00000176170	SPHK1	Sphingosine kinase 1
ENSG00000137767	SQRDL	Sulfide:quinone oxidoreductase
ENSG00000102359	SRPX2	Sushi-repeat-containing protein, X-linked 2
ENSG00000003436	TFPI	Tissue factor pathway inhibitor
ENSG00000105329	TGFB1	Transforming growth factor beta-1
ENSG00000035862	TIMP2	Tissue inhibitor of metalloproteinases 2
ENSG00000137831	UACA	Uveal autoantigen with coiled-coil domains and ankyrin repeats
ENSG00000112715	VEGFA	Vascular endothelial growth factor A

List of the genes differentially regulated in stage 4 adenocarcinomas compared to adenomas and the human orthologs of the genes synergistically regulated by oncogenic Ras and TGF-β in RIE:iRas cells.

Table 7: ARE-containing genes differentially expressed in adenomas vs. stage 4 adenocarcinomas

Ensembl GID	Gene Symbol	Gene Description
ENSG00000179869	ABCA13	ATP-binding cassette sub-family A member 13. [Source:Uniprot/SWISSPROT;Acc:Q86UQ4]
ENSG00000023839	ABCC2	Canalicular multispecific organic anion transporter 1 (ATP-binding cassette sub-family C member 2) (Multidrug resistance-associated protein 2) (Canalicular multidrug resistance protein). [Source:Uniprot/SWISSPROT;Acc:Q92887]
ENSG00000125257	ABCC4	Multidrug resistance-associated protein 4 (ATP-binding cassette sub- family C member 4) (MRP/cMOAT-related ABC transporter) (Multi-specific organic anion transporter-B) (MOAT-B). [Source:Uniprot/SWISSPROT;Acc:O15439]
ENSG00000069431	ABCC9	ATP-binding cassette transporter sub-family C member 9 (Sulfonylurea receptor 2). [Source:Uniprot/SWISSPROT;Acc:O60706]
ENSG00000196177	ACADSB	Short/branched chain specific acyl-CoA dehydrogenase, mitochondrial precursor (EC 1.3.99.-) (SBCAD) (2-methyl branched chain acyl-CoA dehydrogenase) (2-MEBCAD) (2-methylbutyryl-coenzyme A dehydrogenase) (2-methylbutyryl-CoA dehydrogenase). [Source:Uniprot/SWISSPROT;Acc:P45954]
ENSG00000123983	ACSL3	Long-chain-fatty-acid--CoA ligase 3 (EC 6.2.1.3) (Long-chain acyl-CoA synthetase 3) (LACS 3). [Source:Uniprot/SWISSPROT;Acc:O95573]
ENSG00000068366	ACSL4	Long-chain-fatty-acid--CoA ligase 4 (EC 6.2.1.3) (Long-chain acyl-CoA synthetase 4) (LACS 4). [Source:Uniprot/SWISSPROT;Acc:O60488]
ENSG00000184009	ACTG1	Actin, cytoplasmic 2 (Gamma-actin). [Source:Uniprot/SWISSPROT;Acc:P63261]
ENSG00000138107	ACTR1A	Alpha-centractin (Centractin) (Centrosome-associated actin homolog) (Actin-RPV) (ARP1). [Source:Uniprot/SWISSPROT;Acc:P61163]
ENSG00000115170	ACVR1	Activin receptor type-1 precursor (EC 2.7.11.30) (Activin receptor type I) (ACTR-I) (Serine/threonine-protein kinase receptor R1) (SKR1) (Activin receptor-like kinase 2) (ALK-2) (TGF-B superfamily receptor type I) (TSR-I). [Source:Uniprot/SWISSPROT;Acc:Q04771]
ENSG00000123612	ACVR1C	Activin receptor type 1C precursor (EC 2.7.11.30) (ACTR-IC) (Activin receptor-like kinase 7) (ALK-7). [Source:Uniprot/SWISSPROT;Acc:Q8NER5]
ENSG00000135074	ADAM19	ADAM 19 precursor (EC 3.4.24.-) (A disintegrin and metalloproteinase domain 19) (Meltrin beta) (Metalloprotease and disintegrin dentritic antigen marker) (MADDAM). [Source:Uniprot/SWISSPROT;Acc:Q9H013]
ENSG00000008277	ADAM22	ADAM 22 precursor (A disintegrin and metalloproteinase domain 22) (Metalloproteinase-like, disintegrin-like, and cysteine-rich protein 2) (Metalloproteinase-disintegrin ADAM22-3). [Source:Uniprot/SWISSPROT;Acc:Q9P0K1]
ENSG00000140873	ADAMTS18	ADAMTS-18 precursor (EC 3.4.24.-) (A disintegrin and metalloproteinase with thrombospondin motifs 18) (ADAM-TS 18) (ADAM-TS18). [Source:Uniprot/SWISSPROT;Acc:Q8TE60]
ENSG00000154736	ADAMTS5	ADAMTS-5 precursor (EC 3.4.24.-) (A disintegrin and metalloproteinase with thrombospondin motifs 5) (ADAM-TS 5) (ADAM-TS5) (Aggrecanase-2) (ADMP-2) (ADAM-TS 11). [Source:Uniprot/SWISSPROT;Acc:Q9UNA0]
ENSG00000136378	ADAMTS7	ADAM metallopeptidase with thrombospondin type 1 motif, 7 preproprotein [Source:RefSeq_peptide;Acc:NP_055087]
ENSG00000133597	ADCK2	aarF domain containing kinase 2 [Source:RefSeq_peptide;Acc:NP_443085]
ENSG00000170425	ADORA2B	Adenosine A2b receptor. [Source:Uniprot/SWISSPROT;Acc:P29275]
ENSG00000155966	AFF2	AF4/FMR2 family member 2 (Fragile X mental retardation 2 protein) (Protein FMR-2) (FMR2P) (Protein Ox19) (Fragile X E mental retardation syndrome protein). [Source:Uniprot/SWISSPROT;Acc:P51816]
ENSG00000072364	AFF4	AF4/FMR2 family member 4 (ALL1-fused gene from chromosome 5q31) (Major CDK9 elongation factor-associated protein). [Source:Uniprot/SWISSPROT;Acc:Q9UHB7]
ENSG00000119844	AFTPH	Aftiphilin. [Source:Uniprot/SWISSPROT;Acc:Q6ULP2]
ENSG00000162688	AGL	Glycogen debranching enzyme (Glycogen debrancher) [Includes: 4-alpha- glucanotransferase (EC 2.4.1.25) (Oligo-1,4-1,4-glucantransferase); Amylo-alpha-1,6-glucosidase (EC 3.2.1.33) (Amylo-1,6-glucosidase) (Dextrin 6-alpha-D-glucosidase)]. [Source:Uniprot/SWISSPROT;Acc:P35573]
ENSG00000018510	AGPS	Alkyldihydroxyacetonephosphate synthase, peroxisomal precursor (EC 2.5.1.26) (Alkyl-DHAP synthase) (Alkylglycerone-phosphate synthase) (Aging-associated protein 5). [Source:Uniprot/SWISSPROT;Acc:O00116]
ENSG00000144891	AGTR1	Type-1 angiotensin II receptor (AT1) (AT1AR) (AT1BR). [Source:Uniprot/SWISSPROT;Acc:P30556]
ENSG00000118507	AKAP7	A-kinase anchor protein 7 isoform gamma (Protein kinase A-anchoring protein 7 isoform gamma) (A-kinase anchor protein 18). [Source:Uniprot/SWISSPROT;Acc:Q9P0M2]
ENSG00000117020	AKT3	RAC-gamma serine/threonine-protein kinase (EC 2.7.11.1) (RAC-PK-gamma) (Protein kinase Akt-3) (Protein kinase B, gamma) (PKB gamma) (STK-2). [Source:Uniprot/SWISSPROT;Acc:Q9Y243]
ENSG00000184254	ALDH1A3	Aldehyde dehydrogenase 1A3 (EC 1.2.1.5) (Aldehyde dehydrogenase 6) (Retinaldehyde dehydrogenase 3) (RALDH-3). [Source:Uniprot/SWISSPROT;Acc:P47895]
ENSG00000154122	ANKH	Progressive ankylosis protein homolog (ANK). [Source:Uniprot/SWISSPROT;Acc:Q9HCJ1]
ENSG00000001629	ANKIB1	Ankyrin repeat and IBR domain-containing protein 1 (Fragment). [Source:Uniprot/SWISSPROT;Acc:Q9P2G1]
ENSG00000107890	ANKRD26	Ankyrin repeat domain-containing protein 26. [Source:Uniprot/SWISSPROT;Acc:Q9UPS8]
ENSG00000135299	ANKRD6	Ankyrin repeat domain-containing protein 6. [Source:Uniprot/SWISSPROT;Acc:Q9Y2G4]
ENSG00000182287	AP1S2	AP-1 complex subunit sigma-2 (Adapter-related protein complex 1 sigma- 1B subunit) (Sigma-adaptin 1B) (Adaptor protein complex AP-1 sigma-1B subunit) (Golgi adaptor HA1/AP1 adaptin sigma-1B subunit) (Clathrin assembly protein complex 1 sigma-1B small chai [Source:Uniprot/SWISSPROT;Acc:P56377]
ENSG00000152056	AP1S3	AP-1 complex subunit sigma-3 (Adapter-related protein complex 1 sigma- 1C subunit) (Sigma-adaptin 1C) (Adaptor protein complex AP-1 sigma-1C subunit) (Golgi adaptor HA1/AP1 adaptin sigma-1C subunit) (Clathrin assembly protein complex 1 sigma-1C small chai [Source:Uniprot/SWISSPROT;Acc:Q96PC3]
ENSG00000102030	ARD1A	N-terminal acetyltransferase complex ARD1 subunit homolog A (EC 2.3.1.88) (EC 2.3.1.-). [Source:Uniprot/SWISSPROT;Acc:P41227]
ENSG00000104728	ARHGEF10	Rho guanine nucleotide exchange factor 10. [Source:Uniprot/SWISSPROT;Acc:O15013]
ENSG00000116584	ARHGEF2	Rho/Rac guanine nucleotide exchange factor 2 (GEF-H1 protein) (Proliferating cell nucleolar antigen p40). [Source:Uniprot/SWISSPROT;Acc:Q92974]
ENSG00000102606	ARHGEF7	Rho guanine nucleotide exchange factor 7 (PAK-interacting exchange factor beta) (Beta-Pix) (COOL-1) (p85). [Source:Uniprot/SWISSPROT;Acc:Q14155]
ENSG00000049618	ARID1B	AT-rich interactive domain-containing protein 1B (ARID domain- containing protein 1B) (Osa homolog 2) (hOsa2) (p250R) (BRG1-binding protein hELD/OSA1) (BRG1-associated factor 250b) (BAF250B). [Source:Uniprot/SWISSPROT;Acc:Q8NFD5]
ENSG00000032219	ARID4A	AT-rich interactive domain-containing protein 4A (ARID domain- containing protein 4A) (Retinoblastoma-binding protein 1) (RBBP-1). [Source:Uniprot/SWISSPROT;Acc:P29374]
ENSG00000185305	ARL15	ADP-ribosylation factor-like 15 (ARL15), mRNA [Source:RefSeq_dna;Acc:NM_019087]
ENSG00000188042	ARL4C	ADP-ribosylation factor-like protein 4C (ADP-ribosylation factor-like protein 7) (ADP-ribosylation factor-like protein LAK). [Source:Uniprot/SWISSPROT;Acc:P56559]
ENSG00000134108	ARL8B	ADP-ribosylation factor-like protein 8B (ADP-ribosylation factor-like protein 10C) (Novel small G protein indispensable for equal chromosome segregation 1). [Source:Uniprot/SWISSPROT;Acc:Q9NVJ2]
ENSG00000143437	ARNT	Aryl hydrocarbon receptor nuclear translocator (ARNT protein) (Dioxin receptor, nuclear translocator) (Hypoxia-inducible factor 1 beta) (HIF-1 beta). [Source:Uniprot/SWISSPROT;Acc:P27540]
ENSG00000162704	ARPC5	Actin-related protein 2/3 complex subunit 5 (ARP2/3 complex 16 kDa subunit) (p16-ARC). [Source:Uniprot/SWISSPROT;Acc:O15511]
ENSG00000111339	ART4	Ecto-ADP-ribosyltransferase 4 precursor (EC 2.4.2.31) (NAD(P)(+)-- arginine ADP-ribosyltransferase 4) (Mono(ADP-ribosyl)transferase 4) (Dombrock blood group carrier molecule) (CD297 antigen). [Source:Uniprot/SWISSPROT;Acc:Q93070]
ENSG00000117407	ARTN	Artemin precursor (Enovin) (Neublastin). [Source:Uniprot/SWISSPROT;Acc:Q5T4W7]
ENSG00000004848	ARX	Homeobox protein ARX (Aristaless-related homeobox). [Source:Uniprot/SWISSPROT;Acc:Q96QS3]
ENSG00000168387	ASB14	Ankyrin repeat and SOCS box protein 14 (ASB-14). [Source:Uniprot/SWISSPROT;Acc:Q8WXK2]
ENSG00000198363	ASPH	Aspartyl/asparaginyl beta-hydroxylase (EC 1.14.11.16) (Aspartate beta- hydroxylase) (ASP beta-hydroxylase) (Peptide-aspartate beta- dioxygenase). [Source:Uniprot/SWISSPROT;Acc:Q12797]
ENSG00000148219	ASTN2	astrotactin 2 isoform c [Source:RefSeq_peptide;Acc:NP_937830]
ENSG00000157087	ATP2B2	Plasma membrane calcium-transporting ATPase 2 (EC 3.6.3.8) (PMCA2) (Plasma membrane calcium pump isoform 2) (Plasma

		membrane calcium ATPase isoform 2). [Source:Uniprot/SWISSPROT;Acc:Q01814]
ENSG00000118217	ATF6	Cyclic AMP-dependent transcription factor ATF-6 alpha (Activating transcription factor 6 alpha) (ATF6-alpha). [Source:Uniprot/SWISSPROT;Acc:P18850]
ENSG00000145782	ATG12	Autophagy-related protein 12 (APG12-like). [Source:Uniprot/SWISSPROT;Acc:O94817]
ENSG00000085978	ATG16L1	Autophagy-related protein 16-1 (APG16-like 1). [Source:Uniprot/SWISSPROT;Acc:Q676U5]
ENSG00000111676	ATN1	Atrophin-1 (Dentatorubral-pallidoluysian atrophy protein). [Source:Uniprot/SWISSPROT;Acc:P54259]
ENSG00000206190	ATP10A	Probable phospholipid-transporting ATPase VA (EC 3.6.3.1) (ATPVA) (Aminophospholipid translocase VA). [Source:Uniprot/SWISSPROT;Acc:O60312]
ENSG00000118322	ATP10B	Probable phospholipid-transporting ATPase VB (EC 3.6.3.1). [Source:Uniprot/SWISSPROT;Acc:O94823]
ENSG00000068650	ATP11A	Probable phospholipid-transporting ATPase IH (EC 3.6.3.1) (ATPase class I type 11A) (ATPase IS). [Source:Uniprot/SWISSPROT;Acc:P98196]
ENSG00000058063	ATP11B	Probable phospholipid-transporting ATPase IF (EC 3.6.3.1) (ATPase class I type 11B) (ATPase IR). [Source:Uniprot/SWISSPROT;Acc:Q9Y2G3]
ENSG00000101974	ATP11C	Probable phospholipid-transporting ATPase IG (EC 3.6.3.1) (ATPase class I type 11C) (ATPase IG) (ATPase IQ) (ATPase class VI type 11C). [Source:Uniprot/SWISSPROT;Acc:Q8NB49]
ENSG00000174437	ATP2A2	Sarcoplasmic/endoplasmic reticulum calcium ATPase 2 (EC 3.6.3.8) (Calcium pump 2) (SERCA2) (SR Ca(2+)-ATPase 2) (Calcium-transporting ATPase sarcoplasmic reticulum type, slow twitch skeletal muscle isoform) (Endoplasmic reticulum class 1/2 Ca(2+) ATPase). [Source:Uniprot/SWISSPROT;Acc:P16615]
ENSG00000125375	ATP5S	ATP synthase subunit s, mitochondrial precursor (ATP synthase coupling factor B) (Mitochondrial ATP synthase regulatory component factor B). [Source:Uniprot/SWISSPROT;Acc:Q99766]
ENSG00000165240	ATP7A	Copper-transporting ATPase 1 (EC 3.6.3.4) (Copper pump 1) (Menkes disease-associated protein). [Source:Uniprot/SWISSPROT;Acc:Q04656]
ENSG00000123472	ATPAF1	ATP synthase mitochondrial F1 complex assembly factor 1 isoform 1 precursor [Source:RefSeq_peptide;Acc:NP_073582]
ENSG00000124788	ATXN1	Ataxin-1 (Spinocerebellar ataxia type 1 protein). [Source:Uniprot/SWISSPROT;Acc:P54253]
ENSG00000148090	AUH	Methylglutaconyl-CoA hydratase, mitochondrial precursor (EC 4.2.1.18) (AU-specific RNA-binding enoyl-CoA hydratase) (AU-binding protein/enoyl-CoA hydratase). [Source:Uniprot/SWISSPROT;Acc:Q13825]
ENSG00000155096	AZIN1	Antizyme inhibitor 1 (AZI) (Ornithine decarboxylase antizyme inhibitor). [Source:Uniprot/SWISSPROT;Acc:O14977]
ENSG00000169255	B3GALNT1	UDP-GalNAc:beta-1,3-N-acetylgalactosaminyltransferase 1 (EC 2.4.1.79) (Beta-3-GalNAc-T1) (Beta-1,3-galactosyltransferase 3) (Beta-1,3- GalTase 3) (Beta3Gal-T3) (b3Gal-T3) (Galactosylgalactosylglucosylceramide beta-D-acetyl-galactosaminyltransferase) (UDP [Source:Uniprot/SWISSPROT;Acc:O75752]
ENSG00000006453	BAIAP2L1	Brain-specific angiogenesis inhibitor 1-associated protein 2-like protein 1 (BAI1-associated protein 2-like protein 1). [Source:Uniprot/SWISSPROT;Acc:Q9UHR4]
ENSG00000122507	PTHB1_HUMAN	Protein PTHB1 (Parathyroid hormone-responsive B1 gene protein) (Bardet-Biedl syndrome 9 protein). [Source:Uniprot/SWISSPROT;Acc:Q3SYG4]
ENSG00000124243	BCAS4	Breast carcinoma amplified sequence 4. [Source:Uniprot/SWISSPROT;Acc:Q8TDM0]
ENSG00000060982	BCAT1	Branched-chain-amino-acid aminotransferase, cytosolic (EC 2.6.1.42) (BCAT(c)) (ECA39 protein). [Source:Uniprot/SWISSPROT;Acc:P54687]
ENSG00000171791	BCL2	Apoptosis regulator Bcl-2. [Source:Uniprot/SWISSPROT;Acc:P10415]
ENSG00000153094	BCL2L11	Bcl-2-like protein 11 (Bcl2-interacting mediator of cell death). [Source:Uniprot/SWISSPROT;Acc:O43521]
ENSG00000121380	BCL2L14	Apoptosis facilitator Bcl-2-like 14 protein (Apoptosis regulator Bcl- G). [Source:Uniprot/SWISSPROT;Acc:Q9BZR8]
ENSG00000085185	BCORL1	BCL6 co-repressor-like 1 [Source:RefSeq_peptide;Acc:NP_068765]
ENSG00000186716	BCR	Breakpoint cluster region protein (EC 2.7.11.1) (NY-REN-26 antigen). [Source:Uniprot/SWISSPROT;Acc:P11274]
ENSG00000101966	BIRC4	Baculoviral IAP repeat-containing protein 4 (Inhibitor of apoptosis protein 3) (X-linked inhibitor of apoptosis protein) (X-linked IAP) (IAP-like protein) (HILP). [Source:Uniprot/SWISSPROT;Acc:P98170]
ENSG00000204217	BMPR2	Bone morphogenetic protein receptor type-2 precursor (EC 2.7.11.30) (Bone morphogenetic protein receptor type II) (BMP type II receptor) (BMPR-II). [Source:Uniprot/SWISSPROT;Acc:Q13873]
ENSG00000162813	BPNT1	3'(2'),5'-bisphosphate nucleotidase 1 (EC 3.1.3.7) (Bisphosphate 3'- nucleotidase 1) (PAP-inositol-1,4-phosphatase) (PIP). [Source:Uniprot/SWISSPROT;Acc:O95861]
ENSG00000185658	BRWD1	Bromodomain and WD repeat domain-containing protein 1 (WD repeat protein 9). [Source:Uniprot/SWISSPROT;Acc:Q9NSI6]
ENSG00000112763	BTN2A1	Butyrophilin subfamily 2 member A1 precursor. [Source:Uniprot/SWISSPROT;Acc:Q7KYR7]
ENSG00000165424	C10orf56	C10orf56 protein (Fragment). [Source:Uniprot/SPTREMBL;Acc:Q5U5T9]
ENSG00000158636	C11orf30	Protein EMSY. [Source:Uniprot/SWISSPROT;Acc:Q7Z589]
ENSG00000174718	C12orf35	C12orf35 protein (Fragment). [Source:Uniprot/SPTREMBL;Acc:Q4KN17]
ENSG00000134548	C12orf39	Protein C12orf39 precursor [Contains: Putative amidated peptide NWTPQAMLYLKGAQ]. [Source:Uniprot/SWISSPROT;Acc:Q9BT56]
ENSG00000151287	C13orf27	OTTHUMP00000018668. [Source:Uniprot/SPTREMBL;Acc:Q5JUR7]
ENSG00000050130	C14orf100	Medulloblastoma antigen MU-MB-50.4. [Source:Uniprot/SWISSPROT;Acc:Q9P055]
ENSG00000100629	C14orf145	CN145_HUMAN Isoform 2 of Q6ZU80 - Homo sapiens (Human) [Source:Uniprot/Varsplic;Acc:Q6ZU80-2]
ENSG00000196943	C14orf21	Leukotriene B4 receptor 2 (LTB4-R2) (Seven transmembrane receptor BLTR2) (Leukotriene B4 receptor BLT2) (LTB4 receptor JULF2). [Source:Uniprot/SWISSPROT;Acc:Q9NPC1]
ENSG00000151327	C14orf24	Uncharacterized protein C14orf24. [Source:Uniprot/SWISSPROT;Acc:Q8N128]
ENSG00000178761	C15orf17	Uncharacterized protein C15orf17. [Source:Uniprot/SWISSPROT;Acc:Q5XKK7]
ENSG00000141622	RNF165	RING finger protein 165. [Source:Uniprot/SWISSPROT;Acc:Q6ZSG1]
ENSG00000152242	C18orf25	Uncharacterized protein C18orf25. [Source:Uniprot/SWISSPROT;Acc:Q96B23]
ENSG00000131943	C19orf12	C19orf12 protein. [Source:Uniprot/SPTREMBL;Acc:Q9BSL7]
ENSG00000173436	CA151_HUMAN	UPF0327 protein C1orf151. [Source:Uniprot/SWISSPROT;Acc:Q5TGZ0]
ENSG00000159208	C1orf51	Uncharacterized protein C1orf51. [Source:Uniprot/SWISSPROT;Acc:Q8N365]
ENSG00000160679	C1orf77	Uncharacterized protein C1orf77. [Source:Uniprot/SWISSPROT;Acc:Q9Y3Y2]
ENSG00000154429	C1orf96	C1orf96 protein. [Source:Uniprot/SPTREMBL;Acc:Q6P9G2]
ENSG00000139178	C1RL	complement component 1, r subcomponent-like precursor [Source:RefSeq_peptide;Acc:NP_057630]
ENSG00000089177	SNX23_HUMAN	Kinesin-like motor protein C20orf23 (Sorting nexin-23). [Source:Uniprot/SWISSPROT;Acc:Q96L93]
ENSG00000089101	CT026_HUMAN	Uncharacterized protein C20orf26. [Source:Uniprot/SWISSPROT;Acc:Q8NHU2]
ENSG00000172296	C20orf38	Uncharacterized protein C20orf38. [Source:Uniprot/SWISSPROT;Acc:Q9NUV7]
ENSG00000125514	C20orf51	Putative uncharacterized protein C20orf51. [Source:Uniprot/SWISSPROT;Acc:Q9H493]
ENSG00000101247	NP_077025.2	CDNA: FLJ22324 fis, clone HRC05551 (C20orf7 protein). [Source:Uniprot/SPTREMBL;Acc:Q9H6F4]
ENSG00000101230	Q9H599_HUMAN	OTTHUMP00000030295 (Fragment). [Source:Uniprot/SPTREMBL;Acc:Q9H599]
ENSG00000169621	C2orf13	Uncharacterized protein C2orf13. [Source:Uniprot/SWISSPROT;Acc:Q8IW19]
ENSG00000115827	C2orf37	CDNA FLJ13096 fis, clone NT2RP3002166. [Source:Uniprot/SPTREMBL;Acc:Q9H908]
ENSG00000183624	C3orf37	UPF0361 protein DC12. [Source:Uniprot/SWISSPROT;Acc:Q96FZ2]
ENSG00000180611	C3orf59	Uncharacterized protein C3orf59. [Source:Uniprot/SWISSPROT;Acc:Q8IYB1]
ENSG00000120519	C4orf13	C4orf13 protein. [Source:Uniprot/SPTREMBL;Acc:Q8IZ62]
ENSG00000197405	C5AR1	C5a anaphylatoxin chemotactic receptor (C5a-R) (C5aR) (CD88 antigen). [Source:Uniprot/SWISSPROT;Acc:P21730]
ENSG00000181904	C5orf24	CDNA FLJ37562 fis, clone BRCOC2000487. [Source:Uniprot/SPTREMBL;Acc:Q8N1T9]
ENSG00000170085	C5orf25	Uncharacterized protein C5orf25. [Source:Uniprot/SWISSPROT;Acc:Q8NDZ2]
ENSG00000196821	C6orf106	CF106_HUMAN Isoform 2 of Q9H6K1 - Homo sapiens (Human) [Source:Uniprot/Varsplic;Acc:Q9H6K1-2]
ENSG00000135338	C6orf152	
ENSG00000135334	C6orf166	CDNA FLJ10342 fis, clone NT2RM2000837. (Fragment). [Source:Uniprot/SPTREMBL;Acc:Q9NW35]
ENSG00000156928	C7orf30	
ENSG00000153790	C7orf31	
ENSG00000188732	C7orf46	C7orf46 protein. [Source:Uniprot/SPTREMBL;Acc:Q4G0Q7]
ENSG00000147679	C8orf53	CDNA FLJ30717 fis, clone FCBBF2001672. [Source:Uniprot/SPTREMBL;Acc:Q96NJ8]
ENSG00000137135	C9orf100	OTTHUMP00000021341 (C9orf100 protein). [Source:Uniprot/SPTREMBL;Acc:Q5T4R2]
ENSG00000120159	C9orf82	Uncharacterized protein C9orf82. [Source:Uniprot/SWISSPROT;Acc:Q9H8G2]

ENSG00000164989	C9orf93	Uncharacterized protein C9orf93. [Source:Uniprot/SWISSPROT;Acc:Q6TFL3]
ENSG00000178031	C9orf94	Protein C9orf94 precursor. [Source:Uniprot/SWISSPROT;Acc:Q496M8]
ENSG00000178538	CA8	Carbonic anhydrase-related protein (CARP) (CA-VIII). [Source:Uniprot/SWISSPROT;Acc:P35219]
ENSG00000182389	CACNB4	Voltage-dependent L-type calcium channel subunit beta-4 (CAB4) (Calcium channel voltage-dependent subunit beta 4). [Source:Uniprot/SWISSPROT;Acc:O00305]
ENSG00000136436	CALCOCO2	calcium binding and coiled-coil domain 2 [Source:RefSeq_peptide;Acc:NP_005822]
ENSG00000160014	CALM1	Calmodulin (CaM). [Source:Uniprot/SWISSPROT;Acc:P62158]
ENSG00000183049	CAMK1D	Calcium/calmodulin-dependent protein kinase type 1D (EC 2.7.11.17) (CaM kinase ID) (CaM kinase I delta) (CaMKI-delta) (CaM-KI delta) (CaMKI delta) (Camk1D) (CamKI-like protein kinase) (CKLiK). [Source:Uniprot/SWISSPROT;Acc:Q8IU85]
ENSG00000118200	CAMSAP1L1	calmodulin regulated spectrin-associated protein 1-like 1 [Source:RefSeq_peptide;Acc:NP_982284]
ENSG00000105971	CAV2	Caveolin-2. [Source:Uniprot/SWISSPROT;Acc:P51636]
ENSG00000078699	CBFA2T2	Protein CBFA2T2 (MTG8-like protein) (MTG8-related protein 1) (Myeloid translocation-related protein 1) (ETO homologous on chromosome 20) (p85). [Source:Uniprot/SWISSPROT;Acc:O43439]
ENSG00000122565	CBX3	Chromobox protein homolog 3 (Heterochromatin protein 1 homolog gamma) (HP1 gamma) (Modifier 2 protein) (HECH). [Source:Uniprot/SWISSPROT;Acc:Q13185]
ENSG00000100307	CBX7	Chromobox protein homolog 7. [Source:Uniprot/SWISSPROT;Acc:O95931]
ENSG00000183287	CCBE1	collagen and calcium binding EGF domains 1 [Source:RefSeq_peptide;Acc:NP_597716]
ENSG00000152492	CCDC50	Coiled-coil domain-containing protein 50 (Protein Ymer). [Source:Uniprot/SWISSPROT;Acc:Q8IVM0]
ENSG00000176155	CCDC57	coiled-coil domain containing 57 [Source:RefSeq_peptide;Acc:NP_932348]
ENSG00000133773	CCDC59	coiled-coil domain containing 59 [Source:RefSeq_peptide;Acc:NP_054886]
ENSG00000147082	CCNB3	G2/mitotic-specific cyclin-B3. [Source:Uniprot/SWISSPROT;Acc:Q8WWL7]
ENSG00000163468	CCT3	T-complex protein 1 subunit gamma (TCP-1-gamma) (CCT-gamma) (hTRiC5). [Source:Uniprot/SWISSPROT;Acc:P49368]
ENSG00000177575	CD163	Scavenger receptor cysteine-rich type 1 protein M130 precursor (CD163 antigen) (Hemoglobin scavenger receptor) [Contains: Soluble CD163 (sCD163)]. [Source:Uniprot/SWISSPROT;Acc:Q86VB7]
ENSG00000135535	CD164	Putative mucin core protein 24 precursor (Multi-glycosylated core protein 24) (MGC-24) (MUC-24) (CD164 antigen). [Source:Uniprot/SWISSPROT;Acc:Q04900]
ENSG00000163606	CD200R1	Cell surface glycoprotein OX2 receptor precursor (CD200 cell surface glycoprotein receptor). [Source:Uniprot/SWISSPROT;Acc:Q8TD46]
ENSG00000153283	CD96	T-cell surface protein tactile precursor (T cell-activated increased late expression protein) (CD96 antigen). [Source:Uniprot/SWISSPROT;Acc:P40200]
ENSG00000079335	CDC14A	Dual specificity protein phosphatase CDC14A (EC 3.1.3.48) (EC 3.1.3.16) (CDC14 cell division cycle 14 homolog A). [Source:Uniprot/SWISSPROT;Acc:Q9UNH5]
ENSG00000163171	CDC42EP3	Cdc42 effector protein 3 (Binder of Rho GTPases 2) (MSE55-related Cdc42-binding protein). [Source:Uniprot/SWISSPROT;Acc:Q9UKI2]
ENSG00000094804	CDC6	Cell division control protein 6 homolog (CDC6-related protein) (p62(cdc6)) (HsCDC6) (HsCDC18). [Source:Uniprot/SWISSPROT;Acc:Q99741]
ENSG00000123374	CDK2	Cell division protein kinase 2 (EC 2.7.11.22) (p33 protein kinase). [Source:Uniprot/SWISSPROT;Acc:P24941]
ENSG00000176749	CDK5R1	Cyclin-dependent kinase 5 activator 1 precursor (CDK5 activator 1) (Cyclin-dependent kinase 5 regulatory subunit 1) (Tau protein kinase II 23 kDa subunit) (TPKII regulatory subunit) (p23) (p25) (p35) [Contains: Cyclin-dependent kinase 5 activator 1, p35; [Source:Uniprot/SWISSPROT;Acc:Q15078]
ENSG00000129355	CDKN2D	Cyclin-dependent kinase 4 inhibitor D (p19-INK4d). [Source:Uniprot/SWISSPROT;Acc:P55273]
ENSG00000115163	CENPA	Uncharacterized protein C2orf18 precursor. [Source:Uniprot/SWISSPROT;Acc:Q8N357]
ENSG00000114331	CENTB2	Centaurin-beta 2 (Cnt-b2). [Source:Uniprot/SWISSPROT;Acc:Q15057]
ENSG00000047365	CENTD1	Centaurin-delta 1 (Cnt-d1) (Arf-GAP, Rho-GAP, ankyrin repeat and pleckstrin homology domain-containing protein 2) (PARX protein). [Source:Uniprot/SWISSPROT;Acc:Q8WZ64]
ENSG00000001626	CFTR	Cystic fibrosis transmembrane conductance regulator (CFTR) (cAMP- dependent chloride channel) (ATP-binding cassette transporter sub- family C member 7). [Source:Uniprot/SWISSPROT;Acc:P13569]
ENSG00000170791	CHCHD7	Coiled-coil-helix-coiled-coil-helix domain-containing protein 7. [Source:Uniprot/SWISSPROT;Acc:Q9BUK0]
ENSG00000128656	CHN1	N-chimaerin (NC) (N-chimerin) (Alpha chimerin) (A-chimaerin) (Rho- GTPase-activating protein 2). [Source:Uniprot/SWISSPROT;Acc:P15882]
ENSG00000131873	CHSY1	Chondroitin sulfate synthase 1 (EC 2.4.1.175) (Glucuronosyl-N- acetylgalactosaminyl-proteoglycan 4-beta-N-acetylgalactosaminyltransferase 1) (Chondroitin sulfate synthase 1) (N-acetylgalactosaminyl-proteoglycan 3-beta-glucuronosyltransferase 1) (EC 2.4.1 [Source:Uniprot/SWISSPROT;Acc:Q86X52]
ENSG00000099622	CIRBP	Cold-inducible RNA-binding protein (Glycine-rich RNA-binding protein CIRP) (A18 hnRNP). [Source:Uniprot/SWISSPROT;Acc:Q14011]
ENSG00000114737	CISH	Cytokine-inducible SH2-containing protein (CIS) (CIS-1) (Suppressor of cytokine signaling) (SOCS) (Protein G18). [Source:Uniprot/SWISSPROT;Acc:Q9NSE2]
ENSG00000163539	CLASP2	CLIP-associating protein 2 (Cytoplasmic linker-associated protein 2). [Source:Uniprot/SWISSPROT;Acc:O75122]
ENSG00000172243	CLEC7A	dendritic cell-associated C-type lectin 1 isoform a [Source:RefSeq_peptide;Acc:NP_922938]
ENSG00000169504	CLIC4	Chloride intracellular channel protein 4 (Intracellular chloride ion channel protein p64H1). [Source:Uniprot/SWISSPROT;Acc:Q9Y696]
ENSG00000183723	CMTM4	CKLF-like MARVEL transmembrane domain-containing protein 4 (Chemokine- like factor superfamily member 4). [Source:Uniprot/SWISSPROT;Acc:Q8IZR5]
ENSG00000198515	CNGA1	cGMP-gated cation channel alpha 1 (CNG channel alpha 1) (CNG-1) (CNG1) (Cyclic nucleotide-gated channel alpha 1) (Cyclic nucleotide-gated channel, photoreceptor) (Cyclic nucleotide-gated cation channel 1) (Rod photoreceptor cGMP-gated channel subunit alph [Source:Uniprot/SWISSPROT;Acc:P29973]
ENSG00000149970	CNKSR2	Connector enhancer of kinase suppressor of ras 2 (Connector enhancer of KSR2) (CNK2). [Source:Uniprot/SWISSPROT;Acc:Q8WXI2]
ENSG00000125107	CNOT1	CCR4-NOT transcription complex, subunit 1 isoform a [Source:RefSeq_peptide;Acc:NP_057368]
ENSG00000106714	CNTNAP3	Contactin-associated protein-like 3 precursor (Cell recognition molecule Caspr3). [Source:Uniprot/SWISSPROT;Acc:Q9BZ76]
ENSG00000082438	COBLL1	COBL-like 1 [Source:RefSeq_peptide;Acc:NP_055715]
ENSG00000168434	COG7	Conserved oligomeric Golgi complex component 7. [Source:Uniprot/SWISSPROT;Acc:P83436]
ENSG00000123500	COL10A1	Collagen alpha-1(X) chain precursor. [Source:Uniprot/SWISSPROT;Acc:Q03692]
ENSG00000111799	COL12A1	Collagen alpha-1(XII) chain precursor. [Source:Uniprot/SWISSPROT;Acc:Q99715]
ENSG00000187955	COL14A1	Collagen alpha-1(XIV) chain precursor (Undulin). [Source:Uniprot/SWISSPROT;Acc:Q05707]
ENSG00000204262	COL5A2	Collagen alpha-2(V) chain precursor. [Source:Uniprot/SWISSPROT;Acc:P05997]
ENSG00000198612	COPS8	COP9 signalosome complex subunit 8 (Signalosome subunit 8) (SGN8) (JAB1-containing signalosome subunit 8) (COP9 homolog) (hCOP9). [Source:Uniprot/SWISSPROT;Acc:Q99627]
ENSG00000167186	COQ7	Ubiquinone biosynthesis protein COQ7 homolog (Coenzyme Q biosynthesis protein 7 homolog) (Timing protein clk-1 homolog). [Source:Uniprot/SWISSPROT;Acc:Q99807]
ENSG00000203710	CR1	Complement receptor type 1 precursor (C3b/C4b receptor) (CD35 antigen). [Source:Uniprot/SWISSPROT;Acc:P17927]
ENSG00000118260	CREB1	cAMP response element-binding protein (CREB). [Source:Uniprot/SWISSPROT;Acc:P16220]
ENSG00000111269	CREBL2	cAMP responsive element binding protein-like 2 [Source:RefSeq_peptide;Acc:NP_001301]
ENSG00000150938	CRIM1	Cysteine-rich motor neuron 1 protein precursor (CRIM-1) (Cysteine-rich repeat-containing protein S52). [Source:Uniprot/SWISSPROT;Acc:Q9NZV1]
ENSG00000112282	CRSP3	CRSP complex subunit 3 (Cofactor required for Sp1 transcriptional activation subunit 3) (Transcriptional coactivator CRSP130) (Vitamin D3 receptor-interacting protein complex 130 kDa component) (DRIP130) (Activator-recruited cofactor 130 kDa component) (A [Source:Uniprot/SWISSPROT;Acc:Q9ULK4]
ENSG00000198223	CSF2RA	Granulocyte-macrophage colony-stimulating factor receptor alpha chain precursor (GM-CSF-R-alpha) (GMR) (CD116 antigen) (CDw116). [Source:Uniprot/SWISSPROT;Acc:P15509]
ENSG00000113712	CSNK1A1	Casein kinase I isoform alpha (EC 2.7.11.1) (CKI-alpha) (CK1). [Source:Uniprot/SWISSPROT;Acc:P48729]
ENSG00000100181	CSNK1E	Casein kinase I isoform epsilon (EC 2.7.11.1) (CKI-epsilon) (CKIe). [Source:Uniprot/SWISSPROT;Acc:P49674]
ENSG00000101439	CST3	Cystatin C precursor (Neuroendocrine basic polypeptide) (Gamma-trace) (Post-gamma-globulin).

		[Source:Uniprot/SWISSPROT;Acc:P01034]
ENSG00000151792	CTSO	Cathepsin O precursor (EC 3.4.22.42). [Source:Uniprot/SWISSPROT;Acc:P43234]
ENSG00000036257	CUL3	Cullin-3 (CUL-3). [Source:Uniprot/SWISSPROT;Acc:Q13618]
ENSG00000006210	CX3CL1	Fractalkine precursor (CX3CL1) (Neurotactin) (CX3C membrane-anchored chemokine) (Small inducible cytokine D1). [Source:Uniprot/SWISSPROT;Acc:P78423]
ENSG00000163735	CXCL5	Small inducible cytokine B5 precursor (CXCL5) (Epithelial-derived neutrophil-activating protein 78) (Neutrophil-activating peptide ENA- 78) (ENA-78(1-78)) [Contains: ENA-78(8-78); ENA-78(9-78)]. [Source:Uniprot/SWISSPROT;Acc:P42830]
ENSG00000123575	CX039_HUMAN	Uncharacterized protein CXorf39. [Source:Uniprot/SWISSPROT;Acc:Q6PEV8]
ENSG00000055163	CYFIP2	cytoplasmic FMR1 interacting protein 2 [Source:RefSeq_peptide;Acc:NP_055191]
ENSG00000136848	DAB2IP	Disabled homolog 2-interacting protein (DAB2-interacting protein) (DAB2 interaction protein) (ASK-interacting protein 1). [Source:Uniprot/SWISSPROT;Acc:Q5VWQ8]
ENSG00000070190	DAPP1	Dual adapter for phosphotyrosine and 3-phosphotyrosine and 3- phosphoinositide (hDAPP1) (B cell adapter molecule of 32 kDa) (B lymphocyte adapter protein Bam32). [Source:Uniprot/SWISSPROT;Acc:Q9UN19]
ENSG00000043093	DCUN1D1	DCN1-like protein 1 (Defective in cullin neddylation protein 1-like protein 1) (DCUN1 domain-containing protein 1) (Squamous cell carcinoma-related oncogene). [Source:Uniprot/SWISSPROT;Acc:Q96GG9]
ENSG00000150401	DCUN1D2	DCN1-like protein 2 (Defective in cullin neddylation protein 1-like protein 2) (DCUN1 domain-containing protein 2). [Source:Uniprot/SWISSPROT;Acc:Q6PH85]
ENSG00000153317	DDEF1	130 kDa phosphatidylinositol 4,5-biphosphate-dependent ARF1 GTPase- activating protein (PIP2-dependent ARF1 GAP) (ADP-ribosylation factor- directed GTPase-activating protein 1) (ARF GTPase-activating protein 1) (Development and differentiation-enhancing f [Source:Uniprot/SWISSPROT;Acc:Q9ULH1]
ENSG00000124487	DDX3X	ATP-dependent RNA helicase DDX3X (EC 3.6.1.-) (DEAD box protein 3, X- chromosomal) (Helicase-like protein 2) (HLP2) (DEAD box, X isoform). [Source:Uniprot/SWISSPROT;Acc:O00571]
ENSG00000162701	DENND1B	DENN/MADD domain containing 1B [Source:RefSeq_peptide;Acc:NP_659414]
ENSG00000162777	DENND2D	DENN domain-containing protein 2D. [Source:Uniprot/SWISSPROT;Acc:Q9H6A0]
ENSG00000211448	DIO2	Type II iodothyronine deiodinase (EC 1.97.1.10) (Type-II 5' deiodinase) (DIOII) (Type 2 DI) (5DII). [Source:Uniprot/SWISSPROT;Acc:Q92813]
ENSG00000150764	DIXDC1	DIX domain containing 1 isoform b [Source:RefSeq_peptide;Acc:NP_219493]
ENSG00000116652	DLEU2L	Leukemia-associated protein 2 (Deleted in lymphocytic leukemia 2). [Source:Uniprot/SWISSPROT;Acc:O43262]
ENSG00000075711	DLG1	Disks large homolog 1 (Synapse-associated protein 97) (SAP-97) (hDlg). [Source:Uniprot/SWISSPROT;Acc:Q12959]
ENSG00000100206	DMC1	Meiotic recombination protein DMC1/LIM15 homolog. [Source:Uniprot/SWISSPROT;Acc:Q14565]
ENSG00000105993	DNAJB6	DnaJ homolog subfamily B member 6 (Heat shock protein J2) (HSJ-2) (MSJ-1) (HHDJ1) (MRJ). [Source:Uniprot/SWISSPROT;Acc:O75190]
ENSG00000100246	DNAL4	Dynein light chain 4, axonemal. [Source:Uniprot/SWISSPROT;Acc:O96015]
ENSG00000154813	DPH3	CSL-type zinc finger-containing protein 2 (DelGEF-interacting protein 1) (DelGIP1). [Source:Uniprot/SWISSPROT;Acc:Q96FX2]
ENSG00000113657	DPYSL3	Dihydropyrimidinase-related protein 3 (DRP-3) (Unc-33-like phosphoprotein) (ULIP protein) (Collapsin response mediator protein 4) (CRMP-4). [Source:Uniprot/SWISSPROT;Acc:Q14195]
ENSG00000101412	E2F1	Transcription factor E2F1 (E2F-1) (Retinoblastoma-binding protein 3) (RBBP-3) (PRB-binding protein E2F-1) (PBR3) (Retinoblastoma-associated protein 1) (RBAP-1). [Source:Uniprot/SWISSPROT;Acc:Q01094]
ENSG00000145194	ECE2	Endothelin-converting enzyme 2 (EC 3.4.24.71) (ECE-2). [Source:Uniprot/SWISSPROT;Acc:O60344]
ENSG00000116406	EDEM3	ER degradation-enhancing alpha-mannosidase-like 3. [Source:Uniprot/SWISSPROT;Acc:Q9BZQ6]
ENSG00000164176	EDIL3	EGF-like repeat and discoidin I-like domain-containing protein 3 precursor (EGF-like repeats and discoidin I-like domains protein 3) (Developmentally-regulated endothelial cell locus 1 protein) (Integrin-binding protein DEL1). [Source:Uniprot/SWISSPROT;Acc:O43854]
ENSG00000151617	EDNRA	Endothelin-1 receptor precursor (Endothelin A receptor) (ET-A) (hET- AR) (ETA-R). [Source:Uniprot/SWISSPROT;Acc:P25101]
ENSG00000055332	EIF2AK2	Interferon-induced, double-stranded RNA-activated protein kinase (EC 2.7.11.1) (Interferon-inducible RNA-dependent protein kinase) (Protein kinase RNA-activated) (PKR) (p68 kinase) (P1/eIF-2A protein kinase). [Source:Uniprot/SWISSPROT;Acc:P19525]
ENSG00000123908	EIF2C2	Eukaryotic translation initiation factor 2C 2 (eIF2C 2) (eIF-2C 2) (Argonaute-2) (Slicer protein) (PAZ Piwi domain protein) (PPD). [Source:Uniprot/SWISSPROT;Acc:Q9UKV8]
ENSG00000066044	ELAVL1	ELAV-like protein 1 (Hu-antigen R). [Source:Uniprot/SWISSPROT;Acc:Q15717]
ENSG00000105656	ELL	RNA polymerase II elongation factor ELL (Eleven-nineteen lysine-rich leukemia protein). [Source:Uniprot/SWISSPROT;Acc:P55199]
ENSG00000062598	ELMO2	Engulfment and cell motility protein 2 (CED-12 homolog A) (hCED-12A). [Source:Uniprot/SWISSPROT;Acc:Q96JJ3]
ENSG00000135638	EMX1	Homeobox protein EMX1 (Empty spiracles homolog 1) (Empty spiracles- like protein 1). [Source:Uniprot/SWISSPROT;Acc:Q04741]
ENSG00000001561	ENPP4	ectonucleotide pyrophosphatase/phosphodiesterase 4 (putative function) [Source:RefSeq_peptide;Acc:NP_055751]
ENSG00000112796	ENPP5	Ectonucleotide pyrophosphatase/phosphodiesterase 5 precursor (EC 3.1.-.-) (E-NPP5) (NPP-5). [Source:Uniprot/SWISSPROT;Acc:Q9UJA9]
ENSG00000129595	EPB41L4A	Band 4.1-like protein 4A (Protein NBL4). [Source:Uniprot/SWISSPROT;Acc:Q9HCS5]
ENSG00000095203	EPB41L4B	Band 4.1-like protein 4B (Protein EHM2) (FERM-containing protein CG1). [Source:Uniprot/SWISSPROT;Acc:Q9H329]
ENSG00000135999	EPC2	Enhancer of polycomb homolog 2. [Source:Uniprot/SWISSPROT;Acc:Q52LR7]
ENSG00000124882	EREG	Epiregulin precursor. [Source:Uniprot/SWISSPROT;Acc:O14944]
ENSG00000197930	ERO1L	ERO1-like protein alpha precursor (EC 1.8.4.-) (ERO1-Lalpha) (Oxidoreductin-1-Lalpha) (Endoplasmic oxidoreductin-1-like protein) (ERO1-L). [Source:Uniprot/SWISSPROT;Acc:Q96HE7]
ENSG00000134954	ETS1	C-ets-1 protein (p54). [Source:Uniprot/SWISSPROT;Acc:P14921]
ENSG00000006468	ETV1	ETS translocation variant 1 (ER81 protein). [Source:Uniprot/SWISSPROT;Acc:P50549]
ENSG00000149573	EVA1	Epithelial V-like antigen 1 precursor. [Source:Uniprot/SWISSPROT;Acc:O60487]
ENSG00000085276	EVI1	Ecotropic virus integration site 1 protein homolog (EVI-1). [Source:Uniprot/SWISSPROT;Acc:Q03112]
ENSG00000067208	EVI5	Ecotropic viral integration site 5 protein homolog (EVI-5) (Neuroblastoma stage 4S gene). [Source:Uniprot/SWISSPROT;Acc:O60447]
ENSG00000185104	FAF1	FAS-associated factor 1 (Protein FAF1) (hFAF1). [Source:Uniprot/SWISSPROT;Acc:Q9UNN5]
ENSG00000162636	FAM102B	Protein FAM102B. [Source:Uniprot/SWISSPROT;Acc:Q5T8I3]
ENSG00000189057	FAM111B	family with sequence similarity 111, member B (FAM111B), mRNA [Source:RefSeq_dna;Acc:NM_198947]
ENSG00000197712	FAM114A1	family with sequence similarity 114, member A1 (FAM114A1), mRNA [Source:RefSeq_dna;Acc:NM_138389]
ENSG00000171928	FAM18B	Protein FAM18B. [Source:Uniprot/SWISSPROT;Acc:Q9NYZ1]
ENSG00000138172	FAM26B	Protein FAM26B. [Source:Uniprot/SWISSPROT;Acc:Q9HA72]
ENSG00000203667	FAM36A	Protein FAM36A. [Source:Uniprot/SWISSPROT;Acc:Q5RI15]
ENSG00000139146	FAM60A	Protein FAM60A (Tera protein homolog). [Source:Uniprot/SWISSPROT;Acc:Q9NP50]
ENSG00000009780	FAM76A	Protein FAM76A. [Source:Uniprot/SWISSPROT;Acc:Q8TAV0]
ENSG00000026103	FAS	Tumor necrosis factor receptor superfamily member 6 precursor (FASLG receptor) (Apoptosis-mediating surface antigen FAS) (Apo-1 antigen) (CD95 antigen). [Source:Uniprot/SWISSPROT;Acc:P25445]
ENSG00000083857	FAT	Cadherin-related tumor suppressor homolog precursor (Protein fat homolog). [Source:Uniprot/SWISSPROT;Acc:Q14517]
ENSG00000166147	FBN1	Fibrillin-1 precursor. [Source:Uniprot/SWISSPROT;Acc:P35555]
ENSG00000147364	FBXO25	F-box only protein 25. [Source:Uniprot/SWISSPROT;Acc:Q8TCJ0]
ENSG00000164117	FBXO8	F-box only protein 8 (F-box/SEC7 protein FBS). [Source:Uniprot/SWISSPROT;Acc:Q9NRD0]
ENSG00000137714	FDX1	Adrenodoxin, mitochondrial precursor (Adrenal ferredoxin) (Ferredoxin- 1) (Hepatoredoxin). [Source:Uniprot/SWISSPROT;Acc:P10109]
ENSG00000156427	FGF18	Fibroblast growth factor 18 precursor (FGF-18) (zFGF5). [Source:Uniprot/SWISSPROT;Acc:O76093]
ENSG00000105550	FGF21	Fibroblast growth factor 21 precursor (FGF-21). [Source:Uniprot/SWISSPROT;Acc:Q9NSA1]
ENSG00000118972	FGF23	Fibroblast growth factor 23 precursor (FGF-23) (Tumor-derived hypophosphatemia-inducing factor). [Source:Uniprot/SWISSPROT;Acc:Q9GZV9]
ENSG00000111790	FGFR1OP2	FGFR1 oncogene partner 2 [Source:RefSeq_peptide;Acc:NP_056448]

ENSG00000106080	FKBP14	FK506-binding protein 14 precursor (EC 5.2.1.8) (Peptidyl-prolyl cis- trans isomerase) (PPIase) (Rotamase) (22 kDa FK506-binding protein) (FKBP-22). [Source:Uniprot/SWISSPROT;Acc:Q9NWM8]
ENSG00000181027	FKRP	Fukutin-related protein (EC 2.-.-.-). [Source:Uniprot/SWISSPROT;Acc:Q9H9S5]
ENSG00000160688	FLAD1	flavin adenine dinucleotide synthetase isoform 1 [Source:RefSeq_peptide;Acc:NP_079483]
ENSG00000125848	FLRT3	Leucine-rich repeat transmembrane protein FLRT3 precursor (Fibronectin-like domain-containing leucine-rich transmembrane protein 3). [Source:Uniprot/SWISSPROT;Acc:Q9NZU0]
ENSG00000162769	FLVC1_HUMAN	Feline leukemia virus subgroup C receptor-related protein 1 (Feline leukemia virus subgroup C receptor) (hFLVCR). [Source:Uniprot/SWISSPROT;Acc:Q9Y5Y0]
ENSG00000094963	FMO2	Dimethylaniline monooxygenase [N-oxide-forming] 2 (EC 1.14.13.8) (Pulmonary flavin-containing monooxygenase 2) (FMO 2) (Dimethylaniline oxidase 2) (FMO 1B1). [Source:Uniprot/SWISSPROT;Acc:Q99518]
ENSG00000170802	FOXN2	Forkhead box protein N2 (Human T-cell leukemia virus enhancer factor). [Source:Uniprot/SWISSPROT;Acc:P32314]
ENSG00000139445	FOXN4	Forkhead box protein N4. [Source:Uniprot/SWISSPROT;Acc:Q96NZ1]
ENSG00000114861	FOXP1	Forkhead box protein P1. [Source:Uniprot/SWISSPROT;Acc:Q9H334]
ENSG00000171049	FPRL1	FMLP-related receptor I (FMLP-R-I) (Lipoxin A4 receptor) (LXA4 receptor) (Formyl peptide receptor-like 1) (RFP) (HM63). [Source:Uniprot/SWISSPROT;Acc:P25090]
ENSG00000163430	FSTL1	Follistatin-related protein 1 precursor (Follistatin-like 1). [Source:Uniprot/SWISSPROT;Acc:Q12841]
ENSG00000089280	FUS	RNA-binding protein FUS (Oncogene FUS) (Oncogene TLS) (Translocated in liposarcoma protein) (POMp75) (75 kDa DNA-pairing protein). [Source:Uniprot/SWISSPROT;Acc:P35637]
ENSG00000174951	FUT1	Galactoside 2-alpha-L-fucosyltransferase 1 (EC 2.4.1.69) (GDP-L- fucose:beta-D-galactoside 2-alpha-L-fucosyltransferase 1) (Alpha(1,2)FT 1) (Fucosyltransferase 1) (Blood group H alpha 2- fucosyltransferase). [Source:Uniprot/SWISSPROT;Acc:P19526]
ENSG00000138757	G3BP2	Ras-GTPase-activating protein-binding protein 2 (GAP SH3-domain- binding protein 2) (G3BP-2). [Source:Uniprot/SWISSPROT;Acc:Q9UN86]
ENSG00000022355	GABRA1	Gamma-aminobutyric-acid receptor subunit alpha-1 precursor (GABA(A) receptor subunit alpha-1). [Source:Uniprot/SWISSPROT;Acc:P14867]
ENSG00000128683	GAD1	Glutamate decarboxylase 1 (EC 4.1.1.15) (Glutamate decarboxylase 67 kDa isoform) (GAD-67) (67 kDa glutamic acid decarboxylase). [Source:Uniprot/SWISSPROT;Acc:Q99259]
ENSG00000109586	GALNT7	N-acetylgalactosaminyltransferase 7 (EC 2.4.1.-) (Protein-UDP acetylgalactosaminyltransferase 7) (UDP-GalNAc:polypeptide N-acetylgalactosaminyltransferase 7) (Polypeptide GalNAc transferase 7) (GalNAc-T7) (pp-GaNTase 7). [Source:Uniprot/SWISSPROT;Acc:Q86SF2]
ENSG00000165219	GAPVD1	GTPase activating protein and VPS9 domains 1 [Source:RefSeq_peptide;Acc:NP_056450]
ENSG00000180447	GAS1	Growth-arrest-specific protein 1 precursor (GAS-1). [Source:Uniprot/SWISSPROT;Acc:P54826]
ENSG00000117228	GBP1	Interferon-induced guanylate-binding protein 1 (GTP-binding protein 1) (Guanine nucleotide-binding protein 1) (GBP-1) (HuGBP-1). [Source:Uniprot/SWISSPROT;Acc:P32455]
ENSG00000174500	GCET2	Germinal center B-cell expressed transcript 2 protein (Germinal center-associated lymphoma protein) (hGAL). [Source:Uniprot/SWISSPROT;Acc:Q8N6F7]
ENSG00000127554	GFER	Augmenter of liver regeneration (hERV1 protein). [Source:Uniprot/SWISSPROT;Acc:P55789]
ENSG00000131459	GFPT2	Glucosamine--fructose-6-phosphate aminotransferase [isomerizing] 2 (EC 2.6.1.16) (Hexosephosphate aminotransferase 2) (D-fructose-6- phosphate amidotransferase 2) (GFAT 2) (GFAT2). [Source:Uniprot/SWISSPROT;Acc:O94808]
ENSG00000139278	GLIPR1	Glioma pathogenesis-related protein 1 precursor (GliPR 1) (RTVP-1 protein). [Source:Uniprot/SWISSPROT;Acc:P48060]
ENSG00000115419	GLS	Glutaminase kidney isoform, mitochondrial precursor (EC 3.5.1.2) (GLS) (L-glutamine amidohydrolase) (K-glutaminase). [Source:Uniprot/SWISSPROT;Acc:O94925]
ENSG00000087338	GMCL1	Germ cell-less protein-like 1. [Source:Uniprot/SWISSPROT;Acc:Q96IK5]
ENSG00000087258	GNAO1	Guanine nucleotide-binding protein G(o) subunit alpha 2. [Source:Uniprot/SWISSPROT;Acc:P29777]
ENSG00000156052	GNAQ	Guanine nucleotide-binding protein G(q) subunit alpha (Guanine nucleotide-binding protein alpha-q). [Source:Uniprot/SWISSPROT;Acc:P50148]
ENSG00000159921	GNE	Bifunctional UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase (UDP-GlcNAc-2-epimerase/ManAc kinase) [Includes: UDP-N- acetylglucosamine 2-epimerase (EC 5.1.3.14) (Uridine diphosphate-N- acetylglucosamine-2-epimerase) (UDP-GlcNAc-2-epimerase) [Source:Uniprot/SWISSPROT;Acc:Q9Y223]
ENSG00000168243	GNG4	Guanine nucleotide-binding protein G(I)/G(S)/G(O) gamma-4 subunit precursor. [Source:Uniprot/SWISSPROT;Acc:P50150]
ENSG00000109163	GNRHR	Gonadotropin-releasing hormone receptor (GnRH receptor) (GnRH-R). [Source:Uniprot/SWISSPROT;Acc:P30968]
ENSG00000143457	GOLPH3L	GPP34-related protein [Source:RefSeq_peptide;Acc:NP_060648]
ENSG00000111711	GOLT1B	Vesicle transport protein GOT1B (Golgi transport 1 homolog B) (hGOT1a) (Putative NF-kappa-B-activating protein 470). [Source:Uniprot/SWISSPROT;Acc:Q9Y3E0]
ENSG00000108587	GOSR1	Golgi SNAP receptor complex member 1 (28 kDa Golgi SNARE protein) (28 kDa cis-Golgi SNARE p28) (GOS-28). [Source:Uniprot/SWISSPROT;Acc:O95249]
ENSG00000092978	GPATC2	G patch domain-containing protein 2. [Source:Uniprot/SWISSPROT;Acc:Q9NW75]
ENSG00000135387	GPIAP1	GPI-anchored membrane protein 1 (GPI-anchored protein p137) (p137GPI) (Membrane component chromosome 11 surface marker 1). [Source:Uniprot/SWISSPROT;Acc:Q14444]
ENSG00000183484	GPR132	Probable G-protein coupled receptor 132 (G2 accumulation protein). [Source:Uniprot/SWISSPROT;Acc:Q9UNW8]
ENSG00000106070	GRB10	Growth factor receptor-bound protein 10 (GRB10 adaptor protein) (Insulin receptor-binding protein GRB-IR). [Source:Uniprot/SWISSPROT;Acc:Q13322]
ENSG00000177885	GRB2	Growth factor receptor-bound protein 2 (Adapter protein GRB2) (SH2/SH3 adapter GRB2) (Protein Ash). [Source:Uniprot/SWISSPROT;Acc:P62993]
ENSG00000164284	GRPEL2	GrpE protein homolog 2, mitochondrial precursor (Mt-GrpE#2). [Source:Uniprot/SWISSPROT;Acc:Q8TAA5]
ENSG00000180613	GSH2_HUMAN	Homeobox protein GSH-2. [Source:Uniprot/SWISSPROT;Acc:Q9BZM3]
ENSG00000105793	GTPBP10	claudin 12 isoform 1 [Source:RefSeq_peptide;Acc:NP_001036182]
ENSG00000172432	GTPBP2	GTP-binding protein 2. [Source:Uniprot/SWISSPROT;Acc:Q9BX10]
ENSG00000070019	GUCY2C	Heat-stable enterotoxin receptor precursor (GC-C) (Intestinal guanylate cyclase) (EC 4.6.1.2) (STA receptor) (hSTAR). [Source:Uniprot/SWISSPROT;Acc:P25092]
ENSG00000105968	H2AFV	Histone H2AV (H2A.F/Z). [Source:Uniprot/SWISSPROT;Acc:Q71UI9]
ENSG00000103044	HAS3	Hyaluronan synthase 3 (EC 2.4.1.212) (Hyaluronate synthase 3) (Hyaluronic acid synthase 3) (HA synthase 3). [Source:Uniprot/SWISSPROT;Acc:O00219]
ENSG00000138411	HECW2	HECT, C2 and WW domain containing E3 ubiquitin protein ligase 2 [Source:RefSeq_peptide;Acc:NP_065811]
ENSG00000019991	HGF	Hepatocyte growth factor precursor (Scatter factor) (SF) (Hepatopoeitin A) [Contains: Hepatocyte growth factor alpha chain; Hepatocyte growth factor beta chain]. [Source:Uniprot/SWISSPROT;Acc:P14210]
ENSG00000111911	HINT3	histidine triad nucleotide binding protein 3 [Source:RefSeq_peptide;Acc:NP_612638]
ENSG00000160883	HK3	Hexokinase-3 (EC 2.7.1.1) (Hexokinase type III) (HK III). [Source:Uniprot/SWISSPROT;Acc:P52790]
ENSG00000152795	HNRPDL	heterogeneous nuclear ribonucleoprotein D-like [Source:RefSeq_peptide;Acc:NP_112740]
ENSG00000096746	HNRPH3	Heterogeneous nuclear ribonucleoprotein H3 (hnRNP H3) (hnRNP 2H9). [Source:Uniprot/SWISSPROT;Acc:P31942]
ENSG00000153187	HNRPU	Heterogeneous nuclear ribonucleoprotein U (hnRNP U) (Scaffold attachment factor A) (SAF-A) (p120) (pp120). [Source:Uniprot/SWISSPROT;Acc:Q00839]
ENSG00000152413	HOMER1	Homer protein homolog 1. [Source:Uniprot/SWISSPROT;Acc:Q86YM7]
ENSG00000173917	HOXB2	Homeobox protein Hox-B2 (Hox-2H) (Hox-2.8) (K8). [Source:Uniprot/SWISSPROT;Acc:P14652]
ENSG00000110756	HPS5	Hermansky-Pudlak syndrome 5 protein (Alpha-integrin-binding protein 63) (Ruby-eye protein 2 homolog) (Ru2). [Source:Uniprot/SWISSPROT;Acc:Q9UPZ3]
ENSG00000136720	HS6ST1	Heparan-sulfate 6-O-sulfotransferase 1 (EC 2.8.2.-) (HS6ST-1). [Source:Uniprot/SWISSPROT;Acc:O60243]
ENSG00000166598	HSP90B1	Endoplasmin precursor (Heat shock protein 90 kDa beta member 1) (94 kDa glucose-regulated protein) (GRP94) (gp96 homolog) (Tumor rejection antigen 1). [Source:Uniprot/SWISSPROT;Acc:P14625]
ENSG00000120694	HSPH1	Heat-shock protein 105 kDa (Heat shock 110 kDa protein) (Antigen NY- CO-25). [Source:Uniprot/SWISSPROT;Acc:Q92598]
ENSG00000102468	HTR2A	5-hydroxytryptamine 2A receptor (5-HT-2A) (Serotonin receptor 2A) (5- HT-2). [Source:Uniprot/SWISSPROT;Acc:P28223]
ENSG00000142149	HUNK	Hormonally up-regulated neu tumor-associated kinase (EC 2.7.11.1) (Serine/threonine-protein kinase MAK-V) (B19). [Source:Uniprot/SWISSPROT;Acc:P57058]
ENSG00000136273	HUS1_HUMAN	Checkpoint protein HUS1 (hHUS1). [Source:Uniprot/SWISSPROT;Acc:O60921]

ENSG00000119912	IDE	Insulin-degrading enzyme (EC 3.4.24.56) (Insulysin) (Insulinase) (Insulin protease). [Source:Uniprot/SWISSPROT;Acc:P14735]
ENSG00000137080	IFNA21	Interferon alpha-21 precursor (Interferon alpha-F) (LeIF F). [Source:Uniprot/SWISSPROT;Acc:P01568]
ENSG00000142166	IFNAR1	Interferon-alpha/beta receptor alpha chain precursor (IFN-alpha-REC). [Source:Uniprot/SWISSPROT;Acc:P17181]
ENSG00000006652	IFRD1	Interferon-related developmental regulator 1 (Nerve growth factor- inducible protein PC4). [Source:Uniprot/SWISSPROT;Acc:O00458]
ENSG00000159217	IGF2BP1	insulin-like growth factor 2 mRNA binding protein 1 [Source:RefSeq_peptide;Acc:NP_006537]
ENSG00000163453	IGFBP7	Insulin-like growth factor-binding protein 7 precursor (IGFBP-7) (IBP- 7) (IGF-binding protein 7) (MAC25 protein) (Prostacyclin-stimulating factor) (PGI2-stimulating factor) (IGFBP-rP1). [Source:Uniprot/SWISSPROT;Acc:Q16270]
ENSG00000132740	IGHMBP2	DNA-binding protein SMUBP-2 (EC 3.6.1.-) (ATP-dependent helicase IGHMBP2) (Immunoglobulin mu-binding protein 2) (SMUBP-2) (Glial factor 1) (GF-1). [Source:Uniprot/SWISSPROT;Acc:P38935]
ENSG00000136696	IL1F8	Interleukin-1 family member 8 (IL-1F8) (Interleukin-1 eta) (IL-1 eta) (FIL1 eta) (Interleukin-1 homolog 2) (IL-1H2). [Source:Uniprot/SWISSPROT;Acc:Q9NZH7]
ENSG00000115594	IL1R1	Interleukin-1 receptor type I precursor (IL-1R-1) (IL-1RT1) (IL-1R- alpha) (p80) (CD121a antigen). [Source:Uniprot/SWISSPROT;Acc:P14778]
ENSG00000109471	IL2	Interleukin-2 precursor (IL-2) (T-cell growth factor) (TCGF) (Aldesleukin). [Source:Uniprot/SWISSPROT;Acc:P60568]
ENSG00000162891	IL20	Interleukin-20 precursor (IL-20) (Four alpha helix cytokine Zcyto10). [Source:Uniprot/SWISSPROT;Acc:Q9NYY1]
ENSG00000113520	IL4	Interleukin-4 precursor (IL-4) (B-cell stimulatory factor 1) (BSF-1) (Lymphocyte stimulatory factor 1) (Binetrakin) (Pitrakinra). [Source:Uniprot/SWISSPROT;Acc:P05112]
ENSG00000169429	IL8	Interleukin-8 precursor (IL-8) (CXCL8) (Monocyte-derived neutrophil chemotactic factor) (MDNCF) (T-cell chemotactic factor) (Neutrophil- activating protein 1) (NAP-1) (Protein 3-10C) (Granulocyte chemotactic protein 1) (GCP-1) (Monocyte-derived neutrophil [Source:Uniprot/SWISSPROT;Acc:P10145]
ENSG00000104331	IMPAD1	myo-inositol monophosphatase A3 [Source:RefSeq_peptide;Acc:NP_060283]
ENSG00000122641	INHBA	Inhibin beta A chain precursor (Activin beta-A chain) (Erythroid differentiation protein) (EDF). [Source:Uniprot/SWISSPROT;Acc:P08476]
ENSG00000198825	INPP5F	inositol polyphosphate-5-phosphatase F [Source:RefSeq_peptide;Acc:NP_055752]
ENSG00000186480	INSIG1	Insulin-induced gene 1 protein (INSIG-1). [Source:Uniprot/SWISSPROT;Acc:O15503]
ENSG00000127080	IPPK	Inositol-pentakisphosphate 2-kinase (EC 2.7.1.-) (Inositol-1,3,4,5,6- pentakisphosphate 2-kinase) (Ins(1,3,4,5,6)P5 2-kinase) (InsP5 2- kinase) (IPK1 homolog). [Source:Uniprot/SWISSPROT;Acc:Q9H8X2]
ENSG00000140575	IQGAP1	Ras GTPase-activating-like protein IQGAP1 (p195). [Source:Uniprot/SWISSPROT;Acc:P46940]
ENSG00000128604	IRF5	Interferon regulatory factor 5 (IRF-5). [Source:Uniprot/SWISSPROT;Acc:Q13568]
ENSG00000160255	ITGB2	Integrin beta-2 precursor (Cell surface adhesion glycoproteins LFA- 1/CR3/p150,95 subunit beta) (Complement receptor C3 subunit beta) (CD18 antigen). [Source:Uniprot/SWISSPROT;Acc:P05107]
ENSG00000056345	ITGB3	Integrin beta-3 precursor (Platelet membrane glycoprotein IIIa) (GPIIIa) (CD61 antigen). [Source:Uniprot/SWISSPROT;Acc:P05106]
ENSG00000101384	JAG1	Jagged-1 precursor (Jagged1) (hJ1) (CD339 antigen). [Source:Uniprot/SWISSPROT;Acc:P78504]
ENSG00000073614	JARID1A	Jumonji/ARID domain-containing protein 1A (Retinoblastoma-binding protein 2) (RBBP-2). [Source:Uniprot/SWISSPROT;Acc:P29375]
ENSG00000011201	KAL1	Anosmin-1 precursor (Kallmann syndrome protein) (Adhesion molecule- like X-linked). [Source:Uniprot/SWISSPROT;Acc:P23352]
ENSG00000176595	KBTBD11	Kelch repeat and BTB domain-containing protein 11 (Kelch domain- containing protein 7B). [Source:Uniprot/SWISSPROT;Acc:O94819]
ENSG00000170852	KBTBD2	Kelch repeat and BTB domain-containing protein 2 (BTB and kelch domain-containing protein 1). [Source:Uniprot/SWISSPROT;Acc:Q8IY47]
ENSG00000171385	KCND3	Potassium voltage-gated channel subfamily D member 3 (Voltage-gated potassium channel subunit Kv4.3). [Source:Uniprot/SWISSPROT;Acc:Q9UK17]
ENSG00000153885	KCTD15	BTB/POZ domain-containing protein KCTD15. [Source:Uniprot/SWISSPROT;Acc:Q96SI1]
ENSG00000128052	KDR	Vascular endothelial growth factor receptor 2 precursor (EC 2.7.10.1) (VEGFR-2) (Kinase insert domain receptor) (Protein-tyrosine kinase receptor Flk-1) (CD309 antigen). [Source:Uniprot/SWISSPROT;Acc:P35968]
ENSG00000112624	KIAA0240	KIAA0240 (KIAA0240), mRNA [Source:RefSeq_dna;Acc:NM_015349]
ENSG00000196663	KIAA0329	Protein KIAA0329/KIAA0297. [Source:Uniprot/SWISSPROT;Acc:O15040]
ENSG00000107771	KIAA1128	granule cell antiserum positive 14 [Source:RefSeq_peptide;Acc:NP_061872]
ENSG00000087301	KIAA1344	Thioredoxin-domain containing protein KIAA1344 precursor. [Source:Uniprot/SWISSPROT;Acc:Q9P2K2]
ENSG00000119698	KIAA1622	HEAT-like repeat-containing protein isoform 1 [Source:RefSeq_peptide;Acc:NP_478144]
ENSG00000148841	KIAA1754	KIAA1754 (KIAA1754), mRNA [Source:RefSeq_dna;Acc:NM_033397]
ENSG00000137177	KIF13A	Kinesin-like protein KIF13A (Kinesin-like protein RBKIN). [Source:Uniprot/SWISSPROT;Acc:Q9H1H9]
ENSG00000197892	KIF13B	Kinesin-like protein KIF13B (Kinesin-like protein GAKIN). [Source:Uniprot/SWISSPROT;Acc:Q9NQT8]
ENSG00000131437	KIF3A	Kinesin-like protein KIF3A (Microtubule plus end-directed kinesin motor 3A). [Source:Uniprot/SWISSPROT;Acc:Q9Y496]
ENSG00000125498	KIR3DL2	Killer cell immunoglobulin-like receptor 3DL2 precursor (MHC class I NK cell receptor) (Natural killer-associated transcript 4) (NKAT-4) (p70 natural killer cell receptor clone CL-5) (CD158k antigen). [Source:Uniprot/SWISSPROT;Acc:P43630]
ENSG00000183853	KIRREL	Kin of IRRE-like protein 1 precursor (Kin of irregular chiasm-like protein 1) (Nephrin-like protein 1). [Source:Uniprot/SWISSPROT;Acc:Q96J84]
ENSG00000049130	KITLG	Kit ligand precursor (C-kit ligand) (Stem cell factor) (SCF) (Mast cell growth factor) (MGF). [Source:Uniprot/SWISSPROT;Acc:P21583]
ENSG00000172059	KLF11	Krueppel-like factor 11 (Transforming growth factor-beta-inducible early growth response protein 2) (TGFB-inducible early growth response protein 2) (TIEG-2). [Source:Uniprot/SWISSPROT;Acc:O14901]
ENSG00000163884	KLF15	Krueppel-like factor 15 (Kidney-enriched krueppel-like factor). [Source:Uniprot/SWISSPROT;Acc:Q9UIH9]
ENSG00000197776	KLHDC1	Kelch domain-containing protein 1. [Source:Uniprot/SWISSPROT;Acc:Q8N7A1]
ENSG00000187961	KLHL17	Kelch-like protein 17 (Actinfilin). [Source:Uniprot/SWISSPROT;Acc:Q6TDP4]
ENSG00000198642	KLHL9	Kelch-like protein 9. [Source:Uniprot/SWISSPROT;Acc:Q9P2J3]
ENSG00000025800	KPNA6	Importin alpha-7 subunit (Karyopherin alpha-6). [Source:Uniprot/SWISSPROT;Acc:O60684]
ENSG00000133703	KRAS	GTPase KRas (K-Ras 2) (Ki-Ras) (c-K-ras) (c-Ki-ras). [Source:Uniprot/SWISSPROT;Acc:P01116]
ENSG00000186831	KRT17	Keratin, type I cytoskeletal 17 (Cytokeratin-17) (CK-17) (Keratin-17) (K17) (39.1). [Source:Uniprot/SWISSPROT;Acc:Q04695]
ENSG00000162511	LAPTM5	Lysosomal-associated transmembrane protein 5 (Lysosomal-associated multitransmembrane protein 5) (Retinoic acid-inducible E3 protein). [Source:Uniprot/SWISSPROT;Acc:Q13571]
ENSG00000161813	LARP4	La-related protein 4 (La ribonucleoprotein domain family member 4). [Source:Uniprot/SWISSPROT;Acc:Q71RC2]
ENSG00000090661	LASS4	LAG1 longevity assurance homolog 4. [Source:Uniprot/SWISSPROT;Acc:Q9HA82]
ENSG00000178177	LCORL	transcription factor MLR1 [Source:RefSeq_peptide;Acc:NP_710153]
ENSG00000116678	LEPR	Leptin receptor precursor (LEP-R) (OB receptor) (OB-R) (HuB219) (CD295 antigen). [Source:Uniprot/SWISSPROT;Acc:P48357]
ENSG00000168924	LETM1	Leucine zipper-EF-hand-containing transmembrane protein 1, mitochondrial precursor. [Source:Uniprot/SWISSPROT;Acc:O95202]
ENSG00000100097	LGALS1	Galectin-1 (Lectin galactoside-binding soluble 1) (Beta-galactoside- binding lectin L-14-I) (Lactose-binding lectin 1) (S-Lac lectin 1) (Galaptin) (14 kDa lectin) (HPL) (HBL) (Putative MAPK-activating protein MP12). [Source:Uniprot/SWISSPROT;Acc:P09382]
ENSG00000116977	LGALS8	Galectin-8 (Gal-8) (Prostate carcinoma tumor antigen 1) (PCTA-1) (Po66 carbohydrate-binding protein) (Po66-CBP). [Source:Uniprot/SWISSPROT;Acc:O00214]
ENSG00000106852	LHX6	LIM/homeobox protein Lhx6.1 (Lhx6). [Source:Uniprot/SWISSPROT;Acc:Q9UPM6]
ENSG00000050405	LIMA1	LIM domain and actin-binding protein 1 (Epithelial protein lost in neoplasm). [Source:Uniprot/SWISSPROT;Acc:Q9UHB6]
ENSG00000140471	LINS1	lines homolog 1 [Source:RefSeq_peptide;Acc:NP_001035706]
ENSG00000143013	LMO4	LIM domain transcription factor LMO4 (LIM domain only protein 4) (LMO- 4) (Breast tumor autoantigen). [Source:Uniprot/SWISSPROT;Acc:P61968]
ENSG00000072201	LNX1	Ubiquitin ligase LNX (EC 6.3.2.-) (Numb-binding protein 1) (Ligand of Numb-protein X 1). [Source:Uniprot/SWISSPROT;Acc:Q8TBB1]
ENSG00000123684	LPGAT1	Acyl-CoA:lysophosphatidylglycerol acyltransferase 1 (EC 2.3.1.-). [Source:Uniprot/SWISSPROT;Acc:Q92604]

ENSG00000144749	LRIG1	Leucine-rich repeats and immunoglobulin-like domains protein 1 precursor (LIG-1). [Source:Uniprot/SWISSPROT;Acc:Q96JA1]
ENSG00000182504	LRRIQ2	leucine-rich repeats and IQ motif containing 2 [Source:RefSeq_peptide;Acc:NP_078824]
ENSG00000155858	LSM11	U7 snRNA-associated Sm-like protein LSm11. [Source:Uniprot/SWISSPROT;Acc:P83369]
ENSG00000119681	LTBP2	Latent-transforming growth factor beta-binding protein 2 precursor (LTBP-2). [Source:Uniprot/SWISSPROT;Acc:Q14767]
ENSG00000139329	LUM	Lumican precursor (Keratan sulfate proteoglycan lumican) (KSPG lumican). [Source:Uniprot/SWISSPROT;Acc:P51884]
ENSG00000169641	LUZP1	Leucine zipper protein 1. [Source:Uniprot/SWISSPROT;Acc:Q86V48]
ENSG00000187123	LYPD6	LY6/PLAUR domain-containing protein 6 precursor. [Source:Uniprot/SWISSPROT;Acc:Q86Y78]
ENSG00000061337	LZTS1	Leucine zipper putative tumor suppressor 1 (F37/esophageal cancer- related gene-coding leucine-zipper motif) (Fez1). [Source:Uniprot/SWISSPROT;Acc:Q9Y250]
ENSG00000127603	MACF1	Microtubule-actin cross-linking factor 1, isoform 4. [Source:Uniprot/SWISSPROT;Acc:Q96PK2]
ENSG00000197063	MAFG	Transcription factor MafG (V-maf musculoaponeurotic fibrosarcoma oncogene homolog G) (hMAF). [Source:Uniprot/SWISSPROT;Acc:O15525]
ENSG00000081026	MAGI3	membrane-associated guanylate kinase-related 3 isoform 2 [Source:RefSeq_peptide;Acc:NP_690864]
ENSG00000017621	NP_079135.1	PDZ domain containing, X chromosome [Source:RefSeq_peptide;Acc:NP_079135]
ENSG00000172469	MANEA	mannosidase, endo-alpha [Source:RefSeq_peptide;Acc:NP_078917]
ENSG00000139625	MAP3K12	Mitogen-activated protein kinase kinase kinase 12 (EC 2.7.11.25) (Mixed lineage kinase) (Leucine-zipper protein kinase) (ZPK) (Dual leucine zipper bearing kinase) (DLK) (MAPK-upstream kinase) [Source:Uniprot/SWISSPROT;Acc:Q12852]
ENSG00000135341	MAP3K7	Mitogen-activated protein kinase kinase kinase 7 (EC 2.7.11.25) (Transforming growth factor-beta-activated kinase 1) (TGF-beta-activated kinase 1). [Source:Uniprot/SWISSPROT;Acc:O43318]
ENSG00000135525	MAP7	Ensconsin (Microtubule-associated protein 7) (Epithelial microtubule- associated protein of 115 kDa) (E-MAP-115). [Source:Uniprot/SWISSPROT;Acc:Q14244]
ENSG00000137802	MAPKBP1	mitogen-activated protein kinase binding protein 1-like [Source:RefSeq_peptide;Acc:NP_055809]
ENSG00000101367	MAPRE1	Microtubule-associated protein RP/EB family member 1 (APC-binding protein EB1) (End-binding protein 1) (EB1). [Source:Uniprot/SWISSPROT;Acc:Q15691]
ENSG00000145416	MAR1	membrane-associated RING-CH protein I [Source:RefSeq_peptide;Acc:NP_060393]
ENSG00000204406	MBD5	Methyl-CpG-binding domain protein 5 (Methyl-CpG-binding protein MBD5). [Source:Uniprot/SWISSPROT;Acc:Q9P267]
ENSG00000151332	MBIP	MAP3K12-binding inhibitory protein 1 (MAPK upstream kinase-binding inhibitory protein) (MUK-binding inhibitory protein). [Source:Uniprot/SWISSPROT;Acc:Q9NS73]
ENSG00000152601	MBNL1	Muscleblind-like protein (Triplet-expansion RNA-binding protein). [Source:Uniprot/SWISSPROT;Acc:Q9NR56]
ENSG00000139793	MBNL2	muscleblind-like 2 isoform 1 [Source:RefSeq_peptide;Acc:NP_659002]
ENSG00000197971	MBP	Myelin basic protein (MBP) (Myelin A1 protein) (Myelin membrane encephalitogenic protein). [Source:Uniprot/SWISSPROT;Acc:P02686]
ENSG00000140943	MBTPS1	Membrane-bound transcription factor site 1 protease precursor (EC 3.4.21.-) (S1P endopeptidase) (Site-1 protease) (Subtilisin/kexin- isozyme 1) (SKI-1). [Source:Uniprot/SWISSPROT;Acc:Q14703]
ENSG00000065328	MCM10	minichromosome maintenance protein 10 isoform 2 [Source:RefSeq_peptide;Acc:NP_060988]
ENSG00000166823	MESP1	mesoderm posterior 1 [Source:RefSeq_peptide;Acc:NP_061140]
ENSG00000140545	MFGE8	Lactadherin precursor (Milk fat globule-EGF factor 8) (MFG-E8) (HMFG) (Breast epithelial antigen BA46) (MFGM) [Contains: Lactadherin short form; Medin]. [Source:Uniprot/SWISSPROT;Acc:Q08431]
ENSG00000141503	MINK1	Misshapen-like kinase 1 (EC 2.7.11.1) (Mitogen-activated protein kinase kinase kinase kinase 6) (MAPK/ERK kinase kinase kinase 6) (MEK kinase kinase 6) (MEKKK 6) (Misshapen/NIK-related kinase) (GCK family kinase MiNK). [Source:Uniprot/SWISSPROT;Acc:Q8N4C8]
ENSG00000186260	MKL2	MKL/myocardin-like protein 2 (Myocardin-related transcription factor B) (MRTF-B) (Megakaryoblastic leukemia 2). [Source:Uniprot/SWISSPROT;Acc:Q9ULH7]
ENSG00000128585	MKLN1	Muskelin. [Source:Uniprot/SWISSPROT;Acc:Q9UL63]
ENSG00000150051	MKX	mohawk homeobox [Source:RefSeq_peptide;Acc:NP_775847]
ENSG00000005483	MLL5	myeloid/lymphoid or mixed-lineage leukemia 5 [Source:RefSeq_peptide;Acc:NP_061152]
ENSG00000143443	MLLT11	Protein AF1q. [Source:Uniprot/SWISSPROT;Acc:Q13015]
ENSG00000171843	MLLT3	Protein AF-9 (ALL1 fused gene from chromosome 9 protein) (Myeloid/lymphoid or mixed-lineage leukemia translocated to chromosome 3 protein) (YEATS domain-containing protein 3). [Source:Uniprot/SWISSPROT;Acc:P42568]
ENSG00000108960	MMD	Monocyte to macrophage differentiation protein (Progestin and adipoQ receptor family member XI). [Source:Uniprot/SWISSPROT;Acc:Q15546]
ENSG00000196549	MME	Neprilysin (EC 3.4.24.11) (Neutral endopeptidase) (NEP) (Enkephalinase) (Neutral endopeptidase 24.11) (Atriopeptidase) (Common acute lymphocytic leukemia antigen) (CALLA) (CD10 antigen). [Source:Uniprot/SWISSPROT;Acc:P08473]
ENSG00000056586	MNAB	Membrane-associated nucleic acid-binding protein (RING finger protein 164). [Source:Uniprot/SWISSPROT;Acc:Q9HBD1]
ENSG00000114978	MOBK1B	Mps one binder kinase activator-like 1B (Mob1 homolog 1B) (Mob1 alpha) (Mob1A) (Protein Mob4B). [Source:Uniprot/SWISSPROT;Acc:Q9H8S9]
ENSG00000133422	MORC2	MORC family CW-type zinc finger protein 2 (Zinc finger CW-type coiled- coil domain protein 1). [Source:Uniprot/SWISSPROT;Acc:Q9Y6X9]
ENSG00000101928	MOSPD1	Motile sperm domain-containing protein 1. [Source:Uniprot/SWISSPROT;Acc:Q9UJG1]
ENSG00000150054	MPP7	palmitoylated membrane protein 7 [Source:RefSeq_peptide;Acc:NP_775767]
ENSG00000114686	MRPL3	Mitochondrial 39S ribosomal protein L3 (L3mt) (MRP-L3). [Source:Uniprot/SWISSPROT;Acc:P09001]
ENSG00000147065	MSN	Moesin (Membrane-organizing extension spike protein). [Source:Uniprot/SWISSPROT;Acc:P26038]
ENSG00000165929	MTAC2D1	Membrane targeting tandem C2 domain-containing protein 1 (Tandem C2 protein in nucleus) (Tac2-N). [Source:Uniprot/SWISSPROT;Acc:Q8N9U0]
ENSG00000065911	MTHFD2	Bifunctional methylenetetrahydrofolate dehydrogenase/cyclohydrolase, mitochondrial precursor [Includes: NAD-dependent methylenetetrahydrofolate dehydrogenase (EC 1.5.1.15); Methenyltetrahydrofolate cyclohydrolase (EC 3.5.4.9)]. [Source:Uniprot/SWISSPROT;Acc:P13995]
ENSG00000171100	MTM1	Myotubularin (EC 3.1.3.48). [Source:Uniprot/SWISSPROT;Acc:Q13496]
ENSG00000087053	MTMR2	Myotubularin-related protein 2 (EC 3.1.3.-). [Source:Uniprot/SWISSPROT;Acc:Q13614]
ENSG00000170873	MTSS1	Metastasis suppressor protein 1 (Missing in metastasis protein) (Metastasis suppressor YGL-1). [Source:Uniprot/SWISSPROT;Acc:O43312]
ENSG00000129422	MTUS1	mitochondrial tumor suppressor 1 isoform 3 [Source:RefSeq_peptide;Acc:NP_001001927]
ENSG00000120279	MYCT1	myc target 1 [Source:RefSeq_peptide;Acc:NP_079383]
ENSG00000104177	MYEF2	Myelin expression factor 2 (MyEF-2) (MST156). [Source:Uniprot/SWISSPROT;Acc:Q9P2K5]
ENSG00000133026	MYH10	Myosin-10 (Myosin heavy chain, nonmuscle IIb) (Nonmuscle myosin heavy chain IIb) (NMMHC II-b) (NMMHC-IIB) (Cellular myosin heavy chain, type B) (Nonmuscle myosin heavy chain-B) (NMMHC-B). [Source:Uniprot/SWISSPROT;Acc:P35580]
ENSG00000095777	MYO3A	Myosin IIIA (EC 2.7.11.1). [Source:Uniprot/SWISSPROT;Acc:Q8NEV4]
ENSG00000166886	NAB2	NGFI-A-binding protein 2 (EGR-1-binding protein 2) (Melanoma- associated delayed early response protein) (Protein MADER). [Source:Uniprot/SWISSPROT;Acc:Q15742]
ENSG00000114503	NCBP2	Nuclear cap-binding protein subunit 2 (20 kDa nuclear cap-binding protein) (NCBP 20 kDa subunit) (CBP20) (NCBP-interacting protein 1) (NIP1) (Cell proliferation-inducing gene 55 protein). [Source:Uniprot/SWISSPROT;Acc:P52298]
ENSG00000128609	NDUFA5	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 5 (EC 1.6.5.3) (EC 1.6.99.3) (NADH-ubiquinone oxidoreductase 13 kDa-B subunit) (Complex I-13kD-B) (CI-13kD-B) (Complex I subunit B13). [Source:Uniprot/SWISSPROT;Acc:Q16718]
ENSG00000078114	NEBL	Nebulette (Actin-binding Z-disk protein). [Source:Uniprot/SWISSPROT;Acc:O76041]
ENSG00000049759	NEDD4L	E3 ubiquitin-protein ligase NEDD4-like protein (EC 6.3.2.-) (Nedd4-2) (NEDD4.2). [Source:Uniprot/SWISSPROT;Acc:Q96PU5]
ENSG00000119408	NEK6	Serine/threonine-protein kinase Nek6 (EC 2.7.11.1) (NimA-related protein kinase 6) (Protein kinase SID6-1512). [Source:Uniprot/SWISSPROT;Acc:Q9HC98]
ENSG00000165973	NELL1	Protein kinase C-binding protein NELL1 precursor (NEL-like protein 1) (Nel-related protein 1). [Source:Uniprot/SWISSPROT;Acc:Q92832]
ENSG00000102908	NFAT5	Nuclear factor of activated T-cells 5 (T-cell transcription factor NFAT5) (NF-AT5) (Tonicity-responsive enhancer-binding protein) (TonE- binding protein) (TonEBP). [Source:Uniprot/SWISSPROT;Acc:O94916]
ENSG00000147862	NFIB	Nuclear factor 1 B-type (Nuclear factor 1/B) (NF1-B) (NFI-B) (NF-I/B) (CCAAT-box-binding transcription factor) (CTF) (TGGCA-binding protein). [Source:Uniprot/SWISSPROT;Acc:O00712]

		Ninein (hNinein) (Glycogen synthase kinase 3 beta-interacting protein) (GSK3B-interacting protein).
ENSG00000100503	NIN	[Source:Uniprot/SWISSPROT;Acc:Q8N4C6]
ENSG00000136783	NIPSNAP3A	Protein NipSnap3A (NipSnap4) (Target for Salmonella secreted protein C) (TassC). [Source:Uniprot/SWISSPROT;Acc:Q9UFN0]
ENSG00000181368	NM_032947.3	MSTP150 (MST150), mRNA [Source:RefSeq_dna;Acc:NM_032947]
ENSG00000115761	NOL10	Nucleolar protein 10. [Source:Uniprot/SWISSPROT;Acc:Q9BSC4]
ENSG00000146909	NOM1	nucleolar protein with MIF4G domain 1 [Source:RefSeq_peptide;Acc:NP_612409]
ENSG00000164867	NOS3	Nitric-oxide synthase, endothelial (EC 1.14.13.39) (EC-NOS) (NOS type III) (NOSIII) (Endothelial NOS) (eNOS) (Constitutive NOS) (cNOS). [Source:Uniprot/SWISSPROT;Acc:P29474]
ENSG00000074181	NOTCH3	Neurogenic locus notch homolog protein 3 precursor (Notch 3) [Contains: Notch 3 extracellular truncation; Notch 3 intracellular domain]. [Source:Uniprot/SWISSPROT;Acc:Q9UM47]
ENSG00000102763	NP_055873.1	OTTHUMP00000018324 (KIAA0564 protein). [Source:Uniprot/SPTREMBL;Acc:Q5VW08]
ENSG00000160131	NP_001017980.1	
ENSG00000154153	NP_001030022.1	CDNA: FLJ22155 fis, clone HRC00205 (Hypothetical protein FLJ20152). [Source:Uniprot/SPTREMBL;Acc:Q9H6L5]
ENSG00000163945	NP_065945.1	
ENSG00000139597	NP_438169.1	CG018 protein. [Source:Uniprot/SPTREMBL;Acc:Q8WTU5]
ENSG00000181982	NP_775734.1	CDNA FLJ43363 fis, clone NT2RP7017474. [Source:Uniprot/SPTREMBL;Acc:Q6ZUS6]
ENSG00000149308	NPAT	nuclear protein, ataxia-telangiectasia locus [Source:RefSeq_peptide;Acc:NP_002510]
ENSG00000124222	NPEPL1	Syntaxin-16 (Syn16). [Source:Uniprot/SWISSPROT;Acc:O14662]
ENSG00000126368	NR1D1	Orphan nuclear receptor NR1D1 (V-erbA-related protein EAR-1) (Rev- erbA-alpha). [Source:Uniprot/SWISSPROT;Acc:P20393]
ENSG00000119508	NR4A3	Orphan nuclear receptor NR4A3 (Nuclear hormone receptor NOR-1) (Neuron-derived orphan receptor 1) (Mitogen-induced nuclear orphan receptor). [Source:Uniprot/SWISSPROT;Acc:Q92570]
ENSG00000178425	NT5DC1	5'-nucleotidase domain-containing protein 1. [Source:Uniprot/SWISSPROT;Acc:Q5TFE4]
ENSG00000074590	NUAK1	NUAK family SNF1-like kinase 1 (EC 2.7.11.1) (AMPK-related protein kinase 5). [Source:Uniprot/SWISSPROT;Acc:O60285]
ENSG00000120526	NUDCD1	NudC domain containing 1 [Source:RefSeq_peptide;Acc:NP_116258]
ENSG00000112874	NUDT12	Peroxisomal NADH pyrophosphatase NUDT12 (EC 3.6.1.22) (Nucleoside diphosphate-linked moiety X motif 12) (Nudix motif 12). [Source:Uniprot/SWISSPROT;Acc:Q9BQG2]
ENSG00000101888	NXT2	NTF2-related export protein 2 (p15-2 protein). [Source:Uniprot/SWISSPROT;Acc:Q9NPJ8]
ENSG00000173559	OBFC2A	oligonucleotide/oligosaccharide-binding fold containing 2A [Source:RefSeq_peptide;Acc:NP_001026886]
ENSG00000149256	ODZ4	Teneurin-4 (Ten-4) (Tenascin-M4) (Ten-m4) (Protein Odd Oz/ten-m homolog 4). [Source:Uniprot/SWISSPROT;Acc:Q6N022]
ENSG00000087263	OGFOD1	2-oxoglutarate and iron-dependent oxygenase domain containing 1 [Source:RefSeq_peptide;Acc:NP_060703]
ENSG00000184881	OR51B2	Olfactory receptor 51B2 (Odorant receptor HOR5'beta3). [Source:Uniprot/SWISSPROT;Acc:Q9Y5P1]
ENSG00000091039	OSBPL8	Oxysterol-binding protein-related protein 8 (OSBP-related protein 8) (ORP-8). [Source:Uniprot/SWISSPROT;Acc:Q9BZF1]
ENSG00000164823	OSGIN2	Oxidative stress induced growth inhibitor 2 (hT41). [Source:Uniprot/SWISSPROT;Acc:Q9Y236]
ENSG00000145623	OSMR	oncostatin M receptor [Source:RefSeq_peptide;Acc:NP_003990]
ENSG00000081087	OSTM1	Osteopetrosis-associated transmembrane protein 1 precursor. [Source:Uniprot/SWISSPROT;Acc:Q86WC4]
ENSG00000007168	PAFAH1B1	Platelet-activating factor acetylhydrolase IB subunit alpha (PAF acetylhydrolase 45 kDa subunit) (PAF-AH 45 kDa subunit) (PAF-AH alpha) (PAFAH alpha) (Lissencephaly-1 protein) (LIS-1). [Source:Uniprot/SWISSPROT;Acc:P43034]
ENSG00000120727	PAIP2	Polyadenylate-binding protein-interacting protein 2 (Poly(A)-binding protein-interacting protein 2) (PABP-interacting protein 2) (PAIP-2). [Source:Uniprot/SWISSPROT;Acc:Q9BPZ3]
ENSG00000149269	PAK1	Serine/threonine-protein kinase PAK 1 (EC 2.7.11.1) (p21-activated kinase 1) (PAK-1) (P65-PAK) (Alpha-PAK). [Source:Uniprot/SWISSPROT;Acc:Q13153]
ENSG00000156453	PCDH1	Protocadherin-1 precursor (Protocadherin-42) (PC42) (Cadherin-like protein 1). [Source:Uniprot/SWISSPROT;Acc:Q08174]
ENSG00000081842	PCDHAC1	Protocadherin alpha C2 precursor (PCDH-alpha-C2). [Source:Uniprot/SWISSPROT;Acc:Q9Y5I4]
ENSG00000078674	PCM1	pericentriolar material 1 [Source:RefSeq_peptide;Acc:NP_006188]
ENSG00000115252	PDE1A	Calcium/calmodulin-dependent 3',5'-cyclic nucleotide phosphodiesterase 1A (EC 3.1.4.17) (Cam-PDE 1A) (61 kDa Cam-PDE) (hCam-1). [Source:Uniprot/SWISSPROT;Acc:P54750]
ENSG00000178104	PDE4DIP	phosphodiesterase 4D interacting protein isoform 2 [Source:RefSeq_peptide;Acc:NP_001002812]
ENSG00000073417	PDE8A	High-affinity cAMP-specific and IBMX-insensitive 3',5'-cyclic phosphodiesterase 8A (EC 3.1.4.17). [Source:Uniprot/SWISSPROT;Acc:O60658]
ENSG00000100311	PDGFB	Platelet-derived growth factor B chain precursor (PDGF B-chain) (Platelet-derived growth factor beta polypeptide) (PDGF-2) (c-sis) (Becaplermin). [Source:Uniprot/SWISSPROT;Acc:P01127]
ENSG00000163110	PDLIM5	PDZ and LIM domain protein 5 (Enigma homolog) (Enigma-like PDZ and LIM domains protein). [Source:Uniprot/SWISSPROT;Acc:Q96HC4]
ENSG00000198872	PEG10	paternally expressed 10 isoform RF1/2 [Source:RefSeq_peptide;Acc:NP_055883]
ENSG00000139946	PELI2	Protein pellino homolog 2 (Pellino-2). [Source:Uniprot/SWISSPROT;Acc:Q9HAT8]
ENSG00000152684	PELO	Integrin alpha-1 (Laminin and collagen receptor) (VLA-1) (CD49a antigen). [Source:Uniprot/SWISSPROT;Acc:P56199]
ENSG00000049246	PER3	Period circadian protein 3 (hPER3). [Source:Uniprot/SWISSPROT;Acc:P56645]
ENSG00000131779	PEX11B	Peroxisomal membrane protein 11B (Peroxin-11B) (Peroxisomal biogenesis factor 11B) (PEX11beta) (Pex11pbeta). [Source:Uniprot/SWISSPROT;Acc:O96011]
ENSG00000101132	PFDN4	Prefoldin subunit 4 (Protein C-1). [Source:Uniprot/SWISSPROT;Acc:Q9NQP4]
ENSG00000102144	PGK1	Phosphoglycerate kinase 1 (EC 2.7.2.3) (Primer recognition protein 2) (PRP 2). [Source:Uniprot/SWISSPROT;Acc:P00558]
ENSG00000165434	PGM2L1	Phosphoglucomutase-2-like 1 (EC 5.4.2.2) (PMMLP). [Source:Uniprot/SWISSPROT;Acc:Q6PCE3]
ENSG00000013375	PGM3	Phosphoacetylglucosamine mutase (EC 5.4.2.3) (PAGM) (Acetylglucosamine phosphomutase) (N-acetylglucosamine-phosphate mutase) (Phosphoglucomutase 3). [Source:Uniprot/SWISSPROT;Acc:O95394]
ENSG00000109118	PHF12	PHD finger protein 12 (PHD factor 1) (Pf1). [Source:Uniprot/SWISSPROT;Acc:Q96QT6]
ENSG00000135365	PHF21A	PHD finger protein 21A (BRAF35-HDAC complex protein BHC80) (BHC80a). [Source:Uniprot/SWISSPROT;Acc:Q96BD5]
ENSG00000118482	PHF3	PHD finger protein 3. [Source:Uniprot/SWISSPROT;Acc:Q92576]
ENSG00000010318	PHF7	PHD finger protein 7 (Testis development protein NYD-SP6). [Source:Uniprot/SWISSPROT;Acc:Q9BWX1]
ENSG00000006576	PHTF2	putative homeodomain transcription factor 2 [Source:RefSeq_peptide;Acc:NP_065165]
ENSG00000073921	PICALM	Phosphatidylinositol-binding clathrin assembly protein (Clathrin assembly lymphoid myeloid leukemia protein). [Source:Uniprot/SWISSPROT;Acc:Q13492]
ENSG00000100151	PICK1	PRKCA-binding protein (Protein kinase C-alpha-binding protein) (Protein interacting with C kinase 1). [Source:Uniprot/SWISSPROT;Acc:Q9NRD5]
ENSG00000051382	PIK3CB	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit beta isoform (EC 2.7.1.153) (PI3-kinase p110 subunit beta) (PtdIns-3-kinase p110) (PI3K) (PI3Kbeta). [Source:Uniprot/SWISSPROT;Acc:P42338]
ENSG00000065243	PKN2	Serine/threonine-protein kinase N2 (EC 2.7.11.13) (Protein kinase C- like 2) (Protein-kinase C-related kinase 2). [Source:Uniprot/SWISSPROT;Acc:Q16513]
ENSG00000181690	PLAG1	pleiomorphic adenoma gene 1 [Source:RefSeq_peptide;Acc:NP_002646]
ENSG00000122861	PLAU	Urokinase-type plasminogen activator precursor (EC 3.4.21.73) (uPA) (U-plasminogen activator) [Contains: Urokinase-type plasminogen activator long chain A; Urokinase-type plasminogen activator short chain A; Urokinase-type plasminogen activator chain B]. [Source:Uniprot/SWISSPROT;Acc:P00749]
ENSG00000115896	PLCL1	phospholipase C-like 1 [Source:RefSeq_peptide;Acc:NP_006217]
ENSG00000106086	PLEKHA8	pleckstrin homology domain containing, family A (phosphoinositide binding specific) member 8 [Source:RefSeq_peptide;Acc:NP_116028]
ENSG00000141682	PMAIP1	Phorbol-12-myristate-13-acetate-induced protein 1 (PMA-induced protein 1) (Immediate-early-response protein APR). [Source:Uniprot/SWISSPROT;Acc:Q13794]
ENSG00000177666	PNPLA2	patatin-like phospholipase domain containing 2 [Source:RefSeq_peptide;Acc:NP_065109]
ENSG00000085998	POMGNT1	Protein O-linked-mannose beta-1,2-N-acetylglucosaminyltransferase 1 (EC 2.4.1.-) (POMGnT1) (UDP-GlcNAc:alpha-D-mannoside beta-1,2-N- acetylglucosaminyltransferase I.2) (GnT I.2). [Source:Uniprot/SWISSPROT;Acc:Q8WZA1]
ENSG00000133110	POSTN	Periostin precursor (PN) (Osteoblast-specific factor 2) (OSF-2). [Source:Uniprot/SWISSPROT;Acc:Q15063]
ENSG00000151615	POU4F2	POU domain, class 4, transcription factor 2 (Brain-specific homeobox/POU domain protein 3B) (Brn-3B). [Source:Uniprot/SWISSPROT;Acc:Q12837]
ENSG00000186951	PPARA	Peroxisome proliferator-activated receptor alpha (PPAR-alpha). [Source:Uniprot/SWISSPROT;Acc:Q07869]

ENSG00000138032	PPM1B	Protein phosphatase 2C isoform beta (EC 3.1.3.16) (PP2C-beta). [Source:Uniprot/SWISSPROT;Acc:O75688]
ENSG00000058272	PPP1R12A	Protein phosphatase 1 regulatory subunit 12A (Myosin phosphatase- targeting subunit 1) (Myosin phosphatase target subunit 1) (Protein phosphatase myosin-binding subunit). [Source:Uniprot/SWISSPROT;Acc:O14974]
ENSG00000138814	PPP3CA	Serine/threonine-protein phosphatase 2B catalytic subunit alpha isoform (EC 3.1.3.16) (Calmodulin-dependent calcineurin A subunit alpha isoform) (CAM-PRP catalytic subunit). [Source:Uniprot/SWISSPROT;Acc:Q08209]
ENSG00000119414	PPP6C	Serine/threonine-protein phosphatase 6 (EC 3.1.3.16) (PP6). [Source:Uniprot/SWISSPROT;Acc:O00743]
ENSG00000130711	PRDM12	PR domain zinc finger protein 12 (PR domain-containing protein 12). [Source:Uniprot/SWISSPROT;Acc:Q9H4Q4]
ENSG00000142875	PRKACB	cAMP-dependent protein kinase, beta-catalytic subunit (EC 2.7.11.11) (PKA C-beta). [Source:Uniprot/SWISSPROT;Acc:P22694]
ENSG00000108946	PRKAR1A	cAMP-dependent protein kinase type I-alpha regulatory subunit (Tissue- specific extinguisher 1) (TSE1). [Source:Uniprot/SWISSPROT;Acc:P10644]
ENSG00000114302	PRKAR2A	cAMP-dependent protein kinase type II-alpha regulatory subunit. [Source:Uniprot/SWISSPROT;Acc:P13861]
ENSG00000185532	PRKG1	cGMP-dependent protein kinase 1, beta isozyme (EC 2.7.11.12) (cGK 1 beta) (cGKI-beta). [Source:Uniprot/SWISSPROT;Acc:P14619]
ENSG00000183943	PRKX	Serine/threonine-protein kinase PRKX (EC 2.7.11.1) (Protein kinase PKX1). [Source:Uniprot/SWISSPROT;Acc:P51817]
ENSG00000113494	PRLR	Prolactin receptor precursor (PRL-R). [Source:Uniprot/SWISSPROT;Acc:P16471]
ENSG00000141127	PRPSAP2	Phosphoribosyl pyrophosphate synthetase-associated protein 2 (PRPP synthetase-associated protein 2) (41 kDa phosphoribosypyrophosphate synthetase-associated protein) (PAP41). [Source:Uniprot/SWISSPROT;Acc:O60256]
ENSG00000116132	PRRX1	Paired mesoderm homeobox protein 1 (PRX-1) (Paired-related homeobox protein 1) (Homeobox protein PHOX1). [Source:Uniprot/SWISSPROT;Acc:P54821]
ENSG00000126067	PSMB2	Proteasome subunit beta type 2 (EC 3.4.25.1) (Proteasome component C7- I) (Macropain subunit C7-I) (Multicatalytic endopeptidase complex subunit C7-I). [Source:Uniprot/SWISSPROT;Acc:P49721]
ENSG00000136930	PSMB7	Proteasome subunit beta type 7 precursor (EC 3.4.25.1) (Proteasome subunit Z) (Macropain chain Z) (Multicatalytic endopeptidase complex chain Z). [Source:Uniprot/SWISSPROT;Acc:Q99436]
ENSG00000131470	PSMC3IP	TBP-1 interacting protein isoform 1 [Source:RefSeq_peptide;Acc:NP_037422]
ENSG00000188647	PTAR1	PTAR1 protein (Fragment). [Source:Uniprot/SPTREMBL;Acc:Q7Z6K3]
ENSG00000011304	PTBP1	Polypyrimidine tract-binding protein 1 (PTB) (Heterogeneous nuclear ribonucleoprotein I) (hnRNP I) (57 kDa RNA-binding protein PPTB-1). [Source:Uniprot/SWISSPROT;Acc:P26599]
ENSG00000168229	PTGDR	Prostaglandin D2 receptor (Prostanoid DP receptor) (PGD receptor). [Source:Uniprot/SWISSPROT;Acc:Q13258]
ENSG00000050628	PTGER3	Prostaglandin E2 receptor, EP3 subtype (Prostanoid EP3 receptor) (PGE receptor, EP3 subtype) (PGE2-R). [Source:Uniprot/SWISSPROT;Acc:P43115]
ENSG00000171522	PTGER4	Prostaglandin E2 receptor, EP4 subtype (Prostanoid EP4 receptor) (PGE receptor, EP4 subtype). [Source:Uniprot/SWISSPROT;Acc:P35408]
ENSG00000153707	PTPRD	Receptor-type tyrosine-protein phosphatase delta precursor (EC 3.1.3.48) (Protein-tyrosine phosphatase delta) (R-PTP-delta). [Source:Uniprot/SWISSPROT;Acc:P23468]
ENSG00000173482	PTPRM	Receptor-type tyrosine-protein phosphatase mu precursor (EC 3.1.3.48) (Protein-tyrosine phosphatase mu) (R-PTP-mu). [Source:Uniprot/SWISSPROT;Acc:P28827]
ENSG00000172733	PURG	Purine-rich element-binding protein gamma. [Source:Uniprot/SWISSPROT;Acc:Q9UJV8]
ENSG00000177707	PVRL3	Poliovirus receptor-related protein 3 precursor (Nectin-3) (CD113 antigen) (CDw113). [Source:Uniprot/SWISSPROT;Acc:Q9NQS3]
ENSG00000160220	PWP2H	Periodic tryptophan protein 2 homolog. [Source:Uniprot/SWISSPROT;Acc:Q15269]
ENSG00000130508	PXDN	peroxidasin homolog [Source:RefSeq_peptide;Acc:NP_036425]
ENSG00000206539	Q6ZTN9_HUMAN	CDNA FLJ44459 fis, clone UTERU2024656. [Source:Uniprot/SPTREMBL;Acc:Q6ZTN9]
ENSG00000180044	Q8N5S4_HUMAN	
ENSG00000170682	Q96NU5_HUMAN	CDNA FLJ30064 fis, clone ADRGL2000323. [Source:Uniprot/SPTREMBL;Acc:Q96NU5]
ENSG00000198780	Q9H989_HUMAN	CDNA FLJ12919 fis, clone NT2RP2004587, weakly similar to NEUROFILAMENT TRIPLET M PROTEIN. [Source:Uniprot/SPTREMBL;Acc:Q9H989]
ENSG00000112531	QKI	Quaking protein (Hqk). [Source:Uniprot/SWISSPROT;Acc:Q96PU8]
ENSG00000156675	RAB11FIP1	Rab11 family-interacting protein 1 (Rab11-FIP1) (Rab-coupling protein). [Source:Uniprot/SWISSPROT;Acc:Q6WKZ4]
ENSG00000138069	RAB1A	Ras-related protein Rab-1A (YPT1-related protein). [Source:Uniprot/SWISSPROT;Acc:P62820]
ENSG00000080371	RAB21	Ras-related protein Rab-21. [Source:Uniprot/SWISSPROT;Acc:Q9UL25]
ENSG00000069974	RAB27A	Ras-related protein Rab-27A (Rab-27) (GTP-binding protein Ram). [Source:Uniprot/SWISSPROT;Acc:P51159]
ENSG00000184014	RAB6IP1	Rab6-interacting protein 1 (Rab6IP1). [Source:Uniprot/SWISSPROT;Acc:Q6IQ26]
ENSG00000017797	RALBP1	RalA-binding protein 1 (RalBP1) (Ral-interacting protein 1) (76 kDa Ral-interacting protein) (Dinitrophenyl S-glutathione ATPase) (DNP-SG ATPase). [Source:Uniprot/SWISSPROT;Acc:Q15311]
ENSG00000123728	RAP2C	Ras-related protein Rap-2c precursor. [Source:Uniprot/SWISSPROT;Acc:Q9Y3L5]
ENSG00000091428	RAPGEF4	Rap guanine nucleotide exchange factor 4 (cAMP-regulated guanine nucleotide exchange factor II) (cAMP-GEFII) (Exchange factor directly activated by cAMP 2) (Epac 2). [Source:Uniprot/SWISSPROT;Acc:Q8WZA2]
ENSG00000077092	RARB	Retinoic acid receptor beta (RAR-beta) (RAR-epsilon) (HBV-activated protein). [Source:Uniprot/SWISSPROT;Acc:P10826]
ENSG00000123094	RASSF8	Ras association domain-containing protein 8 (Carcinoma-associated protein HOJ-1). [Source:Uniprot/SWISSPROT;Acc:Q8NHQ8]
ENSG00000162437	RAVER2	Ribonucleoprotein PTB-binding 2 (Protein raver-2). [Source:Uniprot/SWISSPROT;Acc:Q9HCJ3]
ENSG00000146587	RBAK	RB-associated KRAB repressor [Source:RefSeq_peptide;Acc:NP_066986]
ENSG00000103479	RBL2	Retinoblastoma-like protein 2 (130 kDa retinoblastoma-associated protein) (PRB2) (P130) (RBR-2). [Source:Uniprot/SWISSPROT;Acc:Q08999]
ENSG00000119707	RBM25	Probable RNA-binding protein 25 (RNA-binding motif protein 25) (RNA- binding region-containing protein 7) (Protein S164). [Source:Uniprot/SWISSPROT;Acc:P49756]
ENSG00000056586	MNAB	Membrane-associated nucleic acid-binding protein (RING finger protein 164). [Source:Uniprot/SWISSPROT;Acc:Q9HBD1]
ENSG00000143344	RGL1	Ral guanine nucleotide dissociation stimulator-like 1 (RalGDS-like 1). [Source:Uniprot/SWISSPROT;Acc:Q9NZL6]
ENSG00000143333	RGS16	Regulator of G-protein signaling 16 (RGS16) (Retinally abundant regulator of G-protein signaling) (RGS-R) (A28-RGS14P). [Source:Uniprot/SWISSPROT;Acc:O15492]
ENSG00000143248	RGS5	Regulator of G-protein signaling 5 (RGS5). [Source:Uniprot/SWISSPROT;Acc:O15539]
ENSG00000143878	RHOB	Rho-related GTP-binding protein RhoB precursor (H6). [Source:Uniprot/SWISSPROT;Acc:P62745]
ENSG00000126858	RHOT1	Mitochondrial Rho GTPase 1 (EC 3.6.5.-) (MIRO-1) (hMiro-1) (Ras homolog gene family member T1) (Rac-GTP-binding protein-like protein). [Source:Uniprot/SWISSPROT;Acc:Q8IXI2]
ENSG00000111785	RIC8B	Synembryn-B (Brain synembrin) (hSyn) (Protein Ric-8B). [Source:Uniprot/SWISSPROT;Acc:Q9NVN3]
ENSG00000101098	RIMS4	Regulating synaptic membrane exocytosis protein 4 (Rab3-interacting molecule 4) (RIM 4) (RIM4 gamma). [Source:Uniprot/SWISSPROT;Acc:Q9H426]
ENSG00000058729	RIOK2	Serine/threonine-protein kinase RIO2 (EC 2.7.11.1) (RIO kinase 2). [Source:Uniprot/SWISSPROT;Acc:Q9BVS4]
ENSG00000104312	RIPK2	Receptor-interacting serine/threonine-protein kinase 2 (EC 2.7.11.1) (RIP-like-interacting CLARP kinase) (Receptor-interacting protein 2) (RIP-2) (CARD-containing interleukin-1 beta-converting enzyme- associated kinase) (CARD-containing IL-1 beta ICE-kina [Source:Uniprot/SWISSPROT;Acc:O43353]
ENSG00000171865	RNASEH1	Ribonuclease H1 (EC 3.1.26.4) (RNase H1) (Ribonuclease H type II). [Source:Uniprot/SWISSPROT;Acc:O60930]
ENSG00000163162	RNF149	ring finger protein 149 [Source:RefSeq_peptide;Acc:NP_775918]
ENSG00000164197	RNF180	ring finger protein 180 [Source:RefSeq_peptide;Acc:NP_848627]
ENSG00000108375	RNF43	Transcription elongation factor SPT4 (hSPT4) (DRB sensitivity-inducing factor small subunit) (DSIF small subunit) (DSIF p14). [Source:Uniprot/SWISSPROT;Acc:P63272]
ENSG00000111880	RNGTT	mRNA capping enzyme (HCE) (HCAP1) [Includes: Polynucleotide 5'- triphosphatase (EC 3.1.3.33) (mRNA 5'-triphosphatase) (TPase); mRNA guanylyltransferase (EC 2.7.7.50) (GTP--RNA guanylyltransferase) (GTase)]. [Source:Uniprot/SWISSPROT;Acc:O60942]
ENSG00000197756	RPL37A	60S ribosomal protein L37a. [Source:Uniprot/SWISSPROT;Acc:P61513]
ENSG00000159216	RUNX1	Runt-related transcription factor 1 (Core-binding factor, alpha 2 subunit) (CBF-alpha 2) (Acute myeloid leukemia 1 protein) (Oncogene AML-1) (Polyomavirus enhancer-binding protein 2 alpha B subunit) (PEBP2-alpha B) (PEA2-alpha B) (SL3-3 enhancer factor 1 [Source:Uniprot/SWISSPROT;Acc:Q01196]

ENSG00000198853	RUSC2	RUN and SH3 domain-containing protein 2. [Source:Uniprot/SWISSPROT;Acc:Q8N2Y8]
ENSG00000163785	RYK	Tyrosine-protein kinase RYK precursor (EC 2.7.10.1). [Source:Uniprot/SWISSPROT;Acc:P34925]
ENSG00000187634	SAMD11	sterile alpha motif domain containing 11 [Source:RefSeq_peptide;Acc:NP_689699]
ENSG00000110075	SAPS3	SAPS domain family, member 3 [Source:RefSeq_peptide;Acc:NP_060782]
ENSG00000085365	SCAMP1	Secretory carrier-associated membrane protein 1 (Secretory carrier membrane protein 1). [Source:Uniprot/SWISSPROT;Acc:O15126]
ENSG00000146285	SCML4	sex comb on midleg-like 4 [Source:RefSeq_peptide;Acc:NP_932347]
ENSG00000196876	SCN8A	Sodium channel protein type 8 subunit alpha (Sodium channel protein type VIII subunit alpha) (Voltage-gated sodium channel subunit alpha Nav1.6). [Source:Uniprot/SWISSPROT;Acc:Q9UQD0]
ENSG00000144306	SCRN3	secernin 3 [Source:RefSeq_peptide;Acc:NP_078859]
ENSG00000164022	SCYE1	Multisynthetase complex auxiliary component p43 [Contains: Endothelial monocyte-activating polypeptide 2 (EMAP-II) (Small inducible cytokine subfamily E member 1)]. [Source:Uniprot/SWISSPROT;Acc:Q12904]
ENSG00000000457	SCYL3	Protein-associating with the carboxyl-terminal domain of ezrin (Ezrin- binding protein PACE-1) (SCY1-like protein 3). [Source:Uniprot/SWISSPROT;Acc:Q8IZE3]
ENSG00000075223	SEMA3C	Semaphorin-3C precursor (Semaphorin E) (Sema E). [Source:Uniprot/SWISSPROT;Acc:Q99985] Semaphorin-6A precursor (Semaphorin VIA) (Sema VIA) (Semaphorin 6A-1) (SEMA6A-1).
ENSG00000092421	SEMA6A	[Source:Uniprot/SWISSPROT;Acc:Q9H2E6]
ENSG00000119231	SENP5	Sentrin-specific protease 5 (EC 3.4.22.-) (Sentrin/SUMO-specific protease SENP5). [Source:Uniprot/SWISSPROT;Acc:Q96HI0]
ENSG00000086475	SEPHS1	Selenide, water dikinase 1 (EC 2.7.9.3) (Selenophosphate synthetase 1) (Selenium donor protein 1). [Source:Uniprot/SWISSPROT;Acc:P49903]
ENSG00000164402	SEPT8	Septin-8. [Source:Uniprot/SWISSPROT;Acc:Q92599]
ENSG00000132824	SERINC3	Serine incorporator 3 (Tumor differentially expressed protein 1). [Source:Uniprot/SWISSPROT;Acc:Q13530]
ENSG00000106366	SERPINE1	Plasminogen activator inhibitor 1 precursor (PAI-1) (Endothelial plasminogen activator inhibitor) (PAI). [Source:Uniprot/SWISSPROT;Acc:P05121]
ENSG00000173349	SFT2D3	Vesicle transport protein SFT2C (SFT2 domain-containing protein 3). [Source:Uniprot/SWISSPROT;Acc:Q587I9]
ENSG00000164466	SFXN1	Sideroflexin-1 (Tricarboxylate carrier protein) (TCC). [Source:Uniprot/SWISSPROT;Acc:Q9H9B4]
ENSG00000126821	SGPP1	Sphingosine-1-phosphate phosphatase 1 (EC 3.1.3.-) (Sphingosine-1- phosphatase 1) (SPPase1) (Spp1) (hSPPase1). [Source:Uniprot/SWISSPROT;Acc:Q9BX95]
ENSG00000198478	SH3BGRL2	SH3 domain-binding glutamic acid-rich-like protein 2 (Fovea-associated SH3 domain-binding protein). [Source:Uniprot/SWISSPROT;Acc:Q9UJC5]
ENSG00000087266	SH3BP2	SH3 domain-binding protein 2 (3BP-2). [Source:Uniprot/SWISSPROT;Acc:P78314]
ENSG00000131370	SH3BP5	SH3 domain-binding protein 5 (SH3 domain-binding protein that preferentially associates with BTK). [Source:Uniprot/SWISSPROT;Acc:O60239]
ENSG00000154447	SH3RF1	SH3 domain containing ring finger 1 [Source:RefSeq_peptide;Acc:NP_065921]
ENSG00000168779	SHOX2	Short stature homeobox protein 2 (Paired-related homeobox protein SHOT) (Homeobox protein Og12X). [Source:Uniprot/SWISSPROT;Acc:O60902]
ENSG00000110013	SIAE	Sialate O-acetylesterase precursor (EC 3.1.1.53) (Sialic acid-specific 9-O-acetylesterase) (H-Lse). [Source:Uniprot/SWISSPROT;Acc:Q9HAT2]
ENSG00000101307	SIRPB1	Signal-regulatory protein beta-1 precursor (SIRP-beta-1) (CD172b antigen). [Source:Uniprot/SWISSPROT;Acc:O00241]
ENSG00000100625	SIX4	Homeobox protein SIX4 (Sine oculis homeobox homolog 4). [Source:Uniprot/SWISSPROT;Acc:Q9UIU6]
ENSG00000018280	SLC11A1	Natural resistance-associated macrophage protein 1 (NRAMP 1). [Source:Uniprot/SWISSPROT;Acc:P49279]
ENSG00000110911	SLC11A2	Natural resistance-associated macrophage protein 2 (NRAMP 2) (Divalent metal transporter 1) (DMT1). [Source:Uniprot/SWISSPROT;Acc:P49281]
ENSG00000074803	SLC12A1	Solute carrier family 12 member 1 (Bumetanide-sensitive sodium- (potassium)-chloride cotransporter 2) (Kidney-specific Na-K-Cl symporter). [Source:Uniprot/SWISSPROT;Acc:Q13621]
ENSG00000112394	SLC16A10	solute carrier family 16, member 10 [Source:RefSeq_peptide;Acc:NP_061063]
ENSG00000165449	SLC16A9	solute carrier family 16 (monocarboxylic acid transporters), member 9 [Source:RefSeq_peptide;Acc:NP_919274]
ENSG00000110436	SLC1A2	Excitatory amino acid transporter 2 (Sodium-dependent glutamate/aspartate transporter 2). [Source:Uniprot/SWISSPROT;Acc:P43004]
ENSG00000102743	SLC25A15	Mitochondrial ornithine transporter 1 (Solute carrier family 25 member 15). [Source:Uniprot/SWISSPROT;Acc:Q9Y619]
ENSG00000085491	SLC25A24	solute carrier family 25 member 24 isoform 2 [Source:RefSeq_peptide;Acc:NP_998816]
ENSG00000155850	SLC26A2	Sulfate transporter (Diastrophic dysplasia protein) (Solute carrier family 26 member 2). [Source:Uniprot/SWISSPROT;Acc:P50443]
ENSG00000125520	SLC2A4RG	SLC2A4 regulator (GLUT4 enhancer factor) (GEF) (Huntington disease gene regulatory region-binding protein 1) (HDBP-1). [Source:Uniprot/SWISSPROT;Acc:Q9NR83]
ENSG00000145740	SLC30A5	zinc transporter ZTL1 isoform 1 [Source:RefSeq_peptide;Acc:NP_075053]
ENSG00000117620	SLC35A3	UDP-N-acetylglucosamine transporter (Golgi UDP-GlcNAc transporter) (Solute carrier family 35 member A3). [Source:Uniprot/SWISSPROT;Acc:Q9Y2D2]
ENSG00000139209	SLC38A4	Sodium-coupled neutral amino acid transporter 4 (Na(+)-coupled neutral amino acid transporter 4) (Amino acid transporter A3) (System A amino acid transporter 3) (System N amino acid transporter 3) (Solute carrier family 38 member 4). [Source:Uniprot/SWISSPROT;Acc:Q969I6]
ENSG00000151012	SLC7A11	Cystine/glutamate transporter (Amino acid transport system xc-) (xCT) (Calcium channel blocker resistance protein CCBR1). [Source:Uniprot/SWISSPROT;Acc:Q9UPY5]
ENSG00000172716	SLFN11	schlafen family member 11 [Source:RefSeq_peptide;Acc:NP_689483]
ENSG00000184564	SLITRK6	SLIT and NTRK-like protein 6 precursor. [Source:Uniprot/SWISSPROT;Acc:Q9H5Y7]
ENSG00000101166	CT045_HUMAN	Uncharacterized protein C20orf45. [Source:Uniprot/SWISSPROT;Acc:Q9Y3B1]
ENSG00000198887	SMC5	SMC5 protein [Source:RefSeq_peptide;Acc:NP_055925]
ENSG00000138041	SMEK2	SMEK homolog 2. [Source:Uniprot/SWISSPROT;Acc:Q5MIZ7]
ENSG00000198742	SMURF1	Smad ubiquitination regulatory factor 1 (EC 6.3.2.-) (Ubiquitin-- protein ligase SMURF1) (Smad-specific E3 ubiquitin ligase 1) (hSMURF1). [Source:Uniprot/SWISSPROT;Acc:Q9HCE7]
ENSG00000099940	SNAP29	Synaptosomal-associated protein 29 (SNAP-29) (Vesicle-membrane fusion protein SNAP-29) (Soluble 29 kDa NSF attachment protein). [Source:Uniprot/SWISSPROT;Acc:O95721]
ENSG00000164975	SNAPC3	snRNA-activating protein complex subunit 3 (SNAPc subunit 3) (snRNA- activating protein complex 50 kDa subunit) (SNAPc 50 kDa subunit) (Small nuclear RNA-activating complex polypeptide 3) (Proximal sequence element-binding transcription factor subunit bet [Source:Uniprot/SWISSPROT;Acc:Q92966]
ENSG00000163877	SNIP1	Smad nuclear-interacting protein 1. [Source:Uniprot/SWISSPROT;Acc:Q8TAD8]
ENSG00000184602	SNN	Stannin (AG8_1). [Source:Uniprot/SWISSPROT;Acc:O75324]
ENSG00000172164	SNTB1	Beta-1-syntrophin (59 kDa dystrophin-associated protein A1 basic component 1) (DAPA1B) (Tax interaction protein 43) (TIP-43) (Syntrophin 2) (BSYN2). [Source:Uniprot/SWISSPROT;Acc:Q13884]
ENSG00000147481	SNTG1	Gamma-1-syntrophin (G1SYN) (Syntrophin 4) (SYN4). [Source:Uniprot/SWISSPROT;Acc:Q9NSN8]
ENSG00000086300	SNX10	Sorting nexin-10. [Source:Uniprot/SWISSPROT;Acc:Q9Y5X0]
ENSG00000120833	SOCS2	Suppressor of cytokine signaling 2 (SOCS-2) (Cytokine-inducible SH2 protein 2) (CIS-2) (STAT-induced STAT inhibitor 2) (SSI-2). [Source:Uniprot/SWISSPROT;Acc:O14508]
ENSG00000112096	SOD2	Superoxide dismutase [Mn], mitochondrial precursor (EC 1.15.1.1). [Source:Uniprot/SWISSPROT;Acc:P04179]
ENSG00000176887	SOX11	Transcription factor SOX-11. [Source:Uniprot/SWISSPROT;Acc:P35716]
ENSG00000203883	SOX18	Transcription factor SOX-18. [Source:Uniprot/SWISSPROT;Acc:P35713]
ENSG00000182957	SPATA13	spermatogenesis associated 13 [Source:RefSeq_peptide;Acc:NP_694568]
ENSG00000101448	SPINLW1	Eppin precursor (Epididymal protease inhibitor) (Serine protease inhibitor-like with Kunitz and WAP domains 1) (WAP four-disulfide core domain protein 7) (Protease inhibitor WAP7). [Source:Uniprot/SWISSPROT;Acc:O95925]
ENSG00000172296	C20orf38	Uncharacterized protein C20orf38. [Source:Uniprot/SWISSPROT;Acc:Q9NUV7]
ENSG00000075142	SRI	Sorcin (22 kDa protein) (CP-22) (V19). [Source:Uniprot/SWISSPROT;Acc:P30626]
ENSG00000138434	SSFA2	Sperm-specific antigen 2 (Cleavage signal-1 protein) (CS-1). [Source:Uniprot/SWISSPROT;Acc:P28290]
ENSG00000114850	SSR3	Translocon-associated protein subunit gamma (TRAP-gamma) (Signal sequence receptor subunit gamma) (SSR-gamma).

		[Source:Uniprot/SWISSPROT;Acc:Q9UNL2]
ENSG00000139874	SSTR1	Somatostatin receptor type 1 (SS1R) (SRIF-2). [Source:Uniprot/SWISSPROT;Acc:P30872]
ENSG00000008513	ST3GAL1	CMP-N-acetylneuraminate-beta-galactosamide-alpha-2,3-sialyltransferase (EC 2.4.99.4) (Beta-galactoside alpha-2,3-sialyltransferase) (Alpha 2,3-ST) (Gal-NAc6S) (Gal-beta-1,3-GalNAc-alpha-2,3-sialyltransferase) (ST3GalIA) (ST3O) (ST3GalA.1) (SIAT4-A) (ST3Ga [Source:Uniprot/SWISSPROT;Acc:Q11201]
ENSG00000184005	ST6GALNAC3	Alpha-N-acetylgalactosaminide alpha-2,6-sialyltransferase 3 (EC 2.4.99.-) (GalNAc alpha-2,6-sialyltransferase III) (ST6GalNAc III) (Sialyltransferase 7C) (STY). [Source:Uniprot/SWISSPROT;Acc:Q8NDV1]
ENSG00000117069	ST6GALNAC5	Alpha-N-acetylgalactosaminide alpha-2,6-sialyltransferase 5 (EC 2.4.99.-) (GalNAc alpha-2,6-sialyltransferase V) (ST6GalNAc V) (GD1 alpha synthase) (Sialyltransferase 7E). [Source:Uniprot/SWISSPROT;Acc:Q9BVH7]
ENSG00000101972	STAG2	Cohesin subunit SA-2 (Stromal antigen 2) (SCC3 homolog 2). [Source:Uniprot/SWISSPROT;Acc:Q8N3U4]
ENSG00000159167	STC1	Stanniocalcin-1 precursor (STC-1). [Source:Uniprot/SWISSPROT;Acc:P52823]
ENSG00000115661	STK16	Serine/threonine-protein kinase 16 (EC 2.7.11.1) (Protein kinase PKL12) (Myristoylated and palmitoylated serine/threonine-protein kinase) (MPSK) (TGF-beta-stimulated factor 1) (TSF-1) (hPSK). [Source:Uniprot/SWISSPROT;Acc:O75716]
ENSG00000104375	STK3	Serine/threonine-protein kinase 3 (EC 2.7.11.1) (STE20-like kinase MST2) (MST-2) (Mammalian STE20-like protein kinase 2) (Serine/threonine-protein kinase Krs-1). [Source:Uniprot/SWISSPROT;Acc:Q13188]
ENSG00000101109	STK4	Serine/threonine-protein kinase 4 (EC 2.7.11.1) (STE20-like kinase MST1) (MST-1) (Mammalian STE20-like protein kinase 1) (Serine/threonine-protein kinase Krs-2). [Source:Uniprot/SWISSPROT;Acc:Q13043]
ENSG00000196182	STK40	Serine/threonine-protein kinase 40 (EC 2.7.11.1) (SINK-homologous serine/threonine-protein kinase). [Source:Uniprot/SWISSPROT;Acc:Q8N2I9]
ENSG00000166263	STXBP4	Syntaxin-binding protein 4 (Syntaxin 4-interacting protein) (Synip). [Source:Uniprot/SWISSPROT;Acc:Q6ZWJ1]
ENSG00000168952	STXBP6	Syntaxin-binding protein 6 (Amisyn). [Source:Uniprot/SWISSPROT;Acc:Q8NFX7]
ENSG00000108375	RNF43	Transcription elongation factor SPT4 (hSPT4) (DRB sensitivity-inducing factor small subunit) (DSIF small subunit) (DSIF p14). [Source:Uniprot/SWISSPROT;Acc:P63272]
ENSG00000143502	SUSD4	Sushi domain-containing protein 4 precursor. [Source:Uniprot/SWISSPROT;Acc:Q5VX71]
ENSG00000198168	SVIP_HUMAN	Small VCP/p97-interacting protein. [Source:Uniprot/SWISSPROT;Acc:Q8NHG7]
ENSG00000135316	SYNCRIP	Heterogeneous nuclear ribonucleoprotein Q (hnRNP Q) (hnRNP-Q) (Synaptotagmin-binding, cytoplasmic RNA-interacting protein) (Glycine- and tyrosine-rich RNA-binding protein) (GRY-RBP) (NS1-associated protein 1). [Source:Uniprot/SWISSPROT;Acc:O60506]
ENSG00000054654	SYNE2	Nesprin-2 (Nuclear envelope spectrin repeat protein 2) (Syne-2) (Synaptic nuclear envelope protein 2) (Nucleus and actin connecting element protein) (Protein NUANCE). [Source:Uniprot/SWISSPROT;Acc:Q8WXH0]
ENSG00000119888	TACSTD1	Tumor-associated calcium signal transducer 1 precursor (Major gastrointestinal tumor-associated protein GA733-2) (Epithelial cell surface antigen) (Epithelial glycoprotein) (EGP) (Adenocarcinoma- associated antigen) (KSA) (KS 1/4 antigen) (Cell surface gl [Source:Uniprot/SWISSPROT;Acc:P16422]
ENSG00000065882	TBC1D1	TBC1 domain family member 1. [Source:Uniprot/SWISSPROT;Acc:Q86TI0]
ENSG00000065491	TBC1D22B	TBC1 domain family member 22B. [Source:Uniprot/SWISSPROT;Acc:Q9NU19]
ENSG00000167202	TBC1D2B	TBC1 domain family, member 2B [Source:RefSeq_peptide;Acc:NP_055894]
ENSG00000183735	TBK1	Serine/threonine-protein kinase TBK1 (EC 2.7.11.1) (TANK-binding kinase 1) (T2K) (NF-kappa-B-activating kinase). [Source:Uniprot/SWISSPROT;Acc:Q9UHD2]
ENSG00000089225	TBX5	T-box transcription factor TBX5 (T-box protein 5). [Source:Uniprot/SWISSPROT;Acc:Q99593]
ENSG00000140262	TCF12	Transcription factor 12 (Transcription factor HTF-4) (E-box-binding protein) (DNA-binding protein HTF4). [Source:Uniprot/SWISSPROT;Acc:Q99081]
ENSG00000101190	TCFL5	Transcription factor-like 5 protein (Cha transcription factor) (HPV-16 E2-binding protein 1) (E2BP-1). [Source:Uniprot/SWISSPROT;Acc:Q9UL49]
ENSG00000187621	TCL6	T-cell leukemia/lymphoma 6 protein (Protein TNG1). [Source:Uniprot/SWISSPROT;Acc:P56846]
ENSG00000187621	TCL6	T-cell leukemia/lymphoma 6 protein (Protein TNG1). [Source:Uniprot/SWISSPROT;Acc:P56846]
ENSG00000184786	TCTE3	t-complex-associated-testis-expressed 3 [Source:RefSeq_peptide;Acc:NP_777570]
ENSG00000135269	TES	Testin (TESS). [Source:Uniprot/SWISSPROT;Acc:Q9UGI8]
ENSG00000108064	TFAM	Transcription factor A, mitochondrial precursor (mtTFA) (Mitochondrial transcription factor 1) (MtTF1) (Transcription factor 6-like 2). [Source:Uniprot/SWISSPROT;Acc:Q00059]
ENSG00000003436	TFPI	Tissue factor pathway inhibitor precursor (TFPI) (Lipoprotein- associated coagulation inhibitor) (LACI) (Extrinsic pathway inhibitor) (EPI). [Source:Uniprot/SWISSPROT;Acc:P10646]
ENSG00000072274	TFRC	Transferrin receptor protein 1 (TfR1) (TfR) (Trfr) (CD71 antigen) (T9) (p90). [Source:Uniprot/SWISSPROT;Acc:P02786]
ENSG00000135966	TGFBRAP1	TGF beta receptor associated protein -1 [Source:RefSeq_peptide;Acc:NP_004248]
ENSG00000186340	THBS2	Thrombospondin-2 precursor. [Source:Uniprot/SWISSPROT;Acc:P35442]
ENSG00000151923	TIAL1	Nucleolysin TIAR (TIA-1-related protein). [Source:Uniprot/SWISSPROT;Acc:Q01085]
ENSG00000138297	TIMM23	Mitochondrial import inner membrane translocase subunit Tim23. [Source:Uniprot/SWISSPROT;Acc:O14925]
ENSG00000008952	TLOC1	Translocation protein SEC62 (Translocation protein 1) (TP-1) (hTP-1). [Source:Uniprot/SWISSPROT;Acc:Q99442]
ENSG00000137462	TLR2	Toll-like receptor 2 precursor (Toll/interleukin 1 receptor-like protein 4) (CD282 antigen). [Source:Uniprot/SWISSPROT;Acc:O60603]
ENSG00000164342	TLR3	Toll-like receptor 3 precursor (CD283 antigen). [Source:Uniprot/SWISSPROT;Acc:O15455]
ENSG00000077147	TM9SF3	Transmembrane 9 superfamily protein member 3 precursor (SM-11044- binding protein) (EP70-P-iso). [Source:Uniprot/SWISSPROT;Acc:Q9HD45]
ENSG00000117500	TMED5	Transmembrane emp24 domain-containing protein 5 precursor. [Source:Uniprot/SWISSPROT;Acc:Q9Y3A6]
ENSG00000066697	C9orf30	transmembrane protein with EGF-like and two follistatin-like domains 1 [Source:RefSeq_peptide;Acc:NP_003683]
ENSG00000160218	TMEM1	Transmembrane protein 1 (Epilepsy holoprosencephaly candidate 1 protein) (EHOC-1) (GT334 protein). [Source:Uniprot/SWISSPROT;Acc:P48553]
ENSG00000157111	TMEM171	Transmembrane protein 171. [Source:Uniprot/SWISSPROT;Acc:Q8WVE6]
ENSG00000176273	TMEM20	Transmembrane protein 20. [Source:Uniprot/SWISSPROT;Acc:Q2M3R5]
ENSG00000172375	TMEM24	Transmembrane protein 24 (Protein DLNB23). [Source:Uniprot/SWISSPROT;Acc:O14523]
ENSG00000112697	TMEM30A	Cell cycle control protein 50A (Transmembrane protein 30A). [Source:Uniprot/SWISSPROT;Acc:Q9NV96]
ENSG00000123610	TNFAIP6	Tumor necrosis factor-inducible protein TSG-6 precursor (TNF- stimulated gene 6 protein) (Hyaluronate-binding protein). [Source:Uniprot/SWISSPROT;Acc:P98066]
ENSG00000120889	TNFRSF10B	Tumor necrosis factor receptor superfamily member 10B precursor (Death receptor 5) (TNF-related apoptosis-inducing ligand receptor 2) (TRAIL receptor 2) (TRAIL-R2) (CD262 antigen). [Source:Uniprot/SWISSPROT;Acc:O14763]
ENSG00000173530	TNFRSF10D	Tumor necrosis factor receptor superfamily member 10D precursor (Decoy receptor 2) (DcR2) (TNF-related apoptosis-inducing ligand receptor 4) (TRAIL receptor 4) (TRAIL-R4) (TRAIL receptor with a truncated death domain) (CD264 antigen). [Source:Uniprot/SWISSPROT;Acc:Q9UBN6]
ENSG00000006327	TNFRSF12A	Tumor necrosis factor receptor superfamily member 12A precursor (Fibroblast growth factor-inducible immediate-early response protein 14) (FGF-inducible 14) (Tweak-receptor) (TweakR) (CD266 antigen). [Source:Uniprot/SWISSPROT;Acc:Q9NP84]
ENSG00000107854	TNKS2	Tankyrase-2 (EC 2.4.2.30) (TANK2) (Tankyrase II) (TNKS-2) (TRF1- interacting ankyrin-related ADP-ribose polymerase 2) (Tankyrase-like protein) (Tankyrase-related protein). [Source:Uniprot/SWISSPROT;Acc:Q9H2K2]
ENSG00000100354	TNRC6B	Trinucleotide repeat-containing 6B protein. [Source:Uniprot/SWISSPROT;Acc:Q9UPQ9]
ENSG00000136205	TNS3	tensin-like SH2 domain containing 1 [Source:RefSeq_peptide;Acc:NP_073585]
ENSG00000141198	TOM1L1	TOM1-like 1 protein (Target of myb-like 1 protein) (Src-activating and signaling molecule protein). [Source:Uniprot/SWISSPROT;Acc:O75674]
ENSG00000169905	TOR1AIP2	Torsin-1A-interacting protein 2 (Lumenal domain-like LAP1). [Source:Uniprot/SWISSPROT;Acc:Q8NFQ8]
ENSG00000198846	TOX_HUMAN	Thymus high mobility group box protein TOX. [Source:Uniprot/SWISSPROT;Acc:O94900]
ENSG00000172315	TP53RK	TP53-regulating kinase (EC 2.7.11.1) (p53-related protein kinase) (Nori-2). [Source:Uniprot/SWISSPROT;Acc:Q96S44]
ENSG00000133112	TPT1	Translationally-controlled tumor protein (TCTP) (p23) (Histamine- releasing factor) (HRF) (Fortilin). [Source:Uniprot/SWISSPROT;Acc:P13693]
ENSG00000144935	TRPC1	Short transient receptor potential channel 1 (TrpC1) (TRP-1 protein). [Source:Uniprot/SWISSPROT;Acc:P48995]
ENSG00000083067	TRPM3	Transient receptor potential cation channel subfamily M member 3 (Long transient receptor potential channel 3) (LTrpC3) (Melastatin-2) (MLSN2). [Source:Uniprot/SWISSPROT;Acc:Q9HCF6]

ENSG00000167723	TRPV3	Transient receptor potential cation channel subfamily V member 3 (TrpV3) (Vanilloid receptor-like 3) (VRL-3). [Source:Uniprot/SWISSPROT;Acc:Q8NET8]
ENSG00000106025	TSPAN12	Tetraspanin-12 (Tspan-12) (Transmembrane 4 superfamily member 12) (Tetraspan NET-2). [Source:Uniprot/SWISSPROT;Acc:O95859]
ENSG00000126216	TUBGCP3	Gamma-tubulin complex component 3 (GCP-3) (Spindle pole body protein Spc98 homolog) (hSpc98) (hGCP3) (h104p). [Source:Uniprot/SWISSPROT;Acc:Q96CW5]
ENSG00000122691	TWIST1	Twist-related protein 1 (H-twist). [Source:Uniprot/SWISSPROT;Acc:Q15672]
ENSG00000128791	TWSG1	twisted gastrulation [Source:RefSeq_peptide;Acc:NP_065699]
ENSG00000136810	TXN	Thioredoxin (Trx) (ATL-derived factor) (ADF) (Surface-associated sulphydryl protein) (SASP). [Source:Uniprot/SWISSPROT;Acc:P10599]
ENSG00000108010	TXNL2	Thioredoxin-like protein 2 (PKC-interacting cousin of thioredoxin) (PKC-theta-interacting protein) (PKCq-interacting protein). [Source:Uniprot/SWISSPROT;Acc:O76003]
ENSG00000137831	UACA	Uveal autoantigen with coiled-coil domains and ankyrin repeats protein. [Source:Uniprot/SWISSPROT;Acc:Q9BZF9]
ENSG00000143569	UBAP2L	Ubiquitin-associated protein 2-like (Protein NICE-4). [Source:Uniprot/SWISSPROT;Acc:Q14157]
ENSG00000033178	UBE1L2	ubiquitin-activating enzyme E1-like 2 [Source:RefSeq_peptide;Acc:NP_060697]
ENSG00000109332	UBE2D3	Ubiquitin-conjugating enzyme E2 D3 (EC 6.3.2.19) (Ubiquitin-protein ligase D3) (Ubiquitin carrier protein D3) (Ubiquitin-conjugating enzyme E2-17 kDa 3) (E2(17)KB 3). [Source:Uniprot/SWISSPROT;Acc:P61077]
ENSG00000184787	UBE2G2	Ubiquitin-conjugating enzyme E2 G2 (EC 6.3.2.19) (Ubiquitin-protein ligase G2) (Ubiquitin carrier protein G2). [Source:Uniprot/SWISSPROT;Acc:P60604]
ENSG00000169139	UBE2V2	Ubiquitin-conjugating enzyme E2 variant 2 (MMS2) (Enterocyte differentiation-associated factor EDAF-1) (Enterocyte differentiation- promoting factor) (EDPF-1) (Vitamin D3-inducible protein) (DDVit 1). [Source:Uniprot/SWISSPROT;Acc:Q15819]
ENSG00000009335	UBE3C	Ubiquitin-protein ligase E3C (EC 6.3.2.-). [Source:Uniprot/SWISSPROT;Acc:Q15386]
ENSG00000122042	UBL3	Ubiquitin-like protein 3 precursor (Membrane-anchored ubiquitin-fold protein) (MUB) (HsMUB) (Protein HCG-1). [Source:Uniprot/SWISSPROT;Acc:O95164]
ENSG00000198258	UBL5	Ubiquitin-like protein 5. [Source:Uniprot/SWISSPROT;Acc:Q9BZL1]
ENSG00000120686	UFM1	Ubiquitin-fold modifier 1 precursor. [Source:Uniprot/SWISSPROT;Acc:P61960]
ENSG00000156467	UQCRB	Ubiquinol-cytochrome c reductase complex 14 kDa protein (EC 1.10.2.2) (Complex III subunit VI) (QP-C). [Source:Uniprot/SWISSPROT;Acc:P14927]
ENSG00000006611	USH1C	Harmonin (Usher syndrome type-1C protein) (Autoimmune enteropathy- related antigen AIE-75) (Antigen NY-CO-38/NY-CO-37) (PDZ-73 protein) (NY-REN-3 antigen). [Source:Uniprot/SWISSPROT;Acc:Q9Y6N9]
ENSG00000135655	USP15	Ubiquitin carboxyl-terminal hydrolase 15 (EC 3.1.2.15) (Ubiquitin thioesterase 15) (Ubiquitin-specific-processing protease 15) (Deubiquitinating enzyme 15) (Unph-2) (Unph4). [Source:Uniprot/SWISSPROT;Acc:Q9Y4E8]
ENSG00000162402	USP24	Ubiquitin carboxyl-terminal hydrolase 24 (EC 3.1.2.15) (Ubiquitin thioesterase 24) (Ubiquitin-specific-processing protease 24) (Deubiquitinating enzyme 24). [Source:Uniprot/SWISSPROT;Acc:Q9UPU5]
ENSG00000055483	USP36	Ubiquitin carboxyl-terminal hydrolase 36 (EC 3.1.2.15) (Ubiquitin thioesterase 36) (Ubiquitin-specific-processing protease 36) (Deubiquitinating enzyme 36). [Source:Uniprot/SWISSPROT;Acc:Q9P275]
ENSG00000135913	USP37	Ubiquitin carboxyl-terminal hydrolase 37 (EC 3.1.2.15) (Ubiquitin thioesterase 37) (Ubiquitin-specific-processing protease 37) (Deubiquitinating enzyme 37). [Source:Uniprot/SWISSPROT;Acc:Q86T82]
ENSG00000071246	VASH1	Vasohibin-1. [Source:Uniprot/SWISSPROT;Acc:Q7L8A9]
ENSG00000112715	VEGFA	Vascular endothelial growth factor A precursor (VEGF-A) (Vascular permeability factor) (VPF). [Source:Uniprot/SWISSPROT;Acc:P15692]
ENSG00000092820	VIL2	Ezrin (p81) (Cytovillin) (Villin-2). [Source:Uniprot/SWISSPROT;Acc:P15311]
ENSG00000100987	VSX1	Visual system homeobox 1 (Transcription factor VSX1) (Retinal inner nuclear layer homeobox protein) (Homeodomain protein RINX). [Source:Uniprot/SWISSPROT;Acc:Q9NZR4]
ENSG00000116874	WARS2	Tryptophanyl-tRNA synthetase, mitochondrial precursor (EC 6.1.1.2) (Tryptophan--tRNA ligase) (TrpRS) ((Mt)TrpRS). [Source:Uniprot/SWISSPROT;Acc:Q9UGM6]
ENSG00000163625	WDFY3	WD repeat and FYVE domain-containing protein 3 (Autophagy-linked FYVE protein) (Alfy). [Source:Uniprot/SWISSPROT;Acc:Q8IZQ1]
ENSG00000162923	WDR26	WD repeat protein 26. [Source:Uniprot/SWISSPROT;Acc:Q9H7D7]
ENSG00000147548	WHSC1L1	Histone-lysine N-methyltransferase NSD3 (EC 2.1.1.43) (Nuclear SET domain-containing protein 3) (WHSC1-like protein 1) (Wolf-Hirschhorn syndrome candidate 1-like protein 1) (Whistle) (WHSC1-like 1 isoform 9 with methyltransferase activity to lysine). [Source:Uniprot/SWISSPROT;Acc:Q9BZ95]
ENSG00000104415	WISP1	WNT1-inducible signaling pathway protein 1 precursor (WISP-1) (Wnt-1- induced secreted protein). [Source:Uniprot/SWISSPROT;Acc:O95388]
ENSG00000124535	WRNIP1	ATPase WRNIP1 (Werner helicase-interacting protein 1). [Source:Uniprot/SWISSPROT;Acc:Q96S55]
ENSG00000151718	WWC2	WW, C2 and coiled-coil domain containing 2 [Source:RefSeq_peptide;Acc:NP_079225]
ENSG00000047597	XK	Membrane transport protein XK (Kx antigen) (Kell complex 37 kDa component) (XK-related protein 1). [Source:Uniprot/SWISSPROT;Acc:P51811]
ENSG00000015153	YAF2	YY1-associated factor 2. [Source:Uniprot/SWISSPROT;Acc:Q8IY57]
ENSG00000181704	YIPF6	Protein YIPF6 (YIP1 family member 6). [Source:Uniprot/SWISSPROT;Acc:Q96EC8]
ENSG00000166860	ZBTB39	Zinc finger and BTB domain-containing protein 39. [Source:Uniprot/SWISSPROT;Acc:O15060]
ENSG00000149289	ZC3H12C	
ENSG00000123200	ZC3H13	Zinc finger CCCH domain-containing protein 13. [Source:Uniprot/SWISSPROT;Acc:Q5T200]
ENSG00000177764	ZCCHC3	Zinc finger CCHC domain-containing protein 3. [Source:Uniprot/SWISSPROT;Acc:Q9NUD5]
ENSG00000153786	ZDHHC7	Palmitoyltransferase ZDHHC7 (EC 2.3.1.-) (Zinc finger DHHC domain- containing protein 7) (DHHC-7) (Zinc finger protein 370). [Source:Uniprot/SWISSPROT;Acc:Q9NXF8]
ENSG00000180787	ZFP3	Zinc finger protein 3 homolog (Zfp-3). [Source:Uniprot/SWISSPROT;Acc:Q96NJ6]
ENSG00000121741	ZMYM2	Zinc finger protein 198 (Zinc finger MYM-type protein 2) (Fused in myeloproliferative disorders protein) (Rearranged in atypical myeloproliferative disorder protein). [Source:Uniprot/SWISSPROT;Acc:Q9UBW7]
ENSG00000186448	ZNF197	Zinc finger protein 197 (ZnF20). [Source:Uniprot/SWISSPROT;Acc:O14709]
ENSG00000197657	ZNF323	Zinc finger protein 323. [Source:Uniprot/SWISSPROT;Acc:Q96LW9]
ENSG00000074657	ZNF532	zinc finger protein 532 [Source:RefSeq_peptide;Acc:NP_060651]
ENSG00000198453	ZNF568	zinc finger protein 568 [Source:RefSeq_peptide;Acc:NP_940941]
ENSG00000198740	ZNF652	zinc finger protein 652 [Source:RefSeq_peptide;Acc:NP_055712]

All genes listed in the table are differentially regulated in human CRC stage 4 adenocarcinomas compared to adenomas and contain an ARE motif in the 3'UTR. Presence of an ARE (AU-rich element) was determined by analysis using the ARE database, ARED3.0 (Bakheet et al., 2006).

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