Journal of Cellular Biochemistry 97:1161–1174 (2006)

PROSPECTS

Tissue Fibrosis and Carcinogenesis: Divergent or Successive Pathways Dictate Multiple Molecular Therapeutic Targets for Oligo Decoy Therapies

Kenneth R. Cutroneo,[1]* Sheryl L. White,[2] Jen-Fu Chiu,[3] and H. Paul Ehrlich[4]

[1]Department of Biochemistry, College of Medicine, 89 Beaumont Avenue, University of Vermont, Burlington, Vermont 05405
[2]Departments of Anatomy and Neurobiology, College of Medicine, University of Vermont, Burlington, Vermont 05405
[3]Department of Anatomy, University of Hong Kong, School of Medicine, Hong Kong, Peoples Republic of China
[4]Division of Plastic Surgery, Penn State University College of Medicine, Hershey, Pennsylvania 17033

Abstract The extracellular matrix (ECM) is composed of several families of macromolecular components: fibrous proteins such as collagens, type I collagen (COL1), type III collagen (COL3), fibronectin, elastin, and glycoconjugates such as proteoglycans and matrix glycoproteins. Their receptors on the cell membrane, most of which in the case of the ECM belong to the integrins, which are heterodimeric proteins composed of α and β chains. COL1 is the major fibrous collagen of bone, tendon, and skin; while COL3 is the more pliable collagen of organs like liver. Focus will not only be given to the regulation of synthesis of several fibrogenic parameters but also modulation of their degradation during growth factor-induced tissue fibrosis and cancer development. Evidence will be provided that certain tissues, which undergo fibrosis, also become cancerous. Why does there exist a divergency between tissues, which undergo frank fibrosis as an endpoint, and those tissues that undergo fibrosis and subsequently are susceptible to carcinogenicity; resulting from the etiological factor(s) causing the initial injury? For example, why does a polyvinyl alcohol (PVA) sponge implant become encapsulated and filled with fibrous tissue then fibrosis tissue growth stops? Why does the subcutaneous injection of a fibrogenic growth factor cause a benign growth and incisional wounding results in fibrosis and ultimately scarring? There are many examples of tissues, which undergo fibrosis as a prerequisite to carcinogenesis. Is there a cause-effect relationship? If you block tissue fibrosis in these precancerous tissues, would you block cancer formation? What are the molecular targets for blocking fibrosis and ultimately carcinogenesis? How can oligo decoys may be used to attenuate carcinogenesis and which oligo decoys specifically attenuate fibrogenesis as a prelude to carcinogenesis? What are other molecular targets for oligo decoy therapy in carcinogenesis? J. Cell. Biochem. 97: 1161–1174, 2006.

Key words: fibrosis; cancer; oligo decoys

TISSUE FIBROSIS AND CARCINOGENESIS

Figure 1 shows the fibrosis pathway involving the initial tissue injury due to an etiological factor(s). For example infection is followed by inflammation, angiogenesis, proliferation of fibroblasts, the transition of fibroblasts into myofibroblasts, the migration of smooth muscle cells into the injured area, and an increase in ECM synthesis. A decrease in ECM degradation results in increased ECM deposition, which are orchestrated by numerous profibrotic growth factors including transforming growth factor-β (TGF-β1), connective tissue growth factor (CTGF), insulin-like growth factor (IGF), and platelet-derived growth factor (PDGF).

Fibrosis is common in the establishment of benign tumors and cancers. What is the biological signal(s) to divert a benign outcome to cancer? In spontaneous mouse C_3H tumors [Cutroneo et al., 1972], there are increases of both prolyl hydroxylase (PH) activity and collagen synthesis. TGF-β1 is the most predominant profibrotic

*Correspondence to: Kenneth R. Cutroneo, Department of Biochemistry, College of Medicine, 89 Beaumont Avenue, University of Vermont, Burlington, VT 05405.
E-mail: kenneth.cutroneo@uvm.edu

Received 7 November 2005; Accepted 10 November 2005
DOI 10.1002/jcb.20750

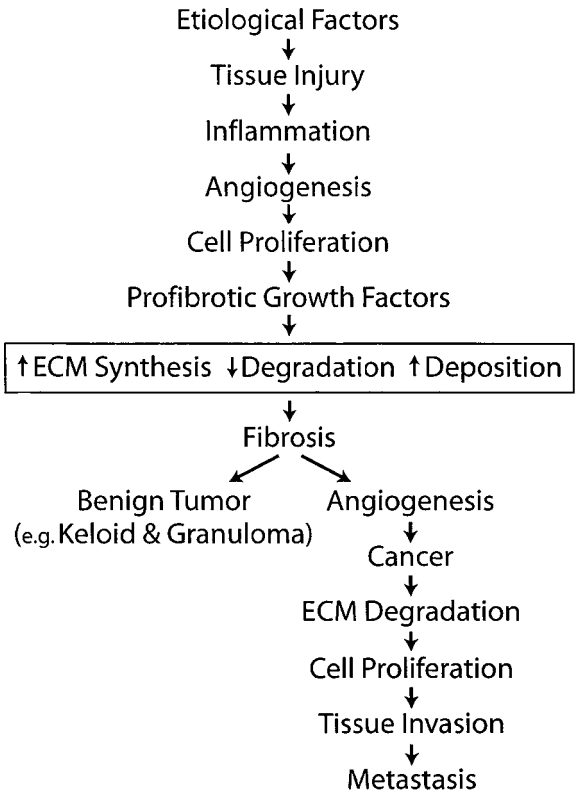

Fig. 1. Schematic diagram of the biochemical and molecular mechanisms of some tissues to form benign fibrous tumors and divergent stages of other tissues to undergo carcinogenesis.

growth factor, causing tissue fibrosis as well as both pro-apoptotic signals and survival signals depending on the type of cancer cell.

A recent review reports various transacting factors, which bind to the promoter(s) of the type I collagen α (*COL1A1*) and/or the *COL1A2* genes that activate the transcription of the gene(s) as molecular targets for oligo decoy gene therapy [Cutroneo, 2005]. Nuclear factor-kappa B (NF-KB) decoy therapy has led to the amelioration of collagen-induced arthritis in rats [Tomita et al., 1999]. ODN (oligo deoxynucleotide) decoy treatment has also proven to be a novel therapeutic in various cardiovascular diseases [Morishita et al., 2001; Kume et al., 2002; Tomita et al., 2003]. Phosphorothioate ODNs containing the TGF-β element found in the distal promoter of the *COL1A1* gene are demonstrated as novel nonsteroidal antifibrotics to inhibit the early progression of schistosomiasis liver fibrosis [Cutroneo and Boros, 2002; Boros et al., 2005].

TGF-β AND SMAD MECHANISMS OF SIGNAL TRANSDUCING GENE EXPRESSION AND CARCINOGENESIS

A comprehensive overview of TGF-β/SMAD signaling pathway is presented diagrammatically in Figure 2. Active TGF-β binds to the TGF-βII receptor that causes activation of the TGF-βI receptor's intrinsic serine threonine activity. As a result Smad 2 and Smad 3 are phosphorylated. These phosphorylated Smads then form a heterometric complex with the unphosphorylated Co-Smad 4. This activated complex translocates into the nucleus, where in combination with certain co-activators turn on TGF-β/SMAD sensitive genes. The Smad 6, 7, and 8 are Smad proteins that feedback and inhibit the TGF-β/SMAD signaling pathway.

Focus will be on the regulation of Smads in relation to carcinogenesis and the regulation of these transcription factor proteins by TGF-β treatment. TGF-β plays a key role in breast cell carcinogenesis, where interfering with TGF-β-induced Smad 3 nuclear accumulation suppresses certain genes involved in tumor invasiveness [Lindemann et al., 2003]. In pancreatic carcinomas, there is a strong correlation between defects in Co-Smad 4 and malignancy [Ramachandra et al., 2002]. In malignant choriocarcinoma cells, there is a resistance to TGF-β signaling and a loss of Smad 3 expression [Xu et al., 2002]. The loss of Smad 3 expression also increases tumorigenicity in human gastric cancers and these tumors

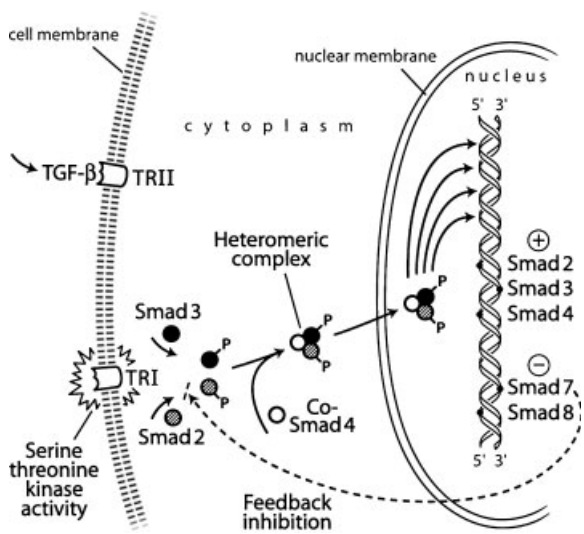

Fig. 2. The TGF-β1/SMAD signaling pathway. Positive (+) and negative (−) Smad protein transcription factor regulators.

do not respond to growth inhibition by TGF-β1 treatment [Han et al., 2004]. However, when Smad 3 is introduced into gastric cancer cells, the responsiveness to TGF-β1 is restored. The overall intact SMAD signaling pathway appears necessary for TGF-β inhibition of the expression of oncogenic genes and the arrest of tumor cell growth [Nicolas and Hill, 2003]. However, in another study, the TGF-β/SMAD signaling pathway was shown functional in primary human ovarian cancer cells [Dunfield et al., 2002]. Therefore, if abnormalities in ovarian cancer cells' response to TGF-β signaling exist, they must be downstream from the Smad proteins. As an example, the loss of c-Myc repression in ovarian cancer is related to TGF-β growth arrest independent of the TGF-β/SMAD signaling pathway [Baldwin et al., 2003].

A prelude to metastasis that is a multi-step process involves local tumor cell tissue invasion and the establishment of cancerous cell invasion at distant sites. Activated Smad 2 and H-ras become elevated when a differentiated squamous carcinoma changes from an epithelial cell type to a migratory mesenchymal tumor cell [Oft et al., 2002]. These findings pinpoint molecular targets for gene therapy in the prevention of tumor metastasis. A dominant negative Smad 2 mutation results in TGF-β resistance in a human carcinoma cell line [Tsang et al., 2002]. The defective Smad 2 protein is not phosphorylated in response to TGF-β1, shows a decrease in forming a complex with Smad 3 and does not associate with Co-Smad 4. Smad 2 is mutated in the G2 human hepatoma cell line which is responsible for its cancerous phenotype [Dumont et al., 2003]. The mutant Smad 2 avoids ubiquitination and proteosome degradation and thus leads to disregulated cell proliferation during tumorigenesis. Generally mutations in the Smad proteins produce a lack of TGF-β signaling tumor growth arrest. Mutations of Smad 2 and Co-4 are common features in human cervical cancers [Maliekal et al., 2003]. There is a divergent effect of TGF-β 2/3 SMAD signaling in various breast cancer cell lines [Tian et al., 2003]. A reduction of Smad 2/3 signaling enhances tumorigenesis.

Depending on the hepatoma cells species, the TGF-β1 effects on cell-cycle arrest and apoptosis are different [Buenemann et al., 2001]. There is also cross-talk with the cellular stress death pathway, which ultimately leads to apoptosis [Kim et al., 2004]. Activin receptor-like kinase-7 induces apoptosis through activation of the TGF-β pathway components that results in the transcription of JNK and p38 activation. Smad 7, an inhibitory Smad, is a member of one of the super families of Smad proteins. The JNK cascade is essential for the potentiation of apoptosis by Smad 7 [Mazars et al., 2001]. TGF-β involvement with Smad 7 plays an active role in causing apoptosis in prostatic carcinoma cells [Landstrom et al., 2000]. Inhibiting Smad 7 expression by ODN antisense in several cell lines eliminated TGF-β1 induction of apoptosis. TGF-β1-mediated prostate cancer cell apoptosis through Smad 7 is observed in several cell lines. The specific activation of the p38 mitogen-activated protein kinase pathway is by TGF-β-activated kinase and mitogen-activated protein kinase 3 [Edlund et al., 2003]. TGF-β1 signaling is blunted by mutant Co-Smad 4 in SNU-216 gastric cancer cells, in which TGF-β1 does not cause an upregulation of TGF-β receptors [Ju et al., 2003]. In pancreatic carcinomas, TGF-β tumor suppressor activity is lost by an inactivation of Co-Smad 4, although Co-Smad 4-deficient cancer cells are partially driven by endogenous TGF-β signaling [Subramanian et al., 2004]. Therefore, TGF-β receptor RI may be a novel therapeutic target for this cancer. Co-Smad 4 deletion in pancreatic adenocarcinoma cells results in tumor invasion. Gene therapy for the restoration of Co-Smad 4 is a powerful tool to reverse this invasive phenotype [Duda et al., 2003]. The expression of Smad 7 in hepatocellular carcinomas (HCC) acts as a resistant mechanism to increased TGF-β1 in late stage hepatocarcinogenesis in advanced HCC without reducing TGF-βII receptors [Park et al., 2004]. Co-Smad 4 expression in stellate cells of HCCs is involved in the host's resistance to hepatocarcinogenesis.

In cell lines derived from thyroid carcinoma, Co-Smad 4 expression is deleted. In addition, Smad 7, the inhibitory Smad protein, blocks the receptors and interrupts the phosphorylation of Smads 2/3. The inhibition of the TGF-β response to Smad 7 overexpression may be a mechanism for tumor aggressiveness observed in undifferentiated thyroid tumors [Cerutti et al., 2003]. A Smad 8 gene alteration detected in a third of breast and colon cancers makes this inhibitory Smad protein a novel tumor marker as well as a potential therapeutic target [Cheng et al., 2004]. Epigenetic silencing of Smad 8

expression by promoter DNA hypermethylation in these cancers directly correlates with a loss of Smad 8 expression.

INFLAMMATION, FIBROSIS, AND CARCINOGENESIS

The inflammatory response to liver injury caused by hepatitis type C and/or B viral infection leads to liver cirrhosis and eventually to HCC [Cutroneo and Chiu, 2003]. This inflammatory response is associated with an increase of the inducible cycloenoxygenase-2 (COX-2) enzyme, which catalyzes the conversion of arachidonic acid to prostaglandins. These lipids inhibit apoptosis and promote tumor growth by increasing neoangiogenesis. The COX-2 enzyme is elevated in various tumors (Table I). Non-steroidal anti-inflammatory therapeutics, for example COX-2 inhibitors, are potent anti-carcinogenic agents (Table II). Prostaglandins are reduced in pulmonary fibrosis due to a decrease expression of the COX-2 enzyme by fibroblasts [Wilborn et al., 1995; Petkova et al., 2003], suggesting the utility of exogenous prostaglandins to suppress the fibrotic response of lung fibrosis.

Increased collagen synthesis is associated with spontaneous mouse mammary gland neoplasms [Cutroneo et al., 1972] and a cloned mouse mammary tumor cell line [Roesel et al., 1978]. PH activity has also been shown to be increased in Reuber hepatomas as compared to normal livers [Cutroneo and Scott, 1979]. Rats with liver cirrhosis that were fed an inhibitor of PH showed reduced fibrosis but the development of neoplasms [Sakaida et al., 1994].

Fibrosis is promoted by an immune response [Vaage, 1992]. The fibrous capsule may halt tumor growth, resulting in the development of a benign tumor, which is shielded from systemic immune mechanisms [Vaage, 1992a,b]. In human breast cancer, fibrotic foci are found in some intraductal carcinomas but not in others [Hasebe et al., 1998]. Fibrotic foci are associated with high tumor aggressiveness. Intraductal carcinomas with diffuse fibrosis are associated with a myoepithelial immunophenotype carcinoma cells [Tamiolakis et al., 2002]. Fibrotic foci in invasive breast carcinoma correlate with carbonic anhydrase, hypoxia, and hypoxia inducible factor (HIF)-driven angiogenesis [Colpaert et al., 2003]. Fibrotic foci may be an indicator of tumor angiogenesis and an independent indicator of early metastasis in lymph node negative breast cancer patients [Colpaert et al., 2001]. However, in another inflammatory breast cancer report, there was a lack of angiogenesis and fibrosis [Shirakawa et al., 2001].

TGF-β-mediated growth inhibition of metastatic mammary carcinoma cells is observed. On the other hand, TGF-β signaling through the SMAD pathway is required for tumor invasion and metastasis [McEarchern et al., 2001]. Both neoplastic and stromal cells of lung adenocarcinomas with stromal fibrosis are invasive cancers with metastatic activity that show the expression of MMP-7 (gelatinase A) and tissue inhibitor of metalloproteinase-2 (TIMP-2) [Kitamura et al., 1999]. A section on matrix metalloproteinases (MMPs), TIMPs, and carcinogenesis will be presented later.

A strong correlation was found between pancreatic fibrosis and pancreatic cancer [for rev. see Menke and Adler, 2002]. The expression and occurrence of fibrotic foci in advanced pancreatic ductal cancers correlates closely to

TABLE I. The Cox-2 Enzyme in Various Cancer Types

Cancer type	References
Pancreatic	Kokawa et al., Cancer 91:333 (2001)
Esophageal	Shirvani et al., Gastroenterol. 118:487 (2000)
Bladder	Yoshimura et al., J. Urology 165:1468 (2001)
Gastric	Ohno et al., Cancer 91:1876 (2001)
Lung	Brabender et al., Ann. Surgery 235:440 (2002)
Colon	Cianchi et al., Gastroenterol. 121:1339 (2001)
Breast	Soslow et al., Cancer 89:2637 (2000)
Head and neck squamous cell carcinomas	Jaeckel et al., Arch. Otolaryngology—Head & Neck Surg. 127:1253 (2001)
Prostate	Yoshimura et al., Cancer 89:589 (2000)
Retinoblastoma	Karim et al., J. Ophthalmol. 129:398 (2000)
Thyroid	Nose et al., Am. J. Clin. Pathol. 117:546 (2002)
Ovarian	Denkert et al., Am. J. Pathol. 160:893 (2002)
Cervix	Sales et al., J. Clin. Endocrinol & Metab. 86:2243 (2001)
Endometrial	Ferrandina et al., Cancer 95:801 (2002)

TABLE II. The Effect of Nonsteroid Drugs as Cox-2 Inhibitors on Various Cancer

Cancer type	Nonsteroidal anti-flammatory drug	References
Hepatocellular carcinoma	NS-398	Cheng et al., Int. J. Cancer 99:755 (2002)
Colon	Celecoxib	Reddy et al., Cancer Res. 60:293 (2000)
Breast	SC-236	Connolly et al., Brit. J. Cancer 87:231 (2002)
Lung	Nimesulide	Hida et al., Clin. Cancer Res. 6:2006 (2000)
Skin	Celecoxib	Orengo et al., Arch. Dermatol. 138:751 (2002)
Prostate	NS-398	Liu et al., J. Urology 164:820 (2000)
Leukemia	NS-398, Nabumetone	Nakanishi et al., Eur. J. Cancer 37:1570 (2001)
Tongue	Nimesulide	Shiotani et al., Cancer Res. 61:1451 (2001)
Oral	Celecoxib	Wang et al., Larybgnscope 112:839 (2002)
Bladder	Celecoxib	Grubbs et al., Cancer Res. 60:5599 (2000)
Neuroectodermal	Celecoxib	Patti et al., Cancer Lett. 189:13 (2002)
Brain	SC-236	Portnow et al., Neuro-Oncol. 4:22 (2002)

poor patient survival. Proteinase-activator receptor-2 expression is presumably related to pancreatic fibrosis and cancer cell invasion of the pancreas [Ikeda et al., 2003].

TGF-β1 is secreted by gastric precancer cells, which causes an increase of collagen production by fibroblasts and the transition of fibroblasts into myofibroblasts [Mahara et al., 1994]. Therefore, a close correlation of fibrosis of various tissues and their potential oncological transformation exists. The induction of fibrosis by profibrotic growth factors (Fig. 1) and most importantly by TGF-β1, can produce apoptosis, cause ECM encapsulation, and the development of a benign tumor or promote cancerous under the control of increased HIF-1 or possibly other factors.

GROWTH FACTORS AND A TRANSCRIPTION FACTOR INVOLVED IN CARCINOGENESIS

Benign prostatic hyperplasia is characterized by proliferation of both epithelial cells and fibroblasts. Fibroblast growth factors (FGF)-2, 7, 9, and 17 [Polnaszek et al., 2004] are expressed in this hyperplastic tissue. There may be a cause-effect relationship between increased FGF-17 and increased epithelial cell proliferation. Is there another factor that is a marker for the benign or the malignant state? Progranulin, a novel autocrine growth factor, is not expressed in benign breast tissue but is expressed in human breast cancers, in which it correlates with the clinicopathological parameters of tumor grade, proliferation index, and to altered p53 expression [Serrero and Ioffe, 2003]. Progranulin offers a potential therapeutic target for gene therapy of human breast cancers.

Cancers thrive in an acidic, hypoxic stress environment, where oxygen tension and glucose levels are low. HIFs are activated in response to hypoxia because of reduced activity of a new class of 2-oxoglutarate dependent oxygenases presumably located in nuclei. Activation of HIFs occurs in various neoplasms. HIFs induce tumor growth and metabolism through maintaining angiogenesis, anaerobic glycolysis, an acidic pH microenvironment, cellular proliferation, differentiation, cell viability, apoptosis, and regulating ECM metabolism. The HIF pathway is a new target(s) for an understanding the oncological state of cells in culture or cancerous tissues in vivo.

The transcription of HIF-1 α and β subunits, the transcription of which is regulated by 3′ hypoxia-responsive elements, α and β, which are found in an enhancer region of the hypoxia-inducible genes like erythropoietin. This glycoprotein hormone, which is expressed in fetal liver and in adult kidney, is involved in red blood cell production [Percy et al., 1997]. The hypoxia-inducible genes are critically dependent on HIF-1 in endothelial and breast cancer cells [Sowter et al., 2003]. Increased expression of erythropoietin and its receptor play a significant role in cervical carcinogenesis, tumor progression, and aggressiveness [Acs et al., 2003]. In endometrial carcinomas, erythropoietin expression is a prognostic and predictive factor of tumor progression and increased aggressiveness [Acs et al., 2004]. Phosphorylation of HIF-1 stabilizes this transcription factor. SiRNA inhibition of mitogen-activated protein kinase phosphatase-1 expression enhances HIF-1 alpha phosphorylation. This increases the transcription of active HIF-1 as well as induces erythropoietin expression [Liu et al., 2003]. Stabilization of

HIF-1 promotes hypoxia-induced gene expression. HIF-1 is destabilized by hydroxylation of the proline residue-564. Prolyl hydroxylation is inhibited by hypoxia and ferrous iron chelators such as desferrioxamine [Chan et al., 2002]. An intriguing paradox is that during tumorigenesis HIF-1 transactivates many genes involved with tumor growth pathways, which were discussed earlier. However, HIF-1 also upregulates antiproliferative and pro-apoptotic genes [Bacon and Harris, 2004]. A molecular mechanism(s) for these divergent functions may provide a better understanding of the progression of cancer and the development of cancer therapeutics. Perhaps the paradox lies in HIF's actions from different cell types.

An analysis of the promoter region of PH α-1 identified a similar motif to the hypoxia-responsive element found in hypoxia-inducible genes which is within a 120-base pair sequence upstream from the start site of the transcription of the *PHα-1* gene. Under hypoxia the HIF-1 is stimulated and bound to the PH α-1 hypoxia response element [Takahashi et al., 2000]. Therefore, PHα-1 is not only essential for collagen synthesis but is also a target gene for HIF-1. Under oxygen, HIF-α subunits of the HIF transcriptional complex are rapidly destroyed by ubiquitylation via the von Hippel-Lindau tumor suppressor ligase complex and the proteosome pathway. This degradation process is inhibited by hypoxia and iron chelators and is enhanced by HIF-prolyl hydroxylation [Jaakkola et al., 2001]. Since there is an absolute requirement for molecular oxygen and the cofactor iron, HIF-PH is a functional cellular oxygen sensor. Cells respond to a mild decline in oxygen tension by undergoing an anaerobic state of respiration and generating lactic acid. The decrease in environmental pH triggers sequestration of von Hipple-Lindau tumor suppressor gene into the nucleolus thereby neutralizing its ability to degrade HIF-1 [Mekhail et al., 2004a,b]. In contrast to these findings, it has been shown that a downregulation of HIF-1 may be obtained by F0X0 4, a member of the forkhead transcription factor super family independent of the von Hipple-Lindau pathway [Tang and Lasky, 2003]. Recent studies suggest that small molecular inhibitors for each class of PHs are possible for individual HIFs [Hirsila et al., 2003]. Inhibitors of HIF-PH may be used as model compounds for the downstream targets, vascular endothelial growth factor (VEGF), and other hypoxia-inducible genes [Ivan et al., 2002]. This would induce adaptive responses to hypoxia, including angiogenesis [Warnecke et al., 2003] and red blood cell production.

There are nuclear factors, hormones, vitamins, and pharmacological agents that regulate HIFs. HIF-1 regulation of erythropoietin production is under the control of NF-KB [Figueroa et al., 2002]. Histone deacetylase 7 is a transcription activator of HIF-1-α [Jeong et al., 2002; Kato et al., 2004]. Vitamin A, an antioxidant, increases HIF-1-α mRNA levels and specifically stimulates erythropoientin production [Jelkmann et al., 1997]. Hormones and therapeutics also have the potential to regulate the levels of HIFs. Estradiol-17β attenuates HIF-1-α induction and erythropoietion production in human hepatoma cells by interfering with the hypoxia increase of HIF-1-α through an estrogen receptor-dependent mechanism [Mukundan et al., 2004]. PH activity is also increased in rat uterus by estradiol-17β treatment [Salvador et al., 1976]. Bleomycin, a glycopeptide anti-neoplastic agent, increases PH activity in human lung fibroblasts and is associated with an increased collagen synthesis and lung fibrosis. Presently this anticancer drug is believed to act through increased DNA breakage, apoptosis, and decreased cancer cell proliferation. It is possible to speculate that an increase of PH in cancer cells in susceptible tissues, for example the testes, could cause HIF-1-564 hydroxylation, which would result in a decreased expression of hypoxia-inducible genes and allow for HIF-1 ubiquitinization, proteosome degradation, and the transition of a cancer cell to a normal cell.

MOLECULAR TARGETS AND GENE THERAPEUTIC STRATEGIES FOR ANTIFIBROSIS AND INHIBITION OF CARCINOGENESIS

Inhibition of the onset and progression of fibrosis [Cutroneo and Boros, 2002] reduces the development of benign tumors that can be surgically removed. Clinically, the surgical removal of keloids is followed by continuous triamcinolone treatment, a potent synthetic glucocorticoid that inhibits collagen synthesis. The review [Cutroneo, In Press] on gene therapy sites common *cis*-elements in the promoters of the *COL1A1* gene and the *COL1A2* gene to attenuate COL1 synthesis.

Phosphorothioate oligo decoys can be synthesized containing the *cis*-element, for example, the TGF-β element, the Sp1 element, the γB1 element, or others. These double-stranded oligodeoxynucleotides (dsODNs) tie up the appropriate transacting-factor and inhibit gene transcription. Smad proteins are a family of transcription factors regulating gene transcription through TGF-β signaling (Fig. 2). As discussed previously, aberrant TGF-β signaling has been associated with decreased fibrosis and carcinogenesis. Therefore, therapeutics directed at the SMAD pathway activation by TGF-β may have clinical potential. There exists crosstalk between TGF-β signaling, γB1 and Smad 3 [Cutroneo, 2005]. There are other ways to inhibit fibrosis besides consensus sequence containing dsODN decoys. One is through antisense ODNs with the AUG codon and a few bases complimentary to the mRNA coding for a specific protein including either CTGF, the immediate fibrotic growth factor expressed by TGF-β1 treatment, TGF-β1, or thrombospondin-1, which converts the latent form of TGF-β to its active form.

Other molecular targets for tumor growth inhibition following fibrosis exist. The Stat 3 containing ODNs inhibit epithelial cell carcinogenesis [Chan et al., 2004]. The CRE-ODN containing the CRE sequence (5′-TGACGTCA-3′) induce tumor growth in cells having a p53 mutation [Park et al., 2001]. In breast cancer cells, the CRE transcription factor ODN, stabilizes and activates p53 which contributes to tumor cell growth inhibition [Lee et al., 2000]. In ovarian cancer cells, CRE ODNs cause growth arrest, suppress cell invasiveness, and promote tumor cell apoptosis [Alper et al., 2001]. A 24-mer single-stranded cAMP response element ODN competes with binding transcription factors and inhibits cAMP-induced gene transcription. The cytostatic action of these ODNs may enhance the antiproliferative effects of conventional cytotoxic agents [Liu et al., 2004]. In mice, the intravenous injection of ds ODNs containing the consensus NF-KB *cis*-element inhibits hepatic metastasis of reticulosarcoma tumors [Kawamura et al., 2001]. The growth and progression of melanomas are dependent on an adequate blood supply through angiogenesis. The key factors in angiogenesis are tumor necrosis factor-alpha (TNF-α) and VEGF. DsODNs containing the consensus Sp1 *cis*-element inhibit angiogenesis and melanoma growth [Novak et al., 2003].

Alternate to control, carcinogenesis and tumor growth is signaling through TGF-β1 to the TGF-βII receptors which will cause the intrinsic serine/threokinase activity of the TGF-βI receptors to activate the SMAD signaling pathway. However, Smad 7, the inhibitory Smad, interferes with TGF-β1 activation of the SMAD signaling pathway. A Smad 3 element dsODN decoy knocks out Smad 7 gene transcription, resulting in TGF-β1 secretion and its activation by proteinases [Cutroneo and Phan, 2003]. The active TGF-β1 then autoinduces more TGF-β1. Theoretically this would maintain tissue fibrosis, the formation of a benign tumor and little ECM degradation, minimal cancer cell proliferation, no tissue invasion or metastasis (Fig. 1).

MMPs AND TIMPs: THE CANCEROUS TUMOR CAPSULE AND ECM DEGRADATION

As a prerequisite to cancer cell proliferation, tissue invasion and finally metastasis, there is a fine tuned complex process involving MMPs and TIMPs. There is a tight association amongst the activity of MMPs, the promotion of tumor invasion and metastasis in endometrial carcinomas [Di Nezza et al., 2002]. The membrane bound type 1-MMP (MT1-MMP), enzyme, which cleaves both collagen and fibrin, is required for tumor cell invasion [Holmbeck et al., 2003]. Low levels of MMP-1 and MT-MMP in advanced colorectal carcinomas are directly related to favorable survival [Bendardaf et al., 2003]. Suppressing the expression of MT1-MMP in fibroblasts or tumor cells blocks cancer cell invasiveness [Sabeh et al., 2004]. Even though other MMPs continue to be expressed, sequence-specific silencing of MT1-MMP by SiRNA suppresses tumor cell migration and invasion [Ueda et al., 2003]. In prostate cancer cells, FGF-1 induces MT1-MMP and MMP-7 which involves signaling and activation of STAT 3 [Udayakumar et al., 2002, 2004]. In human prostate carcinomas, there is an elevation of MMP-26, which is necessary for the activation of pro-MMP-9. This results in the degradation of fibronectin and type IV collagen, which promotes cancer cell invasion [Zhao et al., 2003].

Basic FGF is the major angiogenic factor in ovarian carcinomas. In these cancers, there is

an inverse relationship between tumor invasion and survival with MMP 2, MMP 9, MT1-MMP, and TIMP 2 [Davidson et al., 2002; Drew et al., 2004]. In human gliomas, VEGF simulates angiogenesis and tumor growth [Deryugina et al., 2002; Munaut et al., 2003]. MMP 19 expression in the epidermis is downregulated during transformation but disappears with neoplastic dedifferentiation [Impola et al., 2003; Pendas et al., 2004]. In squamous cell carcinoma cells, a MEK ½ inhibitor or a p38 inhibitor abolishes the induction of MMP-1 and MMP-10 by ultra-violet radiation [Ramos et al., 2004]. When injected into nude mice, MT1-MMP expressing clones form rapid tumor growth and vascularization [Sounni et al., 2002a,b]. The elevation of MT1-MMP promotes selective invasion and increases the growth of malignant melanomas [Iida et al., 2004]. One therapeutic approach to attenuate cancer growth and invasiveness would be to inhibit MT1-MMP expression. MMP-11 (stromelysin-3) expression is increased during breast carcinogenesis [Nakopoulou et al., 2002]. MMP-11 is expressed by breast tumor-associated fibroblasts [Wang and Tetu, 2002]. It appears a host-stromal protease interaction occurs during breast tumorigenesis. In human salivary gland carcinomas, an enhanced activation of pro-MMP-2, which is mediated by MT1-MMP, is implicated in invasion and metastasis of these tumors [Kayano et al., 2004]. In human gastric cancers, there is a correlation between the expression of ETS-related transcription factor E1AF and MMP-7 (matrilysin) [Yamamoto et al., 2004]. A deficiency of MMP-11 in gastric cancer cells inhibits their growth in soft agar and tumorigenicity in nude mice [Deng et al., 2005].

A disintegrin and metalloproteinase (ADAM) is a newly described family of proteinases that have metalloproteinase, disintegrin, and thrombospondin motifs. They have diverse functions such as cleavage of proteoglycans, ECM degradation, angiogenesis inhibition, tissue development, and organogenesis. The presence of ADAM 9 distinguishes cancerous tumors from solid benign pancreatic tumors [Grutzmann et al., 2004]. ADAM 10 degrades the ECM in prostate cancers resulting in cancer cell proliferation and tissue invasion. In benign tumors ADAM-10 is membrane bound and localized in secretory cells, while in cancer cells, it is localized in their nuclei. ADAM 10 is upregulated in prostate cancers by dihydrotestosterone, IGF-1, and epithelial growth factor (EGF) [McCulloch et al., 2004]. Endothelial ADAM is required for angiogenesis and thus could prove a new therapeutic target for the attenuation of development and growth progression of cancers [Trochon et al., 1998]. In liver cancers, the upregulation of both ADAM 9 and 12 is correlated with an increase of MMP 2 expression, tumor aggressiveness, and progression [Le Pabic et al., 2003]. In the urine of breast cancer patients ADAM 12, a gelatinase, is elevated, and correlates with breast cancer progression [Roy et al., 2004]. ADAM 12 is highly expressed in human glioglastomas and plays a role in cancer cell proliferation by the shedding of harpin-binding EGF [Kodama et al., 2004]. In human breast cancer positive nodes, there is an increased expression of ADAM 9 mRNA and protein as compared to node-negative cancers [O'Shea et al., 2003]. Promoter hypermethylation of the *ADAM 23* gene silences this proteinase in breast tumors [Costa et al., 2004].

At least one study reports TIMP-1 stimulates cancer cell growth [Porter et al., 2004]; while another study reports TIMP-1 inhibits gelatinolytic activity in the tumor stroma, stabilizing collagen fibrils. However, TIMP-1 does not inhibit either the malignant conversion of dysplasias into carcinomas or the development of metastasis [Rhee et al., 2004]. TIMP-1 gene transfer in vivo inhibits tumor-associated angiogenesis [Zacchigna et al., 2004]. The upregulation of mitogen-activated protein kinase phosphatase in tumors overexpressing TIMP-2 leads to dephosphorylation of p38 mitogen-activated protein kinase, inhibition of both tumor growth and angiogenesis [Feldman et al., 2004]. TIMP-3 gene therapy causes tumor suppression, has anti-invasive, anti-angiogenic, and pro-apoptic effects [Ahonen et al., 2002]. TIMP-3 increases apoptosis in melanoma cells by stabilizing death receptors and the activation of their apoptotic signaling cascade through caspase-8 [Ahonen et al., 2003]. TIMP-1 overexpression reduces pancreatic cell growth, metastasis, angiogenesis, and increases tumor apoptosis without altering MMP-2 production [Bloomston et al., 2002]. Frequent promoter hypermethylation silences the *TIMP-3* gene and represses TIMP-3 protein synthesis in pancreatic tumors [Wild et al., 2003]. In primary breast cancers, the expression of

TIMP-1 and plasminogen activator inhibitor (*PAI-1*) genes are elevated and are related to cancer development [Castello et al., 2002; Schrohl et al., 2004]. TIMP-1 inhibits apoptosis by the sequential activation of pertussis toxin-sensitive G protein, c-Src, PI 3 kinase, and Akt [Lee et al., 2003]. TIMP-2 gene therapy inhibits RAS-transformed human breast epithelial cells [Ahn et al., 2004]. TIMP-2 and TIMP-4 are potent inhibitors of MMP-26-mediated pro-MMP-9 activation in breast cancer invasion [Zhao et al., 2004]. In colorectal cancers, MMP-1 and PAI-1 correlate with poor tumor pathology [Baker and Leaper, 2003]. In contrast, TIMP-1 in these cancers is highly expressed by myofibroblasts, which is associated with the invasion of colon cancer cells [Holten-Andersen et al., 2005], indicating stromal-cancer cell interactions. In human urothelial carcinomas TIMP-1 is expressed in the cytoplasm of cancer cells and its increased expression indicates more malignancy [Yano et al., 2002]. Hypermethylation of the DNA promoter region of the *TIMP-2* gene affects carcinogenesis by silencing the expression of this gene. This increases cervical cancer progression and development [Ivanova et al., 2004]. Promoter methylation of the *TIMP-3* gene in choriocarcinomas leads to a decrease of the TIMP-3 protein and an increase of carcinogenesis [Feng et al., 2004].

In conclusion, the immediate response to tissue injury caused by mechanical disruption, bacterial infection, or viral infection is inflammation followed by angiogenesis. During this process, myofibroblasts, macrophages, and smooth muscle cells migrate to the site of injury, releasing profibrotic growth factors. The most potent cytokine causing fibrosis is TGF-β1. Persistent TGF-β1 leads to excess fibrosis and the development of benign tumors such as keloids and granulomas. Persistent TGF-β1 expression is associated with hypertropic scars. In contrast, for the formation of malignant tumors, the organism first encapsulates the malignant cells in a fibrous capsule. When the ECM capsule is degraded by an imbalance between MMPs and TIMPs, tumor cells proliferate, invade the surrounding tissue and metastasis occurs. There exist multiple targets for oligo decoy anti-profibrotic growth factor therapy for inhibiting carcinogenesis. These include the TGF-βs, CTGF, IGF, and PDGF. The SMAD activator protein transcription factors are other potential targets which are positive modulators of the TGF-β/SMAD signaling pathway. Knocking out the inhibitory Smads 6, 7, and/or 8, would intensify TGF-β signaling and increase the development of ECM encapsulated tumors. An oligo decoy-mediated decrease of HIF-1 would potentially ameliorate carcinogenesis. Finally fine tuning the MMP/TIMP balance would drastically alter tumor tissue invasion and metastasis.

ACKNOWLEDGMENTS

The authors thank Dr. David W. Yandell, Director of the Vermont Cancer Center of the University of Vermont's College of Medicine and Dr. Brooke T. Mossman, Director of the Environmental Pathology Program for their critical evaluations of this Review. Dr. Mossman and Dr. Cutroneo are members of the Vermont Cancer Center. Dr. Cutroneo is an Emeritus member of the American Association for Cancer Research. Dr. Chiu is a Professor Emeritus of the University of Vermont.

REFERENCES

Acs G, Zhang PJ, McGrath CM, Acs P, McBroom J, Mohyeldin A, Liu S, Lu H, Verma A. 2003. Hypoxia-inducible erythropoietin signaling in squamous dysplasia and squamous cell carcinoma of the uterine cervix and its potential role in cervical carcinogenesis and tumor progression. Am J Pathol 162:1789–1806.

Acs G, Xu X, Chu C, Acs P, Verma A. 2004. Prognostic significance of erythropoietin expression in human endometrial carcinoma. Cancer 100:2376–2386.

Ahn SM, Jeong SJ, Kim YS, Sohn Y, Moon A. 2004. Retroviral delivery of TIMP-2 inhibits H-ras-induced migration and invasion in MCF10A human breast epithelial cells. Cancer Lett 207:49–57.

Ahonen M, Ala-Aho R, Baker AH, George SJ, Grenman R, Saarialho-Kere U, Kahara VM. 2002. Antitumor activity and bystander effect of adenovirally delivered tissue inhibitor of metalloproteinases-3. Molec Ther 5:705–715.

Ahonen M, Poukkula M, Baker AH, Kashiwagi M, Nagase H, Eriksson JE, Kahari VM. 2003. Tissue inhibitor of metalloproteinases-3 induces apoptosis in melanoma cells by stabilization of death receptors. Oncogene 22: 2121–2134.

Alper O, Bergmann-Leitner ES, Abrams S, Cho-Chung YS. 2001. Apoptosis, growth arrest and suppression of invasiveness by CRE-deoxy oligonucleotide in ovarian cancer cells: Protein kinase A downregulation and cytoplasmic export of CRE-binding proteins. Molec & Cell Biochem 218:55–63.

Bacon A, Harris A. 2004. Hypoxia-inducible factors and hypoxic cell death in tumour physiology. Annals of Med 36:530–539.

Baker EA, Leaper DJ. 2003. The plasminogen activator and matrix metalloproteinase systems in colorectal cancer:

Relationship to tumor pathology. Eur J Cancer 39:981–988.

Baldwin RL, Tran H, Karlan BY. 2003. Loss of c-myc repression coincides with ovarian cancer resistance to transforming growth factor beta growth arrest independent of transforming growth factor beta/SMADL signaling. Cancer Res 63:1413–1419.

Bendardaf R, Lamlum H, Vihinen P, Ristamaki R, Laine J, Pyrhonen S. 2003. Low collagenase-1 (MMP-1) and MT1-MMP expression levels are favorable survival markers in advanced colorectal carcinoma. Oncol 65:337–346.

Bloomston M, Shafii A, Zervos EE, Rosemurgy AS. 2002. TIMP-1 overexpression in pancreatic cancer attenuates tumor growth, decreases implantation and metastasis, and inhibits angiogenesis. J Surg Res 102:39–44.

Boros DL, Singh KP, Gerard HC, Hudson AP, White SL, Cutroneo KR. 2005. A novel nonsteroidal antifibrotic oligo decoy containing the TGF-β element found in the COL1A1 gene which regulates murine schistosomiasis liver fibrosis. J Cell Physiol 204:370–374.

Buenemann CL, Willy C, Buchmann A, Schmiechen A, Schwarz M. 2001. Transforming growth factor-beta1-induced SMAD signaling, cell-cycle arrest and apoptosis in hepatoma cells. Carcinogenesis 22:447–452.

Castello R, Estelles A, Vazquez C, Falco C, Espana F, Almenar SM, Fuster C, Aznar J. 2002. Quantitative real-time reverse transcription-PCR assay for urokinase plasminogen activator, plasminogen activator inhibitor type 1 and tissue metalloproteinase inhibitor type 1 gene expression in primary breast cancer. Clin Chem 48: 1288–1295.

Cerutti JM, Ebina N, Matsuo SE, Martins L, Maciel RM, Kimura ET. 2003. Expression of Smad4 and Smad7 in human thyroid follicular carcinoma cell lines. J Endocrin Invest 26:516–521.

Chan DA, Sutphin PD, Denko NC, Giaccia AJ. 2002. Role of prolyl hydroxylation in oncogenically stabilized hypoxia-inducible factor-1 alpha. J Biol Chem 277:40112–40117.

Chan KS, Sano S, Kiguchi K, Anders J, Komazawa N, Takeda J, DiGiovanni J. 2004. Disruption of Stat3 reveals a critical role in both the initiation and the promotion stages of epithelial carcinogenesis. J Clin Invest 114:720–728.

Cheng KH, Ponte JF, Thiagalingam S. 2004. Elucidation of epigenetic inactivation of Smad8 in cancer using targeted expressed gene display. Cancer Res 64:1639–1646.

Colpaert C, Vermeulen P, van Beest P, Goovaerts G, Weyler J, Van Dam P, Dirix L, Van Marck E. 2001. Intratumoral hypoxia resulting in the presence of a fibrotic focus is an independent predictor of early distant relapse in lymph node-negative breast cancer patients. Histopathol 39: 416–425.

Colpaert CG, Vermeulen PB, Fox SB, Harris AL, Dirix LY, Van Marck EA. 2003. The presence of a fibrotic focus in invasive breast carcinoma correlated with the expression of carbonic anhydrase IX and is a marker of hypoxia and poor prognosis. Breast Cancer Res & Treatment 81:137–147.

Costa FF, Verbisck NV, Salim AC, Ierardi DF, Pires LC, Sasahara RM, Sogayar MC, Zanata SM, Mackay A, O'Hare M, Soares F, Simpson AJ, Camargo AA. 2004. Epigenetic silencing of the adhesion molecule ADAM23 is highly frequent in breast tumors. Oncogene 23:1481–1488.

Cutroneo KR. 2005. TGF-β induced fibrosis and SMAD signaling: Oligo decoys as natural therapeutics for inhibition of tissue fibrosis and scarring. Wound Repair Regen In Press.

Cutroneo KR, Boros DL. 2002. Rational basis for oligodeoxynucleotides to inhibit collagen synthesis in lung fibroblasts and primary fibroblasts from liver granulomas of Schistosoma mansoni-infected mice. Cancer Lett 180: 145–151.

Cutroneo KR, Chiu J-F. 2003. Chapter 8. Pandalai SG, editor. Recent Res Devel Cell Biochem, Inflammation, fibrosis and carcinogenesis, Vol. 1. Trivandrum, India: Transworld Research Network. pp 143–150.

Cutroneo KR, Phan SH. 2003. TGF-β1-induced Smad3 binding to the Smad7 gene: Knockout of Smad7 gene transcription by sense phosphorothioate oligos, autoregulation, and effect on TGF-β1 secretion: Bleomycin acts through TGF-β1. J Cell Biochem 89:474–483.

Cutroneo KR, Scott DF. 1979. Elevation of prolyl hydroxylase activity in Reuber hepatomas. Int J Biochem 10:573–576.

Cutroneo KR, Guzman NA, Liebelt AG. 1972. Elevation of peptidylproline hydroxylase activity and collagen synthesis in spontaneous primary mammary cancers of inbred mice. Cancer Res 32:2828–2833.

Davidson B, Goldberg I, Gotlieb WH, Kopolovic J, Ben-Baruch G, Nesland JM, Reich R. 2002. The prognostic value of metalloproteinases and angiogenic factors in ovarian carcinoma. Molec & Cell Endocrinol 187:39–45.

Deng H, Guo RF, Li WM, Zhao M, Lu YY. 2005. Matrix metalloproteinase 11 depletion inhibits cell proliferation in gastric cancer cells. Biochem Biophys Res Commun 326:274–281.

Deryugina EI, Soroceanu L, Strongin AY. 2002. Up-regulation of vascular endothelial growth factor by membrane-type 1 matrix metalloproteinase stimulates human glioma xenoraft growth and angiogenesis. Cancer Res 62:580–588.

Di Nezza LA, Misajon A, Zhang J, Jobling T, Quinn MA, Ostor AG, Nie G, Lopata A, Salamonsen LA. 2002. Presence of active gelatinases in endometrial carcinoma and correlation of matrix metalloproteinase expression with increasing tumor grade and invasion. Cancer 94:1466–1475.

Drew AF, Blick TJ, Lafleur MA, Tim EL, Robbie MJ, Rice GE, Quinn MA, Thompson EW. 2004. Correlation of tumor- and stromal-derived MT1-MMP expression with progression of human ovarian tumors in SCID mice. Gynecol Oncol 95:437–448.

Duda DG, Sunamura M, Lefter LP, Furukawa T, Yokoyama T, Yatsuoka T, Abe T, Inoue H, Motoi F, Egawa S, Matsumo S, Horii A. 2003. Restoration of Smad4 by gene therapy reverses the invasive phenotype in the pancreatic adenocarcinoma cells. Oncogene 22:6857–6864.

Dumont E, Lallemand F, Prunier C, Ferrand N, Guillouzo A, Clement B, Atfi A, Theret N. 2003. Evidence for a role of Smad3 and Smad2 in stabilization of the tumor-derived mutant Smad2.Q407R. J Biol Chem 278:24881–24887.

Dunfield LD, Dwyer EJ, Nachtigal MW. 2002. TGF beta-induced SMAD signaling remains intact in primary human ovarian cancer cells. Endocrinology 143:1174–1181.

Edlund S, Bu S, Schuster N, Aspenstrom P, Heuchel R, Heldin NE, ten Dijke P, Heldin Ch, Landstrom M. 2003. Transforming growth factor-beta1 (TGF-beta)-induced apoptosis of prostate cancer cells involves Smad7-dependent activation of p38 by TGF-beta-activated kinase 1 and mitogen-activated protein kinase kinase 3. Molec Biol Cell 4:529–544.

Feldman AL, Stetler-Stevenson WG, Costouros NG, Knezevic V, Baibakov G, Alexander HR Jr, Lorang D, Hewitt SM, Seo DW, Miller MS, O'Connor S, Libutti SK. 2004. Modulation of tumor-host interactions, angiogenesis, and tumor growth by tissue inhibitor of metalloproteinase 2 via a novel mechanism. Cancer Res 64:4481–4486.

Feng H, Cheung AN, Xue WC, Wang Y, Wang X, Fu S, Wang Q, Ngan HY, Tsao SW. 2004. Down-regulation and promoter methylation of tissue inhibitor of metalloproteinase 3 in choriocarcinoma. Gynecol Oncol 94:375–382.

Figueroa YG, Chan AK, Ibrahim R, Tang Y, Burow ME, Alam J, Scandurro AB, Beckman BS. 2002. NF-kappaB plays a key role in hypoxia-inducible factor-1-regulated erythropoietin gene expression. Exp Hematol 30:1419–1427.

Grutzmann R, Luttges J, Sipos B, Ammerpohl O, Dobrowolski F, Alldinger I, Kersting S, Ockert D, Koch R, Kalthoff H, Schackert HK, Saeger HD, Kloppel G, Pilarsky C. 2004. ADAM9 expression in pancreatic cancer is associated with tumour type and is a prognostic factor in ductal adenocarcinoma. Br J Cancer 90:1053–1058.

Han SU, Kim HT, Seong do H, Kim YS, Park YS, Bang YJ, Yang HK, Kim SJ. 2004. Loss of the Smad3 expression increases susceptibility to tumorigenicity in human gastric cancer. Oncogene 23:1333–1341.

Hasebe T, Tsuda H, Hirohashi S, Shimosato Y, Tsubono Y, Yamamoto H, Mukai K. 1998. Fibrotic focus in infiltrating ductal carcinoma of the breast: A significant histopathological prognostic parameter for predicting the long-term survival of the patients. Breast Cancer Res Treat 49:195–208.

Hirsila M, Koivunen P, Gunzler V, Kivirikko KI, Myllyharju J. 2003. Characterization of the human prolyl-4-hydroxylases that modify the hypoxia-inducible factor. J Biol Chem 278:30772–30780.

Holmbeck K, Bianco P, Birkedal-Hansen H. 2003. MT1-MMP: A collagenase essential for tumor cell invasive growth. Cancer Cell 4:83–84.

Holten-Andersen MN, Hansen U, Brunner N, Nielsen HJ, Illemann M, Nielsen BS. 2005. Localization of tissue inhibitor of metalloproteinases 1 (TIMP-1) in human colorectal adenoma and adenocarcinoma. Int J Cancer 113:198–206.

Iida J, Wilhelmson KL, Price WA, Wilson CM, Pei D, Furcht LT, McCarthy JB. 2004. Membrane type-1 matrix metalloproteinase promotes human melanoma invasion and growth. J Invest Dermatol 122:167–176.

Ikeda O, Egami H, Ishiko T, Ishikawa S, Kamohara H, Hidaka H, Mita S, Ogawa M. 2003. Expression of proteinase-activated receptor-2 in human pancreatic cancer: A possible relation to cancer invasion and induction on fibrosis. Int J of Oncol 22:295–300.

Impola U, Toriseva M, Suomela S, Jeskanen L, Hieta N, Jahkola T, Renman R, Kahari VM, Saarialho-Kere U. 2003. Matrix metalloproteinase-19 is expressed by proliferating epithelium but disappears with neoplastic dedifferentiation. Int J Cancer 103:709–716.

Ivan M, Haberberger T, Gervasi DC, Michelson KS, Gunzler V, Kondo K, Yang H, Sorokina I, Conaway RC, Conaway JW, Kaelin WG Jr. 2002. Biochemical purification and pharmacological inhibition of a mammalian prolyl hydroxylase acting on hypoxia-inducible factor. Proc Natl Acad Sci USA 99:13459–13464.

Ivanova T, Vinokurova S, Petrenko A, Eshilev E, Solovyova N, Kisseljov F, Kisseljova N. 2004. Frequent hypermethylation of 5′ flanking region of TIMP-2 gene in cervical cancer. Int J Cancer 108:882–886.

Jaakkola P, Mole DR, Tian YM, Wilson MI, Gielbert J, Gaskell SJ, Kriegsheim Av, Hebestreit HF, Mukherji M, Schofield CJ, Maxwell PH, Pugh CW, Ratcliffe PJ. 2001. Targeting of HIF-alpha to the von Hippel-Lindau ubiquitylation complex by O_2-regulated prolyl hydroxylation. Science 292:468–472.

Jelkmann W, Pagel H, Hellwig T, Fandrey J. 1997. Effects of antioxidant vitamins on renal and hepatic erythropoietin production. Kidney Int 51:497–501.

Jeong JW, Bae MK, Ahn MY, Kim SH, Sohn TK, Bae MH, Yoo MA, Song EJ, Lee KJ, Kim KW. 2002. Regulation and destabilization of HIF-1 alpha by ARD1-mediated acetylation. Cell 111:709–720.

Ju HR, Jung U, Sonn CH, Yoon SR, Jeon JH, Yang Y, Lee KN, Choi I. 2003. Aberrant signaling of TGF-beta1 by the mutant Smad4 in the gastric cancer cells. Canc Lett 96:197–206.

Kato H, Tamamizu-Kato S, Shibasaki F. 2004. Histone deacetylase 7 associates with hypoxia-inducible factor 1 alpha and increases transcriptional activity. J Biol Chem 279:41966–41974.

Kawamura I, Morishita R, Tsujimoto S, Manda T, Tomoi M, Tomita N, Goto T, Ogihara T, Kaneda Y. 2001. Intravenous injection of oligodeoxynucleotides to the NF-kappaB binding site inhibits hepatic metastasis of M5076 reticulosarcoma in mice. Gene Ther 8:905–912.

Kayano K, Shimada T, Shinomiya T, Nakai S, Hisa Y, Aoki T, Seiki M, Okada Y. 2004. Activation of pro-MMP-2 mediated by MT1-MMP in human salivary gland carcinomas: Possible regulation of pro-MMP-2 activation by TIMP-2. J Pathol 202:403–411.

Kim BC, van Gelder H, Kim TA, Lee HJ, Baik KG, Chun HH, Lee DA, Choi KS, Kim SJ. 2004. Activin receptor-like kinase-7 induces apoptosis through activation of MAPKs in Smad3-dependent mechanism in hepatoma cells. J Biol Chem 279:28458–28465.

Kitamura H, Oosawa Y, Kawano N, Kameda Y, Hayashi H, Nakatani Y, Udaka N, Ito T, Miyazaki K. 1999. Basement membrane patterns, gelatinase A and tissue inhibitor of metalloproteinase-2 expressions, and stromal fibrosis during the development of peripheral lung adenocarcinoma. Human Pathol 30:331–338.

Kodama T, Ikeda E, Okada A, Ohtsuka T, Shimoda M, Shiomi T, Yoshida K, Nakada M, Ohuchi E, Okada Y. 2004. ADAM12 is selectively overexpressed in human glioblastomas and is associated with glioblastoma cell proliferation and shedding of heparin-binding epidermal growth factor. Am J Pathol 165:1743–1753.

Kume M, Komori K, Matsumoto T, Onohara T, Takeuchi K, Yonemitsu Y, Sugimachi K. 2002. Administration of a decoy against the activator protein-1 binding site

suppresses neointimal thickening in rabbit balloon-injured arteries. Circulation 105:1226–1232.
Landstrom M, Heldin NE, Bu S, Hermansson A, Itoh S, ten Dijke P, Heldin CH. 2000. Smad7 mediates apoptosis induced by transforming growth factor beta in prostatic carcinoma cells. Curr Biol 10:535–538.
Le Pabic H, Bonnier D, Wewer UM, Coutand A, Musso O, Baffet G, Clement B, Theret N. 2003. ADAM12 in human liver cancers: TGF-beta-regulated expression in stellate cells is associated with matrix remodeling. Hepatology 37:1056–1066.
Lee YN, Park YG, Choi YH, Cho YS, Cho-Chung YS. 2000. CRE-transcription factor decoy oligonucleotide inhibition of MCF-7 breast cancer cells: Cross-talk with p53 signaling pathway. Biochem 39:4863–4868.
Lee SJ, Yoo HJ, Bae YS, Kim HJ, Lee ST. 2003. TIMP-1 inhibits apoptosis in breast carcinoma cells via a pathway involving pertussis toxin-sensitive G protein and c-Src. Biochem Biophys Res Commun 312:1196–1201.
Lindemann RK, Nordheim A, Dittmer J. 2003. Interfering with TGF-beta-induced Smad3 nuclear accumulation differentially affects TGF-beta-dependent gene expression. Molec Cancer 2:20.
Liu C, Shi Y, Han Z, Pan Y, Liu N, Han S, Chen Y, Lan M, Qiao T, Fan D. 2003. Suppression of the dual-specificity phosphatase MKP-1 enhances HIF-1 transactivation and increases expression of EPO. Biochem Biophys Res Commun 312:780–786.
Liu WM, Scott KA, Shahin S, Propper DJ. 2004. The in vitro effects of CRE-decoy oligonucleotides in combination with conventional chemotherapy in colorectal cancer cell lines. Eur J Biochem 271:2773–2781.
Mahara K, Kato J, Terui T, Takimoto R, Horimoto M, Murakami T, Mogi Y, Watanabe N, Kohgo Y, Niitsu Y. 1994. Transforming growth factor beta 1 secreted from cirrhosis gastric cancer cells is associated with excess collagen deposition in the tissue. Br J Cancer 69:777–783.
Maliekal TT, Antony ML, Nair A, Paulmurugan R, Karunagran D. 2003. Loss of expression and mutations of Smad2 and Smad4 in human cervical cancer. Oncogene 22:4889–4897.
Mazars A, Lallemand F, Prunier C, Marais J, Ferrand N, Pessah M, Cherqui G, Atfi A. 2001. Evidence for a role of the JNK cascade in Smad7-mediated apoptosis. J Biol Chem 276:36797–36803.
McCulloch DR, Akl P, Samaratunga H, Herington AC, Odorico DM. 2004. Expression of the disintegrin metalloprotease, ADAM-10, in prostate cancer and its regulation by dihydrotestosterone, insulin-like growth factor I, and epidermal growth factor in the prostate cancer cell model LNCaP. Clin Cancer Res 10:314–323.
McEarchern JA, Kobie JJ, Mack V, Wu RS, Meade-Tollin L, Arteaga CL, Dumont N, Besselsen D, Seftor E, Hendrix MJ, Katsanis E, Akporiaye ET. 2001. Invasion and metastasis of a mammary tumor involves TGF-beta signaling. Int J Cancer 91:76–82.
Mekhail K, Gunaratnam L, Bonicalzi ME, Lee S. 2004a. HIF activation by pH-dependent nucleolar sequestration of VHL. Nature Cell Biol 6:642–647.
Mekhail K, Khacho M, Gunaratnam L, Lee S. 2004b. Oxygen sensing by H^+: Implications for HIF and hypoxic cell memory. Cell Cycle 3:1027–1029.
Menke A, Adler G. 2002. TGF-beta-induced fibrogenesis of the pancreas. Int J Gastrointestinal Cancer 31:41–46.
Morishita R, Aoki M, Kaneda Y. 2001. Decoy oligodeoxynucleotides as novel cardiovascular drugs for cardiovascular disease. Ann NY Acd Sci 947:294–301.
Mukundan H, Kanagy NL, Resta TC. 2004. 17-beta estradiol attenuates hypoxic induction of HIF-1 alpha and erythropoietin in Hep3B cells. J Cardiovasc Pharmacol 44:93–100.
Munaut C, Noel A, Hougrand O, Foidart JM, Boniver J, Deprez M. 2003. Vascular endothelial growth factor expression correlates with matrix metalloproteinases MT1-MMP, MMP-2 and MMP-9 in human glioblastomas. Int J Cancer 106:848–855.
Nakopoulou L, Panayotopoulou EG, Giannopoulou I, Alexandrou P, Katsarou S, Athanassiadou P, Keramopoulos A. 2002. Stromelysin-3 protein expression in invasive breast cancer: Relation to proliferation, cell survival and patients' outcome. Modern Pathol 15:1154–1161.
Nicolas FJ, Hill CS. 2003. Attenuation of the TGF-beta-SMAD signaling pathway in pancreatic tumor cells confers resistance to TGF-beta-induced growth arrest. Oncogene 22:3698–3711.
Novak EM, Metzger M, Chammas R, da Costa M, Dantas K, Manabe C, Pires J, de Oliveira AC, Bydlowski SP. 2003. Downregulation of TNF-alpha and VEGF expression by Sp1 decoy oligodeoxy-nucleotides in mouse melanoma tumor. Gene Ther 10:1992–1997.
O'Shea C, McKie N, Buggy Y, Duggan C, Hill AD, McDermott E, O'Higgins N, Duffy MJ. 2003. Expression of ADAM-9 mRNA and protein in human breast cancer. Int J Cancer 105:754–761.
Oft M, Akhurst RJ, Balamain A. 2002. Metastasis is driven by sequential elevation of H-ras and Smad2 levels. Nature Cell Biol 4:487–494.
Park YG, Park S, Lim SO, Lee MS, Ryu CK, Kim I, Cho-Chung YS. 2001. Reduction in cyclin D1/Cdk4/retinoblastoma protein signaling by CRE-decoy oligonucleotide. Biochem Biophys Res Commun 281:1213–1219.
Park YN, Chae KJ, Oh BK, Choi J, Choi KS, Park C. 2004. Expression of Smad7 in hepatocellular carcinoma and dysplastic nodules: Resistance mechanism to transforming growth factor-beta. Hepato-Gastroenterol 51:396–400.
Pendas AM, Folgueras AR, Llano E, Caterina J, Frerard F, Rodriguez F, Astudillo A, Noel A, Birkedal-Hansen H, Lopez-Otin C. 2004. Diet-induced obesity and reduced skin cancer susceptibility in matrix metalloproteinase 19-deficient mice. Molec Cell Biol 24:5304–5313.
Percy JJ, McMullin MF, Lappin TRJ. 1997. Sequence analysis of the 3′ hypoxia-responsive element of the human erythropoietin gene in patients with erythrocytosis. Biochem Molec Med 62:132–134.
Petkova DK, Clelland CA, Ronan JE, Lewis S, Knox AJ. 2003. Reduced expression of cyclooxygenase (COX) in idiopathic pulmonary fibrosis and sarcoidosis. Histopathol 43:381–386.
Polnaszek N, Kwabi-Addo B, Wang J, Ittmann M. 2004. FGF17 is an autocrine prostatic epithelial growth factor and is upregulated in benign prostatic hyperplasia. Prostate 60:18–24.
Porter JF, Shen S, Denhardt DT. 2004. Tissue inhibitor of metalloproteinase-1 stimulates proliferation of human

cancer cells by inhibiting a metalloproteinase. Br J Cancer 90:463–470.

Ramachandra M, Atencio I, Rahman A, Vaillancourt M, Zou A, Avanzini J, Wills K, Bookstein R, Shabram P. 2002. Restoration of transforming growth factor beta signaling by functional expression of Smad4 induces anoikis. Cancer Res 62:6045–6051.

Ramos MC, Steinbrenner H, Stuhlmann D, Sies H, Brenneisen P. 2004. Induction of MMP-10 and MMP-1 in a squamous cell carcinoma cell line by ultraviolet radiation. J Biol Chem 385:75–86.

Rhee JS, Diaz R, Korets L, Hodgson JG, Coussens LM. 2004. TIMP-1 alters susceptibility to carcinogenesis. Cancer Res 64:952–961.

Roesel RA, Cutroneo KR, Scott DF, Howard EF. 1978. Collagen synthesis by cloned mouse mammary tumor cells. Cancer Res 38:3269–3275.

Roy R, Wewer UM, Zurakowski D, Pories SE, Moses MA. 2004. ADAM 12 cleaves extracellular matrix proteins and correlates with cancer status and stage. J Biol Chem 279:51323–51330.

Sabeh F, Ota I, Holmbeck K, Birkedal-Hansen H, Soloway P, Balbin M, Lopez-Otin C, Shapiro S, Inada M, Krane S, Allen E, Chung D, Weiss SJ. 2004. Tumor cell traffic through the extracellular matrix is controlled by the membrane-anchored collagenase MT1-MMP. J Cell Biol 167:769–781.

Sakaida I, Kubota M, Kayano K, Takenaka K, Mori K, Okita K. 1994. Prevention of fibrosis reduces enzyme-altered lesions in the rat liver. Carcinogenesis 15:2201–2206.

Salvador RA, Tsai I, Stassen FLH. 1976. On the mechanism of the increase in prolyl hydroxylase activity in the uterus of the rat given estradiol-17β. Biochem Pharmacol 25:907–912.

Schrohl AS, Holten-Andersen MN, Peters HA, Look MP, Meijer-van Gelder ME, Klijn JG, Brunner N, Foekens JA. 2004. Tumor tissue levels of tissue inhibitor of metalloproteinase-1 as a prognostic marker in primary breast cancer. Clin Cancer Res 10:2289–2298.

Serrero G, Ioffe OB. 2003. Expression of PC-cell-derived growth factor in benign and malignant human breast epithelium. Human Pathol 34:1148–1154.

Shirakawa K, Tsuda H, Heike Y, Kato K, Asada R, Inomata M, Sasaki H, Kasumi F, Yoshimoto M, Iwanaga T, Konishi F, Terada M, Wakasugi H. 2001. Absence of endothelial cells, central necrosis, and fibrosis are associated with aggressive inflammatory breast cancer. Cancer Res 61:445–451.

Sounni NE, Baramova EN, Munaut C, Macquoi E, Frankenne F, Foidart JM, Noel A. 2002a. Expression of membrane type 1 matrix metalloproteinase (MT1-MMP) in A2058 melanoma cells is associated with MMP-2 activation and increased tumor growth and vascularization. Int J Cancer 98:23–28.

Sounni NE, Devy L, Hajitou A, Frankenne F, Munaut C, Gilles C, Deroanne C, Thompson EW, Foidart JM, Noel A. 2002b. MT1-MMP expression promotes tumor growth and angiogenesis through an up-regulation of vascular endothelial growth factor expression. FASEB J 16:555–564.

Sowter HM, Raval R, Moore J, Ratcliffe PJ, Harris AL. 2003. Predominant role of hypoxia-inducible transcription factor (Hif)-1alpha versus Hif-2alpha in regulation of the transcriptional response to hypoxia. Cancer Res 63:6130–6134.

Subramanian G, Schwarz RE, Higgins L, McEnroe G, Chakravarty S, Dugar S, Reiss M. 2004. Targeting endogenous transforming growth factor beta receptor signaling in SMAD4-deficient human pancreatic carcinoma cells inhibits their invasive phenotype. Cancer Res 64:5200–5211.

Takahashi Y, Takahashi S, Shiga Y, Yoshimi T, Miura T. 2000. Hypoxic induction of prolyl-4-hydroxylase alpha (I) in cultured cells. J Biol Chem 275:14139–14146.

Tamiolakis D, Papadopoulos N, Cheva A, Lambropoulou M, Kotini A, Jivannakis T, Simopoulos C. 2002. Immunohistochemical expression of alpha-smooth muscle actin in infiltrating ductal carcinoma of the breast with productive fibrosis. Eur J Gynecol Oncol 23:469–471.

Tang TT, Lasky LA. 2003. The forkhead transcription factor FOXO4 induces the down-regulation of hypoxia-inducible factor 1 alpha by a von Hippel-Lindau protein-independent mechanism. J Biol Chem 278:30125–30135.

Tian F, DaCosta Byfield S, Parks WT, Yoo S, Felici A, Tang B, Piek E, Wakefield LM, Roberts AB. 2003. Reduction in Smad2/3 signaling enhances tumorigenesis but suppresses metastasis of breast cancer cell lines. Canc Res 63:8284–8292.

Tomita T, Takeuchi E, Tomita N, Morishita R, Kaneko M, Yamamoto K, Nakase JT, Seki H, Kato K, Kaneda Y, Ochi T. 1999. Suppressed severity of collagen-induced arthritis by in vivo transfection of nuclear factor kappa B decoy oligodeoxynucleotides as a gene therapy. Arth & Rheum 42:2532–2542.

Tomita N, Azuma H, Kaneda Y, Ogihara JT, Morishita R. 2003. Gene therapy with transcription factor decoy oligonucleotides as a potential treatment for cardiovascular diseases. Curr Drug Targets 4:339–346.

Trochon V, Li H, Vasse M, Frankenne F, Thomaidis A, Soria J, Lu H, Gardner C, Soria C. 1998. Endothelial metalloprotease-disintegrin protein (ADAM) is implicated in angiogenesis in vitro. Angiogenesis 2:277–285.

Tsang KJ, Tsang D, Brown TN, Crowe DL. 2002. A novel dominant negative Smad2 mutation in a TGF-beta resistant human carcinoma cell line. Anticancer Res 22:13–19.

Udayakumar TS, Stratton MS, Nagle RB, Bowden GT. 2002. Fibroblast growth factor-1 induced promatrilysin expression through the activation of extracellular-regulated kinases and STAT3. Neoplasia 4:60–67.

Udayakumar TS, Nagle RB, Bowden GT. 2004. Fibroblast growth factor-1 transcriptionally induces membrane type-1 matrix metalloproteinase expression in prostate carcinoma cell line. Prostate 58:66–75.

Ueda J, Kajita M, Suenaga N, Fujii K, Seiki M. 2003. Sequence-specific silencing of MT1-MMP expression suppresses tumor cell migration and invasion: Importance of MT1-MMP as a therapeutic target for invasive tumors. Oncogene 22:8716–8722.

Vaage J. 1992a. Immunologic aspects of fibrosis in mouse mammary carcinomas. Int J Cancer 50:69–74.

Vaage J. 1992b. Fibrosis in immune control of mammary-tumor growth. Int J Cancer 51:325–328.

Wang CS, Tetu B. 2002. Stromelysin-3 expression by mammary tumor-associated fibroblasts under in vitro breast cancer cell induction. Int J Cancer 99:792–799.

Warnecke C, Griethe W, Weidemann A, Jurgensen JS, William C, Bachmann S, Ivashchenko Y, Wagner I, Frei U, Wiesener M, Eckardt KU. 2003. Activation of the hypoxia-inducible factor-pathway and stimulation of angiogenesis by application of prolyl hydroxylase inhibitors. FASEB J 17:1186–1188.

Watanabe I, Hasebe T, Sasaki S, Konishi M, Inoue K, Nakagohri T, Oda T, Mukai K, Kinoshita T. 2003. Advanced pancreatic ductal cancer: Fibrotic focus and beta-catenin expression correlate with outcome. Pancreas 26:326–333.

Wilborn J, Crofford LJ, Burdick MD, Kunkel SL, Strieter RM, Peters-Golden M. 1995. Cultured lung fibroblasts isolated from patients with idiopathic pulmonary fibrosis have a diminished capacity to synthesize prostaglandin E2 and to express cyclooxygenase-2. J Clin Invest 95: 1861–1868.

Wild A, Ramaswamy A, Langer P, Celik I, Fendrich V, Chaloupka B, Simon B, Bartsch DK. 2003. Frequent methylation-associated silencing of the tissue inhibitor of metalloproteinase-3 gene in pancreatic endocrine tumors. J Clin Endocrinol Metabol 88:1367–1373.

Xu G, Chakraborty C, Lala PK. 2002. Restoration of TGF-beta regulation of plasminogen activator inhibitor-1 in Smad3-restituted human choriocarcinoma cells. Biochem Biophys Res Commun 294:1079–1086.

Yamamoto H, Horiuchi S, Adachi Y, Taniguchi H, Nosho K, Min Y, Imai K. 2004. Expression of ets-related transcriptional factor E1AF is associated with tumor progression and over-expression of matrilysin in human gastric cancer. Carcinogenesis 25:325–332.

Yano A, Nakamoto T, Hashimoto K, Usui T. 2002. Localization and expression of tissue inhibitor of metalloproteinase-1 in human urothelial cancer. J Urol 167: 729–734.

Zacchigna S, Zentilin L, Morini M, Dell'Eva R, Noonan DM, Albini A, Giacca M. 2004. AAV-mediated gene transfer of tissue inhibitor of metalloproteinases-1 inhibits vascular tumor growth and angiogenesis in vivo. Cancer Gene Ther 11:73–80.

Zhao YG, Xiao AZ, Newcomer RG, Park HI, Kang T, Chung LW, Swanson MG, Zhau HE, Kurhanewicz J, Sang QX. 2003. Activation of pro-gelitanse B by endometase/matrilysin-2 promotes invasion of human prostate cancer cells. J Biol Chem 278:15056–15064.

Zhao YG, Xiao AZ, Park HI, Newcomer RG, Yan M, Man YG, Heffelfinger SC, Sang QX. 2004. Endometase/matrilysin-2 in human breast ductal carcinoma in situ and its inhibition by tissue inhibitors of metalloproteinases-2 and-4: A putative role in the initiation of breast cancer invasion. Cancer Res 64:590–598.