[Frontiers in Bioscience 8, e245-260, January 1, 2003]

THE ROLE OF TRANSFORMING GROWTH FACTOR-BETA IN PRIMARY BRAIN TUMORS

Jeremy N. Rich

Department of Medicine, Division of Neurology, Duke University Medical Center, Durham, NC 27710

TABLE OF CONTENTS

1. ABSTRACT

Dramatic therapeutic benefits of targeting specific signal transduction pathways in some cancers have pushed rational molecular targeting to the forefront of cutting-edge cancer therapy. The identification and targeting of pathways critical to the phenotype of cancers offers new hope in the treatment of many patients. Transforming growth factor beta (TGF-beta) is a multifunctional cytokine that is frequently expressed in multiple types of malignant brain tumors. TGF-beta exerts a complex set of effects in cancers with an early tumor suppressive effect through growth inhibition but later effects in cancer development that are tumorigenic – including increased tumor cell motility and invasion, induction of angiogenesis, and immune suppression. Early preclinical and clinical studies have shown promise of anti-TGF-beta strategies in the treatment of malignant gliomas suggesting TGF-beta may be a potential new therapeutic target in neuro-oncology.

2. INTRODUCTION

Primary brain tumors, like all cancers, share a relatively restricted set of characteristics critical to the tumor phenotype – proliferation in the absence of external growth stimuli, avoidance of apoptosis and on limits of replication, escape from external growth suppressive forces and the immune response, new blood vessel formation, and ability to spread into normal tissues (reviewed in 1). The molecular determinants of these behaviors are becoming increasingly well understood, and this understanding is providing novel "targeted" therapies. Prominent amongst the signal transduction pathways that play a critical role in a broad range of tumors is the transforming growth factor-beta (TGF-beta) pathway. Malignant gliomas, meningiomas, medulloblastomas, and ependymomas express high levels of TGF-beta ligand (2-7). In glioma cultures, TGF-beta regulates several aspects of the malignant phenotype, including immune escape, angiogenesis, and tumor invasion. The importance of TGF-beta in gliomas is illustrated by the fact that modulation of the TGF-beta function in gliomas has already been proven to be of value in preclinical trials, and early clinical trials to suppress TGF-beta function have been initiated. Several key points are important to understand the role of TGF-beta in malignant brain tumors: 1) The TGF-beta signal transduction pathways are complicated and interact significantly with many other signal transduction pathways, 2) The functional outcome of TGF-beta signal transduction is strongly dependent on cell type and cell state, 3) TGF-beta has an impact on a large number of cellular processes including proliferation, apoptosis, cell-extracellular matrix and cell-cell interactions, immune function and angiogenesis, 4) TGF-beta can act as a tumor suppressor at early stages of cancer development by suppressing growth and a tumor promoter at later stages, and 5) Malignant brain tumors generally have lost growth suppressive effects of TGF-beta but retain TGF-beta mediated effects on angiogenesis, invasion, and immunosuppression. These points will be developed during the course of this review.

3. TRANSFORMING GROWTH FACTOR BETA SIGNAL TRANSDUCTION

3.1. Normal TGF-beta Signal Transduction

Dramatic advances in our understanding of the TGF-beta mediated signal transduction pathway have occurred in the past decade. The TGF-beta pathway is regulated by numerous mechanisms at multiple levels to permit the integration of the activities of a wide spectrum of other signal transduction pathways. Thus, the cellular context critically determines TGF-beta activation and target gene regulation.

TGF-beta represents the prototype of a large set of structurally related polypeptide growth factors that are involved in nearly every cellular activity: TGF-beta superfamily members regulate growth (8-10), differentiation (11-13), angiogenesis (14-16), extracellular interactions (17-22), invasion (23), and immune system function (24-32). TGF-beta family members are grouped into subsets of more closely related factors including the transforming growth factors beta, activins, growth differentiation factors, Mullerian inhibitory substance, and the bone morphogenetic proteins (BMPs) (reviewed in 33). In the central nervous system, both TGF-beta and BMP family members play complex roles in brain development and response to injury, including cell lineage determination and regulation of survival (reviewed in 34-36).

Roberts and Sporn originally described TGF-beta as a factor capable of inducing fibroblast growth in soft agar (reviewed in 37). There are three known isoforms of TGF-beta (TGF-beta 1-3) in mammals. The isoforms have 64-82% similarity at the amino acid level (38-40). TGF-beta ligands elicit similar effects *in vitro*, but the divergent amino acid sequences outside the highly conserved invariant cysteines may contribute to differential biological activities. For example, TGF-beta$_1$ and TGF-beta$_3$ potently inhibit the growth of some cells on which TGF-beta$_2$ has no effect (41-43). Sequence differences at the C-terminus likely mediate the specificity in isoform effects (44). The high affinity binding of TGF-beta$_1$ is eliminated when six amino acids in the C-terminus are replaced with the sequence of TGF-beta$_2$, indicating the C-terminus of TGF-beta isoforms contributes to distinct receptor affinities. Diverse effects may also occur with temporal and tissue specific secretions of the ligand due to control of expression by distinct promoters (45-48).

The activity of the TGF-beta ligand is tightly regulated by multiple mechanisms (reviewed in 49). TGF-beta is modified during secretion by the cleavage of the C-terminus at RRXR sequences by furin-like proteinases (50). The N-terminal region is designated the latency-associated peptide (LAP), while the C-terminus is the active TGF-beta. The LAP non-covalently associates with a 25-kDa active dimer to form a 100-kDa secreted inactive precursor (51). In most cell types, TGF-beta is translated and secreted in a large latent complex in which TGF-beta ligand is bound to the LAP molecule which is in turn associated covalently with one of four latent TGF-beta binding proteins (LTBPs). Interestingly, several human glioma cell lines secrete active TGF-beta ligand (52). Latent TGF-beta becomes bound to components of the extracellular matrix (ECM) through the LTBP to provide a source of readily accessible ligand. Disruption of LTBP expression in a murine model is linked to tumor formation associated with reduced deposition of TGF-beta (53). To elicit biological effects, the TGF-beta ligand must become activated after its initial secretion. An initial step involves proteolytic cleavage of the LTBP molecule by serine proteases, including plasmin. To become active TGF-beta, the latency-associated peptide must also be released or undergo a conformational change to expose the receptor binding site (reviewed in 54). Prominent mechanisms of activation include acidic conditions (common in most malignant tumors), proteases, integrins, or thrombospondin.

Three classes of receptors mediate TGF-beta effects: type I, type II, and accessory receptors. The accessory receptors include betaglycan (termed the type III receptor) and endoglin. Each accessory receptor binds TGF-beta at low affinity and presents it to type I and II receptors to enhance signaling (55, 56). For example, binding of TGF-beta$_2$ to betaglycan appears to increase the affinity of the type II receptor for this isoform (57, 58). Another receptor type, endoglin, can bind TGF-beta ligands as well, but its exact function is unknown. Betaglycan also binds TGF-beta$_1$ and TGF-beta$_3$ (reviewed in 59), as does endoglin (55). However, endoglin has a very low affinity for TGF-beta$_2$.

Type I and type II receptors for TGF-beta belong to a family of transmembrane serine/threonine kinases that initiate a signal transduction cascade upon ligand binding. TGF-beta receptors are glycoproteins with short extracellular regions, a single transmembrane region, and longer cytoplasmic regions with kinase activity (see Figure 1). Both the type I and type II receptors have a kinase region that is consistent with the canonical sequence of serine/threonine kinases, but the receptor types can be structurally distinguished. Phosphorylation of the receptor regulation sequences and the kinase activities of both the type I and II receptors are critical to TGF-beta signaling (60).

The TGF-beta signal transduction cascade is initiated through the binding of the ligand to the type II receptor. Subsequently, the type I receptor is recruited and binds TGF-beta within a receptor complex with the type II receptor (61-65). Of note, the type I receptor cannot bind ligand in the absence of type II receptor. Since TGF-beta dimerizes and each TGF-beta monomer can facilitate the interaction between one type I and one type II receptor, the TGF-beta receptor complex probably contains two type I and two type II receptors to form a heterotetrameric complex (61, 66-69). Complexes of both the type I and type II receptors are required for the type II receptor to phosphorylate the type I receptor. The type II receptor has constitutively active kinase activity and is autophosphorylated on several serine and threonine residues (70-72). When type I and II receptors are in close proximity, serines of the type I receptor are phosphorylated by the type II receptor (60, 73). Phosphorylation of type I receptor is required for initiating the intracellular signaling cascade since type II receptor alone is incapable of transducing the signal but constitutively active type I receptor alone can (74).

Although several proteins have been determined to bind TGF-beta receptors, the TGF-beta signal transduction cascade from the cytoplasm to the nucleus predominantly involves a novel family of proteins called SMADs. The term SMAD is derived from the conjunction of two invertebrate homologues, SMA from C. elegans (75) and MAD (mother against decapentaplegic) in *Drosophila*(76, 77). The SMADs are classified into three groups based on their

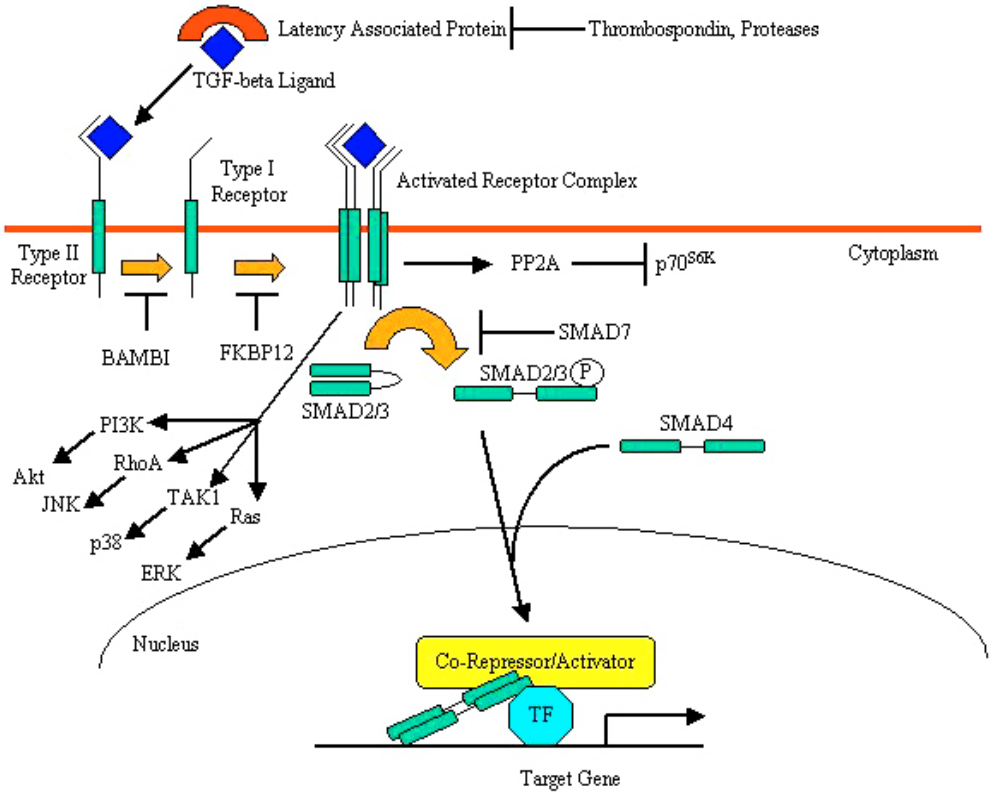

Figure 1. The Transforming Growth Factor Beta Signal Transduction Pathways. Transforming growth factor beta (TGF-beta) is secreted in an inactive latent form that can be activated by proteases. Dimerized TGF-beta ligand induces a heterodimerization of TGF-beta types I and II receptors that is associated with type I receptor phosphorylation and activation. Active TGF-beta type I receptors act on downstream effectors, including the receptor SMADs (R-SMADs) that are posphorylated on the carboxyl terminus to permit binding to the Co-SMAD, SMAD4, and nuclear localization. Nuclear SMAD complexes bind with low affinity to specific SMAD binding elements (SBEs) in the promoter sequences of regulated genes. Transcription complexes are formed with transcriptional binding partners and transcriptional activators/ inhibitors to specifically induce or repress gene expression.

structure and function: the receptor-regulated or pathway-specific SMADs (R-SMADs), the common SMADs (Co-SMADs), and the inhibitory SMADs (I-SMADs). The R-SMADs and Co-SMADs have Mad homology domains, designated MH1 and MH2 regions, joined by a proline-rich linker region. A MH2 region is also present in the carboxyl terminus of the I-SMADs that also have an amino terminus homologous among I-SMADs.

The R-SMADs function as the downstream effectors of TGF-beta receptors through a direct interaction with activated type I receptor. SMAD 2 and 3 specifically mediate the signals induced by TGF-beta and activin (78 - 84). Phosphorylation of R-SMADs induces release from the receptor, relief of auto-inhibitory folding, binding to SMAD 4 (the Co-SMAD), and translocation to the nucleus where transcription regulation is initiated. The I-SMADs (predominantly SMAD 7 in TGF-beta signal) appear to act predominantly in competition with the R-SMADs for receptor binding.

Nuclear localization of an active SMAD complex initiates the regulation of target genes. SMADs bind to particular DNA sequences (CAGAC -- named SBEs, SMAD binding elements) (85). SMADs bind with low affinity to these sequences and SBEs are found throughout the genome. SMAD complex binding to particular promoter sequences is determined by transcriptional binding partners (e.g. AP-1 family members) with subsequent recruitment of co-activators and co-repressors (reviewed in 86). In turn, transcription regulators such as HATs (histone acetyltransferases such as CBP/p300, which act to add acetyl groups to histones resulting in opening of genomic structure for increased transcription) or HDACs (histone deacetylases, which act to repress transcription through removal of acetyl groups on histones) are recruited. Thus, SMADs can act in either an activating or repressive fashion on gene expression dependent on other components of the transcription complex.

The activity of the SMAD pathway is terminated through a number of mechanisms including ubiquitinylation and proteosomic destruction of the SMADs. Specific E3 ligases including SMURF1 and SMURF2 act to increase SMAD and TGF-beta receptor degradation (87 - 91).

Table 1. The Biphasic Role of Transforming Growth Factor Beta in Cancer

Target Cell	Normal Epithelium (Tumor Suppression)	Cancer (Tumor Promotion)
Cell-of-origin (autocrine)	Growth inhibition	Epithelial-to-mesenchymal transition (EMT)
	Apoptosis	Growth stimulation
	Induced senescence	Increased motility
	Maintenance of tissue environment	Increased invasion
Stroma (paracrine)		Increased MMP expression
		Increased angiogenesis
		Immune suppression

The Biphasic Role of Transforming Growth Factor Beta in Cancer. In cancer formation, transforming growth factor beta (TGF-beta) plays a complex role with tumor suppressive effects at early stages of cancer development through growth suppression and tumor enhancement in malignant cancers with both autocrine and paracrine effects on growth, invasion, angiogenesis, and immunosuppression.

3.2. Regulation of TGF-beta Signal Transduction By Other Pathways

TGF-beta signal transduction is heavily influenced by many other cellular pathways (reviewed in 92) – including the Ras (93), Vitamin D (94, 95), wnt (96), phosphatidylinositol 3-kinase (97), interleukin-6 (IL-6) (98), interferon-gamma (IFN-gamma) (99, 100) among others. Non-TGF-beta pathways regulate TGF-beta signal intensity, selection of gene targets and biologic outcome. Of additional complexity, it appears that in some cell types in some cellular conditions, TGF-beta may activate SMAD-independent pathways permitting alternative physiological outcomes or self-regulation of the SMAD pathway.

Several of the pathways that interact directly with the TGF-beta signal pathway are prominent in oncogenesis of brain tumors, including the MAPK pathways. Activation of growth factor receptors increases Ras mediated pathway activity that acts in multiple ways to either increase or decrease SMAD-mediated transcription. MAPK can directly phosphorylate the R-SMADs on a middle, linker region (93). Additionally, MAPK plays a role in the DNA-binding partners of the SMADs through AP-1 family members, jun and fos, and can act to modulate TGF-beta receptor function. Other pathways, including the wnt (96), estrogen (101), interferon-gamma (102), and vitamin D (94, 95) pathways also directly influence TGF-beta function. Some of these pathways act through the induction of SMAD7 (102), one of the inhibitory SMADs.

TGF-beta receptor activation can induce activity of PP2A (103), RhoA (104), ERK, and p38 (105). These pathways have their own effects and can act on the transcription complex formed with the SMADs. Thus, TGF-beta can act by SMAD-independent mechanisms that can cooperate with, inhibit, or act separately from the traditional TGF-beta signal transduction pathways.

Finally, a number of oncogenes have been discovered that act on TGF-beta function, including Ski and SnoN (reviewed in 106). Ski and SnoN can directly interact with SMAD2 and SMAD3 to block TGF-beta-mediated transcriptional activity in two ways: Ski/SnoN displace other transcriptional partners from a transcriptional complex and Ski/SnoN recruit histone deacetylases (HDAC) to repress transcription (107). Ski and SnoN levels exhibit a biphasic relationship with TGF-beta receptor activation – initially Ski and SnoN proteins are decreased through SMURF-mediated ubiquitinylation and proteasome degradation but transcriptional regulation increases levels later suggesting a role in signal termination (108 - 110).

3.3. TGF-beta: Tumor Suppressor or Tumor Enhancer?

The role of TGF-beta in cancer is complex and has been called "paradoxical" (111, 112). The TGF-beta ligand is a tumor suppressor gene early in cancer due to its ability to prevent cellular proliferation and induce apoptosis in some cell types (See Table 1). In malignant cancers TGF-beta acts as a tumor promoter as TGF-beta can mediate a number of effects that support tumor growth – including angiogenesis, increased cell matrix component elaboration, and immunosuppression. The switch of the dominant effects caused by TGF-beta has been the subject of a tremendous amount of study. No one mechanism appears to explain the mechanism by which cancers lose their growth inhibitory response to TGF-beta. Rather, the large number of detailed mechanisms suggests that the inactivation of the tumor suppressive aspects of TGF-beta is critical in a broad range of cancers.

In general, disruption of the normal TGF-beta function can either completely block TGF-beta signaling or selectively target aspects that are growth suppressive. The complete loss of TGF-beta signal transduction is uncommon in most cancers and has not been shown to occur in brain tumors. A mechanism by which TGF-beta function can be disrupted in some cancers, such as colon cancer, involves the loss of expression of the type II receptor (113). This occurs frequently in tumors with abnormal mismatch repair mechanisms. Interestingly, tumors with loss of type II TGF-beta receptor expression often have a favorable prognosis (114).

Most types of cancer employ mechanisms that partially disrupt the normal signaling of TGF-beta, including hyperactive MAPK activity, altered SMAD expression or function, and alterations in target genes (reviewed in 115). Each of these mechanisms may play a role in brain tumors, particularly gliomas. Malignant gliomas have increased Ras/MAPK activity due to increased activity of tyrosine kinase growth factor receptors

not mutant forms of Ras. SMAD mutations have not been found in brain tumors but expression may decline with increasing tumor grade in gliomas. Finally, the genes that are transcriptionally regulated by TGF-beta canbe targeted for deletion or mutation. This will be discussed in the following sections.

3.4 TGF-beta Components in Primary Brain Tumors

Primary brain tumors include a broad range of over one hundred tumor types. These tumors are generally named based on the presumed cell of origin: gliomas (including astrocytomas and oligodendrogliomas) from glia, ependymomas from ependymal cells, and meningiomas from arachnoid cap cell lining the meninges. The cell-of-origin of medulloblastomas and primitive neuroectodermal tumors (PNETs) remain unclear. Although there are many phenotypic differences between these cancer types, primary brain tumors share many common traits among themselves and with other cancer types. Prominent among the requirements for cancer formation and growth include the abilities to avoid growth inhibitory signals, escape immune responses, invade normal structures, and form new blood vessels (reviewed in 1). Each of these functions is impacted by TGF-beta signal transduction. It should, therefore, be expected that many primary brain tumors have altered TGF-beta activity.

All three TGF-beta ligands are expressed in malignant brain tumors, including gliomas, medulloblastomas, ependymomas, and meningiomas (2 - 7, 116 - 118). It is unclear which isoform(s) is most important in brain tumor pathophysiology but most studies have examined the $beta_1$ and $beta_2$ ligands. Interpretation of TGF-beta expression in studies using only cell lines can falsely overestimate TGF-beta expression as one study found that passage of astroglial cell lines increased TGF-$beta_2$ expression levels (119). TGF-beta is also expressed by areas of normal brain in response to the formation of gliomas as measured in a C6 glioma model (120). This reaction by the normal brain is not surprising as TGF-beta is an important regulator of the activity of reactive astrocytes. TGF-beta expression by reactive astrocytes appears to permit an increase in the invasion of these cells and elaboration of new extracellular matrix components while blunting the immune response and astrocyte proliferation (121, 122). Both thrombospondin, which acts to activate latent TGF-beta, and TGF-beta expression have been linked to increased tumor malignancy in gliomas (123). Other mechanisms of TGF-beta activation also are activated in glioma cell lines – the furin-like proteases process pro-TGF-beta to an active form and can be blocked with specific inhibitors (124). TGF-beta-binding protein expression is increased in a carmustine-resistant cell line (125).

Expression of TGF-beta cell surface receptors has been studied in a number of brain tumors. Gliomas, medulloblastomas, primitive neuroectodermal tumors (PNETs), meningiomas and ependymomas tumor samples and cell lines express the TGF-beta type I and II receptors as well as the ligand, which suggests the presence of an autocrine loop (2-7, 117, 126, 127). The accessory receptor endoglin is expressed in tumor endothelium, including gliomas and medulloblastomas (128, 129). No clear link exists between glioma receptor expression level and the tumor malignancy. It does not appear that loss of receptor expression is an important mechanism of resistance to TGF-beta-mediated growth inhibition in glioma cell lines (126, 127).

Smad mutations have not been found in gliomas or medulloblastomas (130, 131) and no systematic evaluation of SMAD expression has found a strong relationship between SMAD expression and response to TGF-beta (126, 127) ? One recent study of different glioma grades by RT-PCR suggests that the expression of SMAD2, 3, and 4 may decrease with increasing glioma tumor grade (5). In an experimental glioma model, we found that the stable expression of a constitutively active form of TGF-$beta_1$ ligand induced decreased expression of SMAD2 and SMAD3 with retained expression of SMAD4 and TGF-beta receptors (J. Rich, unpublished observations).

Ras activity is increased in malignant gliomas in the absence of Ras mutations (132). The relationship of Ras activation in gliomas and resistance to TGF-beta growth inhibition has not been evaluated. Other oncogenes – Ski, SnoN, BF1 – that impact on TGF-beta function have not been evaluated in brain tumors.

4. TGF-BETA AND CELLULAR PROLIFERATION

TGF-beta inhibits the growth of most types of epithelial cells while increasing the proliferation of mesenchymal cells. Thus, the frequent epithelial-to-mesenchymal transition (EMT) associated with cancer development may play an important role in modulating the response to TGF-beta (112). In fact, TGF-beta can induce EMT itself. TGF-beta generally induces a G_0/G_1 cell cycle arrest in most epithelial cell types through the ability to increase the expression or activity of particular cyclin-dependent kinase inhibitors that act to bind and inactivate the cyclin-cyclin dependent kinase (CDK) complexes that regulate cell cycle progression. In some cell types, TGF-beta can induce either cell cycle arrest followed by apoptosis or apoptosis as a primary response. Immune system components are the most prominent cell type in which apoptosis plays a dominant role.

Most primary cell types in the central nervous system are growth inhibited by TGF-beta and may be induced to undergo apoptosis. Astrocytes, the presumed precursor cell of the majority of gliomas, are significantly growth inhibited by TGF-beta treatment (126, 133). This growth inhibition is associated with the induction of the cyclin-dependent kinase inhibitor $p15^{INK4B}$ and a G_0/G_1 cell cycle arrest (126). Further, TGF-beta inhibits astrocyte cell proliferation largely through a SMAD3 pathway as astrocytes derived from mice with disrupted SMAD3 expression failed to exhibit full growth inhibition when treated with TGF-beta (126). Oligodendroglial precursors can undergo apoptosis upon TGF-beta treatment associated with induction of the cyclin-dependent kinase inhibitors $p21^{Waf1}$ and $p27^{Kip1}$ (134). FGF can protect these cells

from the TGF-beta induced apoptotic (134). The role of TGF-beta in neuronal survival or apoptosis is dependent on cellular context. Cerebellar granule neurons undergo apoptosis when exposed to TGF-beta except when exposed to high potassium concentrations (135). Differentiated neurons may actually be protected from apoptotic stimuli by TGF-beta (136). Additionally, TGF-beta can potentiate the neural cell survival mediated by other growth factors (reviewed in 137).

Most glioma cell lines have lost TGF-beta-mediated growth inhibition and some are growth stimulated in response to TGF-beta (126, 127, 138, 139). Two studies have shown that some glioma cell lines may undergo apoptosis if treated with or expressing TGF-beta although this appears to be an exception (140, 141). The loss of growth inhibitory response to TGF-beta in gliomas is associated with increased grade of malignancy (142, 143). Most glioma cell lines do not growth inhibit upon TGF-beta treatment and this is associated with the mutation or promoter methylation of p15INK4B (126), which is frequently co-deleted with the p16INK4A tumor suppressor gene. Many gliomas appear to have a critical target gene (p15INK4B) disrupted as a mechanism of TGF-beta resistance (126). A subset of glioma cell lines is growth stimulated upon TGF-beta treatment. These lines may be hyperdiploid and the resistance may involve PDGF elaboration (144) although other studies have not found this link (126, 127). Other brain tumor cell lines (medulloblastoma, PNET, ependymomas) exhibit resistance to TGF-beta growth inhibition and can be stimulated in their growth as well (7). A study of meningiomas revealed expression of TGF-beta ligand and receptors yet continued sensitivity to TGF-beta-mediated growth inhibition (117). Many issues regarding TGF-beta effects of cell survival remain unanswered. For example, TGF-beta downregulates expression of the tumor suppressor gene Pten/MMAC1/TEP1 (145). It is possible that TGF-beta plays a role in suppressing *PTEN* function in tumors that retain a wild-type Pten genotype.

5. TGF-BETA AND TUMOR MICROENVIRONMENT

There is an increasing recognition of the critical role that the microenvironment plays in the development and growth of nascent tumors (reviewed in 146). Surrounding stromal elements including normal stromal cells, extracellular matrix, and blood vessels largely dictate the survival and growth characteristics of tumors. TGF-beta plays a central role in the regulation of the relationship between a cell and its environment. TGF-beta induces the expression of many components of the extracellular matrix (ECM) as well as cellular adhesion factors. TGF-beta acts in ECM remodeling through regulation of matrix metalloproteinases (MMPs). Additionally, TGF-beta acts in a paracrine fashion to regulate stromal cells, blood vessels, and local immune response. The net result of these interactions in malignant cancers is increased tumor cell invasion and angiogenesis.

5.1. Tumor Cell Invasion

Tumor invasion and metastasis play critical roles in most types of brain tumors. Gliomas rarely metastasize outside of the central nervous system (CNS) but these tumors are highly invasive. Glioma invasion into normal brain prevents curative surgical resection and ultimately leads to failure of "local" therapies directed at the control of the primary site of tumor occurrence. Invasion occurs at an early stage in glioma development. Medulloblastomas, PNETs, and ependymomas are frequently invasive into normal brain and can metastasize both within and outside of the CNS. Invasion into normal brain is a hallmark of the conversion of a meningioma to a malignant phenotype. Although many of the genetic alterations that dysregulate the cell processes of growth and death involved in tumor initiation have been elucidated in recent years, less progress has been made in the complex but critical processes of tumor invasion, metastasis, and angiogenesis. Study of TGF-beta, however, has led to increased understanding of many of these processes. Of note, the regulation of the tumor microenvironment by TGF-beta can permit increased tumor invasion either directly or indirectly.

Experiments with glioma cell lines have generally shown that TGF-beta treatment mediates an invasive glioma phenotype (140, 147). TGF-$beta_1$ induces glioma invasion more readily than TGF-$beta_2$, likely due to differential receptor binding. The effects of TGF-beta involve multiple related mechanisms. TGF-beta upregulates the expression of many components of the ECM that act in tumor invasion, including plasminogen activator inhibitor-1 (PAI-1) (126) and secreted protein, acidic and rich in cysteine (SPARC) (1148) among others. PAI-1 expression has been linked to increased tumor grade in gliomas (149) as well as tumor cell invasion and angiogenesis. SPARC has been linked to a role in glioma cell invasion (150 and J. Rich, unpublished observations) and metastases in medulloblastoma (151). TGF-beta can also act to remodel the ECM through the induction of the matrix metalloproteinases (MMPs), MMP-2 (gelatinase A) and MMP-9 (gelatinase B), in gliomas (152). MMPs can breakdown component of the ECM and cell adhesion molecules, e.g. E-cadherin, permitting increased tumor invasion and motility. The TGF-beta also acts to increase expression of regulators of cell adhesion in gliomas including neural cell adhesion molecule L1 (153) and integrin $alpha_v beta_3$ (the vitronectin cellular receptor) (154). Each of these molecules can mediate an invasive phenotype in gliomas. Integrin $alpha_v beta_3$ is expressed at the leading edge of invading tumors (155), and inhibition of $alpha_v beta_3$ can prevent glioma invasion (154, 156). Integrin $alpha_2$ may also mediate aspects of TGF-beta regulation of glioma invasion as blocking antibodies to $alpha_2$ integrin reversed TGF-beta-mediated invasion (157). TGF-beta can also regulate intracellular pathways that regulate cellular response to interactions with the cellular environment. For example, TGF-beta treatment of U-251MG cells plated on fibronectin increased the expression of paxillin (158), which acts as an adaptor molecule at focal adhesions.

While many studies examine the function of TGF-beta on isolated tumor cell lines, a dominant role of TGF-beta in mediating a tumorigenic phenotype may come from the ability of TGF-beta to alter the behavior of surrounding cells via paracrine mechanisms. For example,

stromal cells secrete much of the tumor-associated MMPs. In fact, the stromal elements may be the primary target of tumor-generated TGF-beta.

5.2. Angiogenesis

Malignant gliomas represent one of the most vascularized cancers with vascular proliferation a hallmark of malignancy. The process of new blood vessel formation is called angiogenesis. The importance of angiogenesis in glioma outcome has been recognized and has been a recent target of therapeutic intervention. In fact, there is mounting evidence that many traditional cancer therapies – including radiation and chemotherapy – target blood vessels. Thus, it is important to increase understanding of the mechanisms regulating vascular proliferation.

TGF-beta has long been recognized to play a role in angiogenesis. The role of TGF-beta in angiogenesis was first recognized after a study in which injection of TGF-beta into newborn mice caused collagen deposition and new blood vessel formation at the site of injection (14). However, the effect of TGF-beta on blood vessel growth is complex with very different effects on measures of angiogenesis in different assays. Endothelial cells are directly growth inhibited by TGF-beta (159). In two-dimensional cultures that mimic microvascular cells at the distal tip of blood vessels, TGF-$beta_1$ inhibits proliferation, increases fibronectin and collagen expression, and decreases tight junction formation. On the other hand, three-dimensional cultures that mimic more mature vessels show that TGF-$beta_1$ has little effect on proliferation but increases tight junction formation and deposition of matrix components (reviewed in 160). Thus, TGF-beta may permit the selective maturation of blood vessels. It is important to remember, however, that most angiogenic assays are not performed from tumor-derived endothelium that may have different biological properties.

The role of TGF-beta in regulating pericyte behavior is less well understood. The co-culture of pericytes and endothelial cells induces the expression of activated TGF-beta (161). Treatment of mesenchymal cells with TGF-beta induces a differentiation towards pericytes (162).

TGF-beta regulates angiogenesis also through the regulation of multiple angiogenic factors. Prominently, TGF-beta can induce VEGF in human glioma cell lines (126). Additionally, expression of MMPs, integrins, PAI-1, and ECM proteins (collagen, fibronectin, laminin, von Willebrand Factor, and vitronectin) as a result of exposure to TGF-beta can induce angiogenesis.

6. TGF-BETA IMMUNE SUPPRESSION

Despite the fact that the brain is an immune privileged organ, CNS neoplasms initiate both local and systemic immunosuppression in the course of their development (reviewed in 163 - 165). The systemic immunosuppressive effects of gliomas in particular have been recognized for decades (166 - 168). Patients with malignant gliomas have decreased cell-mediated immune responses with T-cell lymphopenia, signaling defects in T-cell signaling, and impaired monocytic function associated with low class I and II MHC expression (reviewed in 163 - 165). Patients diagnosed with medulloblastomas have also been found to have systemic lymphopenia associated with tumor recurrence (169). In addition to the systemic immune dysregulation associated with these tumors, local immune reactions are altered despite the presence of infiltrating immune cells. The mechanisms involved in this immunosuppression are likely multi-factorial – including interleukin-10 (IL-10), prostaglandin E_2 (PGE_2), sialic acid-containing glycosphingolipids termed GANGs, and HLA-G (reviewed in 163 - 165) -- but also appear to involve TGF-beta. In fact, the recognition of the role of TGF-$beta_2$ in glioma-mediated immunosuppression antedated its identification as a specific protein (170). TGF-beta plays a pleiotropic role in regulating the immune system including B and T cell, macrophage, and dendritic cell involvement (reviewed in 171). Genetic models have provided evidence of TGF-beta function in immune development. For example, disruption of TGF-$beta_1$ expression is associated with a severe, multi-organ inflammatory process in mice that survive past birth (29, 32). Disruption of TGF-beta effector SMAD3 function leads to inappropriate local immunity (172). As a general concept, TGF-beta induces a block on immune cellular proliferation and differentiation with a particular effect on T cells.

The immune reaction to a tumor is a complex, multi-step process. The initial arm of immune attack on tumors involves the presentation of tumor-associated antigens by antigen presenting cells (APCs) that include dendritic cells, microglia, and macrophage. Naïve, immature APCs are highly efficient in endocytosis but are poor presenters of antigen due to low expression of surface MHC I and II molecules and of co-stimulatory molecules. As APCs mature, they acquire improved capabilities in antigen presentation but lose efficient endocytosis capabilities. APCs can then present antigen to and activate CD4+ helper T lymphocytes. Activated helper T cells elaborate critical cytokines, including interleukin-2 (IL-2), interferon-gamma (IFN-gamma), and tumor necrosis factor-alpha (TNF-alpha) that can activate CD+ cytotoxic T lymphocytes that act as an effector arm to mediate tumor killing.

TGF-beta can act at multiple stages in the cellular immune response process to inhibit the appropriate immune response. TGF-beta inhibits the activation of macrophages and their elaboration of pro-inflammatory cytokines (173). TGF-beta can also inhibit the maturation of dendritic cells into their activated forms (174). Thus, T cells exposed to TGF-beta are not appropriately activated (174) and may undergo apoptosis. TGF-beta also blocks migration of immature dendritic cells in the epidermis known as Langerhans cells from the skin to the lymph node preventing initiation of systemic antigen presentation (175). In addition, the normal upregulation of MHC class II molecules in antigen presentation is blocked with TGF-beta treatment (176).

T cells represent a prominent target of TGF-beta-mediated immune regulation. TGF-beta derived from

gliomas can decrease the function of glioma-derived lymphocytes (177). TGF-beta inhibits the proliferation of T cells *in vitro* (28), in part through the inhibition of IL-2 expression (178). TGF-beta also prevents the activation of naïve T cells in the presence of low levels of antigen. Importantly, TGF-beta can block the differentiation of naïve T cells exposed to optimal levels of antigen into appropriate helper $CD4^+$ and cytotoxic $CD8^+$ cells (179). Thus, these cells are not permitted to adopt a functional state to initiate the full immune response. In particular, a subset of helper T cells critical to initiation of the humoral response designated T_H2 cells are prevented from developing in the presence of TGF-beta. T_H2 cell development is controlled by the expression of Gata-3, the expression of which is inhibited by TGF-beta (179, 180). T_H1 cells that are involved in the cellular immune arm are also inhibited in development by TGF-beta but somewhat less significantly (180).

Thus, multiple arms of the immune response to tumors can be blocked by TGF-beta. Of interest, not only do cancers such as gliomas secrete TGF-beta, but also apoptotic cells and regulatory T cells can secrete TGF-beta. Immune dysfunction has been detected in patients with malignant gliomas consistent with TGF-beta-mediated effects (166- 168). Overall patients can exhibit a systemic immune dysfunction associated with a T cell lymphopenia (166, 181). Further, glioma cells express low levels of MHC class II molecules in response to TGF-beta (182). The role of TGF-beta function in glioma-associated immunosuppression has been shown by studies using antisense oligonucleotides. Gliomas with disrupted TGF-beta expression induce an increased response to lymphokine-activated killer (LAK) cells and tumor control (183, 184).

7. THERAPEUTIC IMPLICATIONS OF TGF-BETA

TGF-beta plays an important tumorigenic role in many of the behaviors of primary brain tumors. Therefore, it is to be expected that neutralizing TGF-beta function will likely offer several advantages in tumor control: decreasing invasion and angiogenesis and inducing an increased immune response. Several interventions have been developed to target TGF-beta activity. Current studies have involved three general approaches: decreasing TGF-beta expression, inhibiting TGF-beta release, or binding active TGF-beta. Expression of antisense oligonucleotides can decrease RNA translation and protein production of the targeted sequence. Constitutive antisense treatment to TGF-beta in a 9L glioma rat model permitted increased tumor-directed immune response and tumor control (183). An antisense oligonucleotide directed to TGF-beta1 has shown preclinical efficacy to increase immunological response to the treated glioma but increased glioma invasion *in vitro* (147, 184). A clinical phase I/II dose escalation trial of antisense oligonucleotide directed to TGF-beta2 (AP12009) determined that the therapy is tolerated and may offer some efficacy with intratumoral injection (185).

Additional therapies involve indirect methods to target TGF-beta secretion or removal. TGF-beta is a common end pathway in many fibrotic diseases. Thus, several agents that have been used in fibrotic diseases appear to function through effects on TGF-beta production. TGF-beta secretion or responsiveness may be modulated by the angiotensin system. Angiotensin-converting-enzyme inhibitors (186) and angiotensin II receptor blockers (187) may offer the ability to block TGF-beta activity but treatment of a renal cell carcinoma cell line with captopril did not alter TGF-beta production but reversed resistance to TGF-beta growth inhibition (188). Captopril treatment decreased glioma cell line invasion *in vitro* associated with a decrease in MMP-2 and MMP-9 expression but the role of TGF-beta in these effects is unclear (189). An anti-allergic agent, N-[3,4-dimethoxycinnamoyl]-anthranilic acid (tranilast), acts to block TGF-beta1 production and blocks growth factor effects on stromal tissues. Treatment of glioma cell lines with tranilast decreases TGF-beta production and *in vitro* measures on glioma invasion (190).

Multiple proteins have been utilized to bind and prevent formation of active TGF-beta complexes. These include soluble type II and III receptors and decorin. Soluble receptors are the extracellular portions of the native receptors. They function by competing with the full-length receptor (191, 192). Decorin and biglycan are both small leucine-rich proteoglycans found in the cartilage (193). Both decorin and biglycan bind TGF-beta but only decorin decreased TGF-beta-mediated fibrosis in a lung fibrosis model (194). Decorin expression in a C6 glioma cell line abrogated tumor growth due at least in part to an immune response as corticosteroid treatment reversed the decorin-mediated effects (195). Decorin may mediate its effects through a non-TGF-beta dependent pathway as neutralizing anti-TGF-beta antibodies failed to recapitulate the results of tumor control achieved with decorin (196).

Small molecule kinase inhibitors are an exciting novel therapeutic modality in oncology. Inhibitors of the TGF-beta receptors are in early preclinical development (197, 198) and may offer the best option in targeting TGF-beta function in chronic therapy, as delivery may be significantly less of a problem than some of the other mentioned therapies. Some caution to all anti-TGF-beta therapies is warranted as they may cause significant systemic side effects due to the critical role that TGF-beta plays in many normal physiological processes.

8. CONCLUSIONS

The complexity of TGF-beta signal transduction and biological activities is daunting but TGF-beta is a critical target in multiple CNS malignancies. The components of the TGF-beta pathway and other signal transduction pathways that interact with TGF-beta signal are rapidly being elucidated and are frequently expressed in human brain tumors. The cell specific nature of the TGF-beta pathway and function demands a thorough examination of TGF-beta pathway components in primary nervous system tissues and the corresponding malignancies. Activation of the TGF-beta pathway in malignant brain tumors is associated with a variety of effects conducive to tumor growth, including tumor cell

invasion, neoangiogenesis, and immune suppression. The future may hold significant advances in brain tumor control through targeted therapies of TGF-beta function likely in combination with other therapeutic modalities.

9. ACKNOWLEDGMENTS

J.N.R. is the recipient of NIH K08 NS02055 and is supported by the Pediatric Brain Tumor Foundation of the United States, Accelerate Brain Cancer Cure, and Finding The Cures in Glioblastoma. Xiao-Fan Wang and Darell Bigner provided helpful discussions.

10. REFERENCES

1. Hanahan D, & R. A. Weinberg: The hallmarks of cancer. *Cell* 100, 57-70 (2000)
2. Constam DB, J. Philipp, U.V. Malipiero, P.T. Dijke, M. Schachner, & A. Fontana: Differential expression of transforming growth factor-ß1, -ß2, and -ß3 by glioblastoma cells, astrocytes, and microglia. *J Immunology* 148, 1404-1410 (1992)
3. Stiles JD, P.T. Ostrow, L.L. Balos, S.J. Greenberg, R. Plunkett, W. Grand, & R.R. Heffner: Correlation of endothelin-1 and transforming growth factor-ß1 with malignancy and vascularity in human gliomas. *J Neuropath Exp Neurol* 56, 435-439 (1997)
4. Ilyin SE, Gonzalez-Gomez I, Gilles FH, Plata-Salaman CR. Interleukin-1 alpha (IL-1 alpha), IL-1 beta, IL-1 receptor type I, IL-1 receptor antagonist, and TGF-beta 1 mRNAs in pediatric astrocytomas, ependymomas, and primitive neuroectodermal tumors. *Mol Chem Neuropathol* 33, 125-137 (1998)
5. Kjellman C, S.P. Olofsson, O. Hansson, T. Von Schantz, M. Lindvall, I. Nilsson, L.G. Salford, H.O. Sjogren, & B. Widegren: Expression of TGF-beta isoforms, TGF-beta receptors, and SMAD molecules at different stages of human glioma. *Int J Cancer* 89, 251-258 (2000)
6. Boyle-Walsh E, M. Birch, J.A. Gallagher, V. Speirs, M.C. White, A. Shenkin, & W.D. Fraser: RT-PCR detection of cytokine transcripts in a series of cultured human meningiomas. *J Pathol* 178, 442-446 (1996)
7. Jennings MT, I.T. Kaariainen, L. Gold, R.J. Maciunas, & P.A. Commers: TGF beta 1 and TGF beta 2 are potential growth regulators for medulloblastomas, primitive neuroectodermal tumors, and ependymomas: evidence in support of an autocrine hypothesis. *Hum Pathol* 25, 464-75 (1994)
8. Boyd F, & J. Massague. Transforming Growth Factor-beta inhibition of epithelial cell proliferation linked to the expression of a 53-kDa membrane receptor. *J Biol Chem* 264, 2272-2278 (1989)
9. Sporn MB, A.B. Roberts, L.M. Wakefield, & R.K. Assoian: Transforming growth factor-beta: biological function and chemical structure. *Science* 233, 532-534 (1986)
10. Yingling JM, P. Das, C. Savage, M. Zhang, R.W. Padgett, & X.F. Wang: Mammalian dwarfins are phosphorylated in response to TGF-beta and are implicated in control of cell growth. *Proc Natl Acad Sci USA* 93, 8940-8944 (1996)
11. Massague J, S. Cheifetz, T. Endo, & B. Nadal-Ginard: Type beta transforming growth factor is an inhibitor of myogenic differentiation. *Proc Natl Acad Sci USA* 83, 8206-8210 (1986)
12. Masui T, L.M. Wakefield, J.F. Lechner, M.A. LaVeck, M.B. Sporn, & C.C. Harris: Type beta transforming growth factor is the primary differentiation-inducing serum factor for normal human bronchial epithelial cells. *Proc Natl Acad Sci USA* 83, 2438-2442 (1986)
13. Olson EN, E. Sternberg, J.S. Hu, G. Spizz, & C. Wilcox: Regulation of myogenic differentiation by type beta transforming growth factor. *J Cell Biol* 103, 1799-1805 (1986)
14. Roberts AB, M.B. Sporn, R.K. Assoian, J.M. Smith, N.S. Roche, L.M. Wakefield, U.I. Heine, L.A. Liotta, V. Falanga, J.H. Kehrl, et al.: Transforming growth factor type beta: rapid induction of fibrosis and angiogenesis in vivo and stimulation of collagen formation in vitro. *Proc Natl Acad Sci USA* 83, 4167-4171 (1986)
15. Yang EY & H.L. Moses: Transforming growth factor beta 1-induced changes in cell migration, proliferation, and angiogenesis in the chicken chorioallantoic membrane. *J Cell Biol* 111, 731-741 (1990)
16. Yang X, L.H. Castilla, X. Xu, C. Li, J. Gotay, M. Weinstein, P.P. Liu, & C.X. Deng: Angiogenesis defects and mesenchymal apoptosis in mice lacking Smad5. *Development* 126, 1571-1580 (1999)
17. Broder WA, & N.A. Noble: Transforming growth factor beta in tissue fibrosis. *N Engl J Med* 331, 1286-1292 (1994)
18. Broder WA, & E. Ruoslahti: Transforming growth factor-beta in disease: the dark side of tissue repair. *J Clin Invest* 90, 1-7 (1992)
19. Edwards DR, G. Murphy, J.J. Reynolds, S.E. Whitham, A.J.P. Docherty, P. Angel, & J.K. Heath: Transforming growth factor beta modulates the expression of collagenase and metalloproteinase inhibitor. *EMBO J* 6, 1899-1904 (1987)
20. Fine A, & R.H. Goldstein: The effect of transforming growth factor-beta on cell proliferation and collagen formation by lung fibroblasts. *J Biol Chem* 262, 3897-3902 (1987)
21. Ignotz RA, & J. Massague: Transforming growth factor-beta stimulates the expression of fibronectin and collagen and their incorporation into the extracellular matrix. *J Biol Chem* 261, 4337-4345 (1986)
22. Polyak K, J. Kato, M.J. Solomon, C.J. Sherr, J. Massague, J.M. Roberts, & A. Koff: p27Kip1, a cyclin-cdk inhibitor, links transforming growth factor-beta and contact inhibition to cell cycle arrest. *Genes Dev* 8, 9-22 (1994)
23. Welch DR, Fabra A, Nakajima M. Transforming growth factor beta stimulates mammary adenocarcinoma cell invasion and metastatic potential. *Proc Natl Acad Sci USA* 87, 7678-7682 (1990)
24. Dang H, A.G. Geiser, J.J. Letterio, T. Nakabayashi, L. Kong, G. Fernandes, & N. Talal: SLE-like autoantibodies and Sjogren's syndrome-like lymphoproliferation in TGF-beta knockout mice. *J Immunol* 155, 3205-3212 (1995)
25. Diebold RJ, M.J. Eis, M. Yin, I. Ormsby, G.P. Boivin, B.J. Darrow, J.E. Saffitz, & T. Doetschman: Early-onset multifocal inflammation in the transforming growth factor

beta1-null mouse is lymphocyte mediated. *Proc Natl Acad Sci USA* 92, 12215-12219 (1995)
26. Geiser AG, J.J. Letterio, A.B. Kulkarni, S. Karlsson, A.B. Roberts, & M.B. Sporn: Transforming growth factor beta 1 (TGF-beta 1) controls expression of major histocompatibility genes in the postnatal mouse: aberrant histocompatibility antigen expression in the pathogenesis of the TGF-beta1 null mouse phenotype. *Proc Natl Acad Sci USA* 90, 9944-9948 (1993)
27. Kehrl JH, A.B. Roberts, L.M. Wakefield, S. Jakowlew, M.B. Sporn, & A.S. Fauci: Transforming growth factor beta is an important immunomodulatory protein for human B lymphocytes. *J Immunol* 137, 3855-3860 (1986)
28. Kehrl JH, L.M. Wakefield, A.B. Roberts, S. Jakowlew, M. Alvarez-Mon, R. Derynck, M.B. Sporn, & A.S. Fauci: Production of transforming growth factor beta by human T lymphocytes and its potential role in the regulation of T cell growth. *J Exp Med* 163, 1037-1050 (1986)
29. Kulkarni AB, C.G. Huh, D. Becker, A. Geiser, M. Lyght, K.C. Flanders, A.B. Roberts, M.B. Sporn, J.M. Ward, & S. Karlsson: Transforming growth factor beta1 null mutation in mice causes excessive inflammatory response and early death. *Proc Natl Acad Sci USA* 90, 770-774 (1993)
30. Kulkarni AB, & S. Karlsson: Inflammation and TGF-beta1: lessons from the TGF-beta1 null mouse. *Res Immunol* 148, 453-456 (1997)
31. Letterio JJ, A.G. Geiser, A.B. Kulkarni, H. Dang, L. Kong, T. Nakabayashi, C.L. Mackall, R.E. Gress, & A.B. Roberts: Autoimmunity associated with TGF-beta1-deficiency in mice is dependent on MHC class II antigen expression. *J Clin Invest* 98, 2109-2119 (1996)
32. Shull MM, I. Ormsby, A.B. Kier, S. Pawlowski, R.J. Diebold, M. Yin, R. Allen, C. Sidman, G. Proetzel, & D. Calvin: Targeted disruption of the mouse transforming growth factor-beta 1 gene results in multifocal inflammatory disease. *Nature* 359, 693-699 (1992)
33. Kingsley DM: The TGF-beta superfamily: New members, new receptors, and new genetic tests of function in different organisms. *Genes Dev* 8, 133-146 (1994)
34. Munoz-Sanjuan I & A.H. Brivanlou: Neural induction, the default model and embryonic stem cells. *Nat Rev Neurosci* 3, 271-280 (2002)
35. Bottner M, K. Krieglstein, & K. Unsicker: The transforming growth factor-betas: structure, signaling, and roles in nervous system development and functions. *J Neurochem* 75, 2227-2240 (2000)
36. Zhao B & J.P. Schwartz: Involvement of cytokines in normal CNS development and neurological diseases: recent progress and perspectives. *J Neurosci Res* 52, 7-16 (1998)
37. Roberts AB, & MB Sporn: The transforming growth factor-beta's. In: Handbook of Experimental Pharmacology, Peptide Growth Factors and their Receptors. Eds: Sporn MB, Roberts AB, Springer, Heidelberg, Germany (1990)
38. Archer SJ, A. Bax, A.B. Roberts, M.B. Sporn, Y. Ogawa, K.A. Piez, J.A. Weatherbee, M.L. Tsang, R. Lucas, B.L. Zheng, J. Wenker, & D.A. Torchia: Transforming growth factor beta 1: secondary structure as determined by heteronuclear magnetic resonance spectroscopy. *Biochemistry* 32, 1164-1171 (1993)
39. Daopin S, K.A. Piez, Y. Ogawa, & D.R. Davies: Crystal structure of transforming growth factor beta2: An unusual fold for the superfamily. *Science* 257, 369-373 (1992)
40. Schlunegger MP, & M.G. Grutter: An unusual feature revealed by the crystal structure of 2.2 A resolution of human transforming growth factor beta2. Nature 358, 430-434 (1992)
41. Jennings JC, S. Mohan, T.A. Linkhart, R. Widstrom, & D.J. Baylink: Comparison of the biological actions of TGF beta-1 and TGF beta-2: differential activity in endothelial cells. *J Cell Physiol* 137, 167-172 (1988)
42. Ohta M, J.S. Greenberger, P. Anklesaria, A. Bassols, & J. Massague: Two forms of transforming growth factor-beta distinguished by multipotential haematopoietic progenitor cells. *Nature* 329, 539-541 (1987)
43. Suardet L, A.C. Gaide, J.M. Calmes, B. Sordat, J.C. Givel, J.F. Eliason, & N. Odartchenko: Responsiveness of three newly established human colorectal cancer cell lines to transforming growth factors beta 1 and beta 2. *Cancer Res* 52, 3705-3712 (1992)
44. Qian SW, J.K. Burmester, M.L. Tsang, J.A. Weatherbee, A.P. Hinck, D.J. Ohlsen, M.B. Sporn, & A.B. Roberts: Binding affinity of transforming growth factor-beta for its type II receptor is determined by the C-terminal region of the molecule. *J Biol Chem* 271, 30656-30662 (1996)
45. Geiser AG, S.J. Kim, A.B. Roberts, & M.B. Sporn: Characterization of the mouse transforming growth factor-beta 1 promoter and activation by the Ha-ras oncogene. *Mol Cell Biol* 11, 84-92 (1991)
46. Kim SJ, A. Glick, M.B. Sporn, & A.B. Roberts: Characterization of the promoter region of the human transforming growth factor-beta 1 gene. *J Biol Chem* 264, 402-408 (1989)
47. Lafyatis R, R. Lechleider, S.J. Kim, S. Jakowlew, A.B. Roberts, & M.B. Sporn: Structural and functional characterization of the transforming growth factor beta 3 promoter. A cAMP-responsive element regulates basal and induced transcription. *J Biol Chem* 265, 19128-19136 (1990)
48. Noma T, A.B. Glick, A.G. Geiser, M.A. O'Reilly, J. Miller, A.B. Roberts, & M.B. Sporn: Molecular cloning and structure of the human transforming growth factor-beta 2 gene promoter. *Growth Factors* 4, 247-255 (1991)
49. Koli K, J. Saharinen, M. Hyytiainen, C. Penttinen, & J. Keski-Oja: Latency, activation, and binding proteins of TGF-beta. *Microsc Res Tech* 52, 354-362 (2001)
50. Dubois CM, M.H. Laprise, F. Blanchette, L.E. Gentry, & R. Leduc: Processing of transforming growth factor beta 1 precursor by human furin convertase. *J Biol Chem* 270, 10618-10624 (1995)
51. Gentry LE, M.N. Lioubin, A.F. Purchio, & H. Marquardt: Molecular events in the processing of recombinant type 1 pre-pro-transforming growth factor beta to the mature polypeptide. *Mol Cell Biol* 8, 4162-4168 (1988)
52. Olofsson A, K. Miyazono, T. Kanzaki, P. Colosetti, U. Engstrom, & C.H. Heldin: Transforming growth factor-beta 1, -beta 2, and -beta 3 secreted by a human glioblastoma cell line. Identification of small and different forms of large latent complexes. *J Biol Chem* 267, 19482-19488 (1992)
53. Sterner-Kock A, I.S. Thorey, K. Koli, F. Wempe, J. Otte, T. Bangsow, K. Kuhlmeier, T. Kirchner, S. Jin, J.

Keski-Oja, & H. Von Melchner: Disruption of the gene encoding the latent transforming growth factor-beta binding protein 4 (LTBP-4) causes abnormal lung development, cardiomyopathy, and colorectal cancer. *Genes Dev* 16, 2264-2273 (2002)
54. Khalil N: TGF-beta: from latent to active. *Microbes Infect* 1, 1255-1263 (1999)
55. Cheifetz S, T. Bellon, C. Cales, S. Vera, C. Bernabeu, J. Massague, & M. Letarte: Endoglin is a component of the transforming growth factor beta receptor system in human endothelial cells. *J Biol Chem* 267, 17225-17228 (1992)
56. Lopez-Casillas F, S. Cheifetz, J. Doody, J.L. Andres, W.S. Lane, & J. Massague: Structure and expression of the membrane proteoglycan betaglycan, a component of the TGF-beta receptor system. *Cell* 67, 785-795 (1991)
57. Lopez-Casillas F, J.L. Wrana, & J. Massague: Betaglycan presents ligand to the TGF-beta signaling receptor. *Cell* 73, 1435-1444 (1993)
58. Lopez-Casillas F, H.M. Payne, J.L. Andres, & J. Massague: Betaglycan can act as a dual modulator of TGF-beta access to signaling receptors: mapping of ligand binding and GAG attachment sites. *J Cell Biol* 124, 557-568 (1994)
59. Massague J, S. Cheifetz, F.T. Boyd, & J.L. Andres: TGF-beta receptors and TGF-beta binding proteoglycans: Recent progress in identifying their functional properties. *Ann NY Acad Sci* 593, 59-72 (1990)
60. Wrana JL, L. Attisano, R. Wieser, F. Ventura, & J. Massague: Mechanism of activation of the TGF-beta receptor. *Nature* 370, 341-347 (1994)
61. Chen RH, & R. Derynck: Homomeric interactions between type II transforming growth factor-beta receptors. *J Biol Chem* 269, 22868-22874 (1994)
62. Ebner R, R.H. Chen, L. Shum, S. Lawler, T.F. Zioncheck, A. Lee, A.R. Lopez, & R. Derynck: Cloning of a type I TGF-beta receptor and its effect on TGF-beta binding to the type II receptor. *Science* 260, 1344-1348 (1993)
63. Franzen P, P. ten Dijke, H. Ichijo, H. Yamashita, P. Schulz, C.H. Heldin, & K. Miyazono: Cloning of a TGF-beta type I receptor that forms a heteromeric complex with the TGF-beta type II receptor. *Cell* 75, 681-692 (1993)
64. Henis YI, A. Moustakas, H.Y. Lin, & H.F. Lodish: The types II and III transforming growth factor beta receptors form homo-oligomers. *J Cell Bio* 126, 139-154 (1994)
65. Wrana JL, L. Attisano, J. Carcamo, A. Zentella, J. Doody, M. Laiho, X.F. Wang, & J. Massague: TGF-beta signals through a heteromeric protein kinase receptor complex. *Cell* 71, 1003-1014 (1992)
66. Attisano L, J. Carcamo, F. Ventura, F.M.B. Weis, J. Massague, & J.L. Wrana: Identification of human activin and TGF-beta type I receptors that form heteromeric kinase complexes with type II receptors. *Cell* 75, 671-680 (1993)
67. Lu KX, & H.F. Lodish: Signaling by chimeric erythropoietin- TGF-beta receptors: Homodimerization of the cytoplasmic domain of the type I TGF-beta receptor and heterodimerization with the type II receptor are both required for intracellular signal transduction. *EMBO J* 15, 4485-4496 (1996)
68. Rodriguez C, F. Chen, R.A. Weinberg, & H.F. Lodish: Cooperative binding of transforming growth factor (TGF)-beta2 to the types I and II TGF-beta receptors. *J Biol Chem* 270, 15919-15922 (1995)
69. Weis-Garcia F, & J. Massague: Complementation between kinase-defective and activation-defective receptors TGF-beta reveals a novel form of receptor cooperativity essential for signaling. *EMBO J* 15, 276-289 (1996)
70. Lawler S, X.H. Feng, R.H. Chen, E.M. Maruoka, C.W. Turck, I. Griswold-Prenner, & R. Derynck: The type II transforming growth factor-beta receptor autophosphorylates not only on serine and threonine but also on tyrosine residues. *J Biol Chem* 272, 14850-14859 (1997)
71. Lin HY, X.F. Wang, E. Ng-Eaton, R.A. Weinberg, & H.F. Lodish: Expression cloning of the TGF-beta type II receptor, a functional transmembrane serine/threonine kinase. *Cell* 68, 775-785 (1992)
72. Nakamura T, K. Sugino, N. Kurosawa, M. Sawai, K. Takio, Y. Eto, S. Iwashita, M. Muramatsu, K. Titani, & H. Sugino: Isolation and characterization of activin receptor from mouse embryonal carcinoma cells: Identification of its serine/threonine/tyrosine protein kinase activity. *J Biol Chem* 267, 18924-18928 (1992)
73. Souchelnytskyi S, K. Tamaki, U. Engstrom, C. Wernstedt, P. ten Dijke, & C.H. Heldin: Phosphorylation of ser465 and Ser467 in the C terminus of Smad2 mediates interaction with Smad4 and is required for transforming growth factor-beta signaling. *J Bio Chem* 272, 28107-28115 (1997)
74. Weiser R, J.L. Wrana, & J. Massague: GS domain mutations that constitutively activate TGFbetaR-I, the downstream signaling component in the TGF-beta receptor complex. *EMBO J* 14, 2199-2208 (1995)
75. Savage C, P. Das, A.L. Finelli, S.R. Townsend, C.Y. Sun, S.E. Baird, & R.W. Padgett: Caenorhabditis elegans genes sma-2, sma-3, and sma-4 define a conserved family of transforming growth factor beta pathway components. *Proc Natl Acad Sci USA* 93, 790-794 (1996)
76. Raftery LA, V. Twombly, K. Wharton, & W.M. Gelbart: Genetic screens to identify elements of the decapentaplegic signaling pathway in Drosphila. *Genetics* 139, 241-254 (1995)
77. Sekelsky JJ, S.J. Newfeld, L.A. Raftery, E.H. Chartoff, & W.M. Gelbart: Genetic characterization and cloning of mothers against dpp, a gene required for decapentaplegic function in Drosophila melanogaster. *Genetics* 139, 1347-1358 (1995)
78. Baker J, & R.M. Harland: A novel mesoderm inducer, Madr2, functions in the activin signal transduction pathway. *Genes Dev* 10, 1880-1889 (1996)
79. Chen Y, J.J. Lebrun, & W. Vale: Regulation of transforming growth factor beta- and activin-induced transcription by mammalian Mad proteins. *Proc Natl Acad Sci USA* 93, 12992-12997 (1996)
80. Eppert K, S.W. Scherer, H. Ozcelik, R. Pirone, P. Hoodless, H. Kim, L.W. Tsui, B. Bapat, S. Gallinger, I.L. Andrulis, G.H. Thomsen, J.L. Wrana, & L. Attisano: MADR2 maps to 18q21 and encodes a TGFbeta-regulated MAD-related protein that is functionally mutated in colorectal carcinoma. *Cell* 86, 543-552 (1996)
81. Graff JM, A. Bansal, & D.A. Melton: Xenopus Mad proteins transduce distinct subsets of signals for the TGF beta superfamily. *Cell* 85, 479-487 (1996)
82. Nakao A, T. Imamura, S. Souchelnytskyi, M. Kawabata, A. Ishisaki, E. Oeda, K. Tamaki, J. Hanai, C.H.

Heldin, K. Miyazono, & P. ten Dijke: TGF-beta receptor-mediated signalling through Smad2, Smad3 and Smad4. *EMBO J* 16, 5252-5262 (1997)
83. Nakao A, E. Roijer, T. Imamura, S. Souchelnytskyi, G. Stenman, C.H. Heldin, & P. ten Dijke: Identification of Smad2, a human Mad-related protein in the transforming growth factor beta signaling pathway. J Biol Chem 272, 2896-2900 (1997)
84. Zhang Y, X.H. Feng, R.Y. Wu, & R. Derynck: Receptor-associated Mad homologues synergize as effectors of the TGF-beta response. *Nature* 383, 168-172 (1996)
85. Jonk LJ, S. Itoh, C.H. Heldin, P. ten Dijke, & W. Kruijer: Identification and functional characterization of a Smad binding element (SBE) in the JunB promoter that acts as a transforming growth factor-beta, activin, and bone morphogenetic protein-inducible enhancer. *J Biol Chem* 273, 21145-21152 (1998)
86. Ten Dijke P, M.J. Goumans, F. Itoh, & S. Itoh: Regulation of cell proliferation by Smad proteins. *J Cell Physiol* 191, 1-16 (2002)
87. Zhu H, P. Kavsak, S. Abdollah, J.L. Wrana, & G.H. Thomsen: A SMAD ubiquitin ligase targets the BMP pathway and affects embryonic pattern formation. *Nature* 400, 687-693 (1999)
88. Zhang Y, C. Chang, D.J. Gehling, A. Hemmati-Brivanlou, & R. Derynck: Regulation of Smad degradation and activity by Smurf2, an E3 ubiquitin ligase. *Proc Natl Acad Sci USA* 98, 974-979 (2001)
89. Kavsak P, R.K. Rasmussen, C.G. Causing, S. Bonni, H. Zhu, G.H. Thomsen, & J.L. Wrana: Smad7 binds to Smurf2 to form an E3 ubiquitin ligase that targets the TGF beta receptor for degradation. *Mol Cell* 6, 1365-1375 (2000)
90. Ebisawa T, M. Fukuchi, G. Murakami, T. Chiba, K. Tanaka, T. Imamura, & K. Miyazono: Smurf1 interacts with transforming growth factor-beta type I receptor through Smad7 and induces receptor degradation. *J Biol Chem* 276, 12477-12480 (2001)
91. Podos SD, K.K. Hanson, Y.C. Wang, & E.L. Ferguson: The DSmurf ubiquitin-protein ligase restricts BMP signaling spatially and temporally during Drosophila embryogenesis. *Dev Cell* 1, 567-78 (2001)
92. Zhang Y, & R. Derynck: Regulation of Smad signalling by protein associations and signalling crosstalk. *Trends Cell Biol* 9, 274-279 (1999)
93. Kretzschmar M, J. Doody, I. Timokhina, & J. Massague: A mechanism of repression of TGFbeta/ Smad signaling by oncogenic Ras. *Genes Dev* 13, 804-816 (1999)
94. Yanagisawa J, Y. Yanagi, Y. Masuhiro, M. Suzawa, M. Watanabe, K. Kashiwagi, T. Toriyabe, M. Kawabata, K. Miyazono, & S. Kato: Convergence of transforming growth factor-beta and vitamin D signaling pathways on SMAD transcriptional coactivators. *Science* 283, 1317-1321 (1999)
95. Subramaniam N, G.M. Leong, T.A. Cock, J.L. Flanagan, C. Fong, J.A. Eisman, & A.P. Kouzmenko: Cross-talk between 1,25-dihydroxyvitamin D3 and transforming growth factor-beta signaling requires binding of VDR and Smad3 proteins to their cognate DNA recognition elements. *J Biol Chem* 276, 15741-15746 (2001)
96. Labbe E, A. Letamendia, & L. Attisano: Association of Smads with lymphoid enhancer binding factor 1/T cell-specific factor mediates cooperative signaling by the transforming growth factor-beta and wnt pathways. *Proc Natl Acad Sci USA* 97, 8358-8363 (2000)
97. Bakin AV, A.K. Tomlinson, N.A. Bhowmick, H.L. Moses, & C.L. Arteaga: Phosphatidylinositol 3-kinase function is required for transforming growth factor beta-mediated epithelial to mesenchymal transition and cell migration. *J Biol Chem* 275, 36803-36810 (2000)
98. Yanagisawa M, K. Nakashima, T. Takizawa, W. Ochiai, H. Arakawa, & T. Taga: Signaling crosstalk underlying synergistic induction of astrocyte differentiation by BMPs and IL-6 family of cytokines. *FEBS Lett* 489, 139-143 (2001)
99. Ulloa L, J. Doody, & J. Massague: Inhibition of transforming growth factor-beta/SMAD signalling by the interferon-gamma/STAT pathway. *Nature* 397, 710-713 (1999)
100. Ghosh AK, W. Yuan, Y. Mori, Sj. Chen, & J. Varga: Antagonistic regulation of type I collagen gene expression by interferon-gamma and transforming growth factor-beta. Integration at the level of p300/CBP transcriptional coactivators. *J Biol Chem* 276, 11041-11048 (2001)
101. Yamamoto T, F. Saatcioglu, & T. Matsuda: Cross-talk between bone morphogenic proteins and estrogen receptor signaling. *Endocrinology* 143, 2635-2642 (2002)
102. Ulloa L, J. Doody, & J. Massague: Inhibition of transforming growth factor-beta/SMAD signalling by the interferon-gamma/STAT pathway. *Nature* 397, 710-713 (1999)
103. Petritsch C, H. Beug, A. Balmain, & M. Oft: TGF-beta inhibits p70 S6 kinase via protein phosphatase 2A to induce G(1) arrest. *Genes Dev* 14, 3093-3101 (2000)
104. Bhowmick NA, M. Ghiassi, A. Bakin, M. Aakre, C.A. Lundquist, M.E. Engel, C.L. Arteaga, & H.L. Moses: Transforming growth factor-beta1 mediates epithelial to mesenchymal transdifferentiation through a RhoA-dependent mechanism. *Mol Biol Cell* 12, 27-36 (2001)
105. Hanafusa H, J. Ninomiya-Tsuji, N. Masuyama, M. Nishita, J. Fujisawa, H. Shibuya, K. Matsumoto, & E. Nishida: Involvement of the p38 mitogen-activated protein kinase pathway in transforming growth factor-beta-induced gene expression. *J Biol Chem* 274, 27161-7 (1999)
106. Liu X, Y. Sun, R.A. Weinberg, & H.F. Lodish: Ski/Sno and TGF-beta signaling. *Cytokine Growth Factor Rev* 12, 1-8 (2001)
107. Stroschein SL, W. Wang, S. Zhou, Q. Zhou, & K. Luo: Negative feedback regulation of TGF-beta signaling by the SnoN oncoprotein. *Science* 286, 771-774 (1999)
108. Sun Y, X. Liu, E. Ng-Eaton, H.F. Lodish, & R.A. Weinberg: SnoN and Ski protooncoproteins are rapidly degraded in response to transforming growth factor beta signaling. *Proc Natl Acad Sci USA* 96, 12442-12447 (1999)
109. Bonni S, H.R. Wang, C.G. Causing, P. Kavsak, S.L. Stroschein, K. Luo, & J.L.Wrana: TGF-beta induces assembly of a Smad2-Smurf2 ubiquitin ligase complex that targets SnoN for degradation. *Nat Cell Biol* 3, 587-595 (2001)
110. Stroschein SL, S. Bonni, J.L. Wrana, & K. Luo: Smad3 recruits the anaphase-promoting complex for

ubiquitination and degradation of SnoN. *Genes Dev* 15, 2822-2836 (2001)
111. Chang H-L, N. Gillett, I. Figari, A.R. Lopez, M.A. Palladino, & R. Derynck: Increased transforming growth factor ß expression inhibits cell proliferation in vitro, yet increases tumorigenicity and tumor growth of Meth A sarcoma cells. *Cancer Res* 53, 4391-4398 (1993)
112. Cui W, D.J. Fowlis, S. Bryson, E. Duffie, H. Ireland, A. Balmain, & R.J. Akhurst: TGF-ß1 inhibits the formation of benign skin tumors, but enhances progression to invasive spindle carcinomas in transgenic mice. *Cell* 86, 531-542 (1996).
113. Markowitz S, J. Wang, L. Myeroff, R. Parsons, L. Sun, J. Lutterbaugh, R.S. Fan, E. Zborowska, K.W. Kinzler, & B. Vogelstein. Inactivation of the type II TGF-beta receptor in colon cancer cells with microsatellite instability. *Science* 268, 1336-1338 (1995)
114. Watanabe T, T.T. Wu, P.J. Catalano, T. Ueki, R. Satriano, D.G. Haller, A.B. Benson 3rd, & S.R. Hamilton: Molecular predictors of survival after adjuvant chemotherapy for colon cancer. *N Engl J Med* 344, 1196-1206 (2001)
115. Wakefield LM, & A.B. Roberts. TGF-beta signaling: positive and negative effects on tumorigenesis. *Curr Opin Genet Dev* 12, 22-29 (2002)
116. Mapstone T, M. McMichael, & D. Goldthwait: Expression of platelet-derived growth factors, transforming growth factors, and the ros gene in a variety of primary human brain tumors. *Neurosurgery* 28, 216-222 (1991)
117. Johnson MD, C.F. Federspiel, L.I. Gold, & H.L. Moses: Transforming growth factor-beta and transforming growth factor beta-receptor expression in human meningioma cells. *Am J Pathol* 141, 633-642 (1992)
118. Boyle-Walsh E, M. Birch, J.A. Gallagher, V. Speirs, M.C. White, A. Shenkin, & W.D. Fraser: RT-PCR detection of cytokine transcripts in a series of cultured human meningiomas. *J Pathol* 178, 442-446 (1996)
119. Anderson RC, J.B. Elder, M.D. Brown, C.E. Mandigo, A.T. Parsa, P.D. Kim, P. Senatus, D.E. Anderson, & J.N. Bruce: Changes in the immunologic phenotype of human malignant glioma cells after passaging in vitro. *Clin Immunol* 102, 84-95 (2002)
120. Ilyin SE, D. Gayle, I. Gonzalez-Gomez, M.E. Miele, & C.R. Plata-Salaman: Brain tumor development in rats is associated with changes in central nervous system cytokine and neuropeptide systems. *Brain Res Bull* 48, 363-73 (1999)
121. Lindholm D, E. Castren, R. Kiefer, F. Zafra, & H. Thoenen: Transforming growth factor-beta 1 in the rat brain: increase after injury and inhibition of astrocyte proliferation. *J Cell Biol* 117, 395-400 (1992)
122. Morganti-Kossmann MC, T. Kossmann, M.E. Brandes, S.E. Mergenhagen, & S.M. Wahl: Autocrine and paracrine regulation of astrocyte function by transforming growth factor-beta. *J Neuroimmunol* 39, 163-173 (1992)
123. Kawataki T, H. Naganuma, A. Sasaki, H. Yoshikawa, K. Tasaka, H. Nukui: Correlation of thrombospondin-1 and transforming growth factor-beta expression with malignancy of glioma. *Neuropathology* 20, 161-169 (2000)
124. Leitlein J, S. Aulwurm, R. Waltereit, U. Naumann, B. Wagenknecht, W. Garten, M. Weller, & M. Platten: Processing of immunosuppressive pro-TGF-beta 1,2 by human glioblastoma cells involves cytoplasmic and secreted furin-like proteases. *J Immunol* 166, 7238-43 (2001)
125. Norman SA, S.N. Rhodes, S. Treasurywala, D.B. Hoelzinger, J. Rankin Shapiro, & A.C. Scheck: Identification of transforming growth factor-beta1-binding protein overexpression in carmustine-resistant glioma cells by MRNA differential display. *Cancer* 89, 850-862 (2000)
126. Rich JN, M. Zhang, M.B. Datto, D.D. Bigner, & X.F. Wang. Transforming growth factor-beta-mediated p15(INK4B) induction and growth inhibition in astrocytes is SMAD3-dependent and a pathway prominently altered in human glioma cell lines. *J Biol Chem* 274, 35053-35058 (1999)
127. Piek E, U. Westermark, M. Kastemar, C.-H. Heldin, E.J. van Zoelen, M. Nister, & P. Ten Dijke: Expression of transforming-growth-factor (TGF)-ß receptors and Smad proteins in glioblastoma cell lines with distinct responses to TGF-ß1. *Int J Cancer* 80, 756-763 (1999)
128. Bodey B, B. Bodey Jr, S.E. Siegel, & H.E. Kaiser: Upregulation of endoglin (CD105) expression during childhood brain tumor-related angiogenesis. Anti-angiogenic therapy. *Anticancer Res* 18, 1485-1500 (1998)
129. Balza E, P. Castellani, A. Zijlstra, D. Neri, L. Zardi, & A. Siri: Lack of specificity of endoglin expression for tumor blood vessels. *Int J Cancer* 94, 579-85 (2001)
130. Riggins GJ, K.W. Kinzler, B. Vogelstein, & S. Thiagalingam: Frequency of Smad gene mutations in human cancers. *Cancer Res* 57, 2578-2580 (1997)
131. Zurawel RH, C. Allen, S. Chiappa, W. Cato, J. Biegel, P. Cogen, F. de Sauvage, & C. Raffel: Analysis of PTCH/SMO/SHH pathway genes in medulloblastoma. *Genes Chromosomes Cancer* 27, 44-451 (2000)
132. Feldkamp MM, N. Lau, L. Roncari, & A. Guha: Isotype-specific Ras. GTP-levels predict the efficacy of farnesyl transferase inhibitors against human astrocytomas regardless of Ras mutational status. *Cancer Res* 61, 4425-4431 (2001)
133. Toru-Delbauffe D, D. Baghdassarian-Chalaye, J.M. Gavaret, F. Courtin, M. Pomerance, & M. Pierre: Effects of transforming growth factor-beta1 on astroglial cells in culture. *J Neurochem* 54, 1056-1061 (1990)
134. Yu C, M. Takeda, & B. Soliven: Regulation of cell cycle proteins by TNF-alpha and TGF-beta in cells of oligodendroglial lineage. *J Neuroimmunol* 108, 2-10 (2000)
135. de Luca A, M. Weller, & A. Fontana: TGF-beta-induced apoptosis of cerebellar granule neurons is prevented by depolarization. *J Neurosci* 16, 4174-4185 (1996)
136. Zhu Y, G.Y. Yang, B. Ahlemeyer, L. Pang, X.M. Che, C. Culmsee, S. Klumpp, & J. Krieglstein: Transforming growth factor-beta 1 increases bad phosphorylation and protects neurons against damage. *J Neurosci* 22, 3898-3909 (2002)
137. Unsicker K & K. Krieglstein: Co-activation of TGF-ss and cytokine signaling pathways are required for neurotrophic functions. *Cytokine Growth Factor Rev* 11, 97-102 (2000)
138. Helseth E, G. Unsgaard, A. Dalen, & V. Randi: The effects of type beta transforming growth factor on

proliferation and epidermal growth factor receptor expression in a human glioblastoma cell line. *J Neurooncol* 6, 269-276 (1988)
139. Helseth E, G. Unsgaard, A. Dalen, & V. Randi: Effects of type beta transforming growth factor in combination with retinoic acid or tumor necrosis factor on proliferation of a human glioblastoma cell line and clonogenic cells from freshly resected human brain tumors. *Cancer Immunol Immunotherapy* 26, 273-279 (1988)
140. Merzak A, S. McCrea, S. Koocheckpour, & G.J. Pilkington: Control of human glioma cell growth, migration and invasion in vitro by transforming growth factor-ß1. *Br J Cancer* 70, 199-203 (1994)
141. Marushige K & Y. Marushige: Induction of apoptosis by transforming growth factor-ß1 in glioma and trigeminal neurinoma cells. *Anticancer Res* 14, 2419-2424 (1994)
142. Jachimczak P, B. Hessdorfer, K. Faebel-Schulte, C. Wismeth, W. Brysch, K.-H. Schlingensiepen, A. Bauer, A. Blesch, & U. Bogdahn: Transforming growth factor-ß-mediated autocrine growth regulation of gliomas as detected with phosphothioate antisense oligonucleotides. *Intl J Cancer* 65, 332-337 (1996)
143. Jennings MT, R.J. Maciunas, R. Carver, C.C. Bascom, P. Juneau, K. Misulius, & H.L. Moses: TGFß1 and TGFß2 are potential growth regulators for low-grade and malignant gliomas in vitro: evidence in support of an autocrine hypothesis. *Intl J Cancer* 49, 129-139 (1991)
144. Jennings MT, C.E. Hart, P.A. Commers, J.A. Whitlock, D. Martincic, R.J. Maciunas, P.L. Moots, & T.M. Shehab: Transforming growth factor beta as a potential tumor progression factor among hyperdiploid glioblastoma cultures: evidence for the role of platelet-derived growth factor. *J Neurooncol* 31, 233-54 (1997)
145. Li DM & H. Sun: TEP1, encoded by a candidate tumor suppressor locus, is a novel protein tyrosine phosphatase regulated by transforming growth factor beta. *Cancer Res* 57, 2124-2129 (1997)
146. Bissell MJ & D. Radisky: Putting tumours in context. *Nature Rev Cancer* 1, 46-54 (2001)
147. Paulus W, I. Baur, C. Huettner, B. Schmauber, W. Roggendorf, K.H. Schlingensiepen, & W. Brysch: Effects of transforming growth factor--ß1 on collagen synthesis, integrin expression, adhesion and invasion of glioma cells. *J Neuropath Exp Neurol* 54, 236-244 (1995)
148. Wrana JL, M. Maeno, B. Hawrylyshyn, K.L. Yao, C. Domenicucci, & J. Sodek: Differential effects of transforming growth factor-beta on the synthesis of extracellular matrix proteins by normal fetal rat calvarial bone cell populations. *J Cell Biol* 106, 915-924 (1988)
149. Muracciole X, S. Romain, H. Dufour, J. Palmari, O. Chinot, L. Ouafik, F. Grisoli, D.F. Branger, & P.M. Martin: PAI-1 and EGFR expression in adult glioma tumors: toward a molecular prognostic classification. *Int J Radiat Oncol Biol Phys* 52, 592-598 (2002)
150. Golembieski WA, S. Ge, K. Nelson, T. Mikkelsen, & S.A. Rempel: Increased SPARC expression promotes U87 glioblastoma invasion in vitro. *Int J Dev Neurosci* 17, 463-472 (1999)
151. MacDonald TJ, K.M. Brown, B. LaFleur, K. Peterson, C. Lawlor, Y. Chen, R.J. Packer, P. Cogen, & D.A. Stephan: Expression profiling of medulloblastoma: PDGFRA and the RAS/MAPK pathway as therapeutic targets for metastatic disease. *Nat Genet* 29, 143-52 (2001)
152. Rooprai HK, A. Kandanearatachi, G. Rucklidge, & G.J. Pilkington: Influence of putative antiinvasive agents on matrix metalloproteinase secretion by human neoplastic glia in vitro. *Ann N Y Acad Sci* 878, 654-657 (1999)
153. Tsuzuki T, S. Izumoto, T. Ohnishi, S. Hiraga, N. Arita, & T. Hayakawa: Neural cell adhesion molecule L1 in gliomas: correlation with TGF-beta and p53. *J Clin Pathol* 51, 13-17 (1998)
154. Platten M, W. Wick, C. Wild-Bode, S. Aulwurm, J. Dichgans, & M. Weller: Transforming growth factors beta(1) (TGF-beta(1)) and TGF-beta(2) promote glioma cell migration via Up-regulation of alpha(V)beta(3) integrin expression. *Biochem Biophys Res Commun* 268, 607-611 (2000)
155. Gladson CL & D.A. Cheresh: Glioblastoma expression of vitronectin and the alpha v beta 3 integrin. Adhesion mechanism for transformed glial cells. *J Clin Invest* 88, 1924-1932 (1991)
156. Paulus, W. & Tonn, J. Basement membrane invasion of glioma cells mediated by integrin receptors. *J Neurosurg* 80, 515-519 (1994)
157. Miyake K, S. Kimura, M. Nakanishi, A. Hisada, M. Hasegawa, S. Nagao, & Y. Abe: Transforming growth factor-beta1 stimulates contraction of human glioblastoma cell-mediated collagen lattice through enhanced alpha2 integrin expression. *J Neuropathol Exp Neurol* 59, 18-28 (2000)
158. Han X, J.E. Stewart Jr, S.L. Bellis, E.N. Benveniste, Q. Ding, K. Tachibana, J.R. Grammer, & C.L. Gladson: TGF-beta1 up-regulates paxillin protein expression in malignant astrocytoma cells: requirement for a fibronectin substrate. *Oncogene* 20, 7976-7986 (2001)
159. Muller G, J. Behrens, U. Nussbaumer, P. Bohlen, & W. Birchmeier: Inhibitory action of transforming growth factor beta on endothelial cells. *Proc Natl Acad Sci USA* 84, 5600-5604 (1987)
160. Madri JA & S Sankar: The biphasic effects of transforming growth factor beta in angiogenesis. In: Tumor angiogenesis. Eds: Bickell R, Lewis E, & Ferrara N, Oxford University Press, NY (1997)
161. Antonelli-Orlidge A, K.B. Saunders, S.R. Smith, & P.A. D'Amore: An activated form of transforming growth factor beta is produced by cocultures of endothelial cells and pericytes. *Proc Natl Acad Sci USA* 6, 4544-4548 (1989)
162. Darland DC & P.A. D'Amore: TGF beta is required for the formation of capillary-like structures in three-dimensional cocultures of 10T1/2 and endothelial cells. *Angiogenesis* 4, 11-20 (2001)
163. Parney IF, C. Hao, & K.C. Petruk: Glioma immunology and immunotherapy. Neurosurgery 46, 778-791 (2000)
164. Pollack IF, H. Okada, & W.H. Chambers: Exploitation of immune mechanisms in the treatment of central nervous system cancer. *Semin Pediatr Neurol* 7, 131-143 (2000)
165. Dix AR, W.H. Brooks, T.L. Roszman, & L.A. Morford: Immune defects observed in patients with primary malignant brain tumors. *J Neuroimmunol* 100, 216-232 (1999)
166. Brooks WH, M.G. Netsky, D.E. Normansell, & D.A. Horwitz: Depressed cell-mediated immunity in patients

with primary intracranial tumors. Characterization of a humoral immunosuppressive factor. *J Exp Med* 136, 1631-1647 (1972)
167. Mahaley MS Jr, W.H. Brooks, T.L. Roszman, D.D. Bigner, L. Dudka, & S. Richardson: Immunobiology of primary intracranial tumors. Part 1: studies of the cellular and humoral general immune competence of brain-tumor patients. *J Neurosurg* 46, 467-476 (1977)
168. Brooks WH, T.L. Roszman, M.S. Mahaley, & R.E. Woosley: Immunobiology of primary intracranial tumours. II. Analysis of lymphocyte subpopulations in patients with primary brain tumours. *Clin Exp Immunol* 29, 61-66 (1977)
169. Tomita T, & M. Ammirati: Reduction of absolute lymphocyte count in children with recurrent medulloblastoma. *Am J Dis Child* 138, 392-394 (1984)
170. Schwyzer M & A. Fontana: Partial purification and biochemical characterization of a T cell suppressor factor produced by human glioblastoma cells. *J Immunol* 134, 1003-1009 (1985)
171. Gorelik L & R.A. Flavell: Transforming growth factor-beta in T-cell biology. *Nature Rev Immunol* 2, 46-53 (2002)
172. Yang X, J.J. Letterio, R.J. Lechleider, L. Chen, R. Hayman, H. Gu, A.B. Roberts, & C. Deng: Targeted disruption of SMAD3 results in impaired mucosal immunity and diminished T cell responsiveness to TGF-beta. *EMBO J* 18, 1280-1291 (1999)
173. Bogdan C & C. Nathan: Modulation of macrophage function by transforming growth factor beta, interleukin-4, and interleukin-10. *Ann N Y Acad Sci* 685, 713-739 (1993)
174. Yamaguchi Y, H. Tsumura, M. Miwa, & K. Inaba: Contrasting effects of TGF-beta 1 and TNF-alpha on the development of dendritic cells from progenitors in mouse bone marrow. *Stem Cells* 15, 144-153 (1997)
175. Ogata M, Y. Zhang, Y. Wang, M. Itakura, Y.Y. Zhang, A. Harada, S. Hashimoto, & K. Matsushima: Chemotactic response toward chemokines and its regulation by transforming growth factor-beta1 of murine bone marrow hematopoietic progenitor cell-derived different subset of dendritic cells. *Blood* 93, 3225-3232 (1999)
176. Nandan D & N.E. Reiner: TGF-beta attenuates the class II transactivator and reveals an accessory pathway of IFN-gamma action. *J Immunol* 158, 1095-1101 (1997)
177. Kuppner MC, Hamou MF, Sawamura Y, Bodmer S, de Tribolet N. Inhibition of lymphocyte function by glioblastoma-derived transforming growth factor beta 2. *J Neurosurg* 71, 211-217 (1989)
178. Brabletz T, I. Pfeuffer, E. Schorr, F. Siebelt, T. Wirth, & E. Serfling: Transforming growth factor beta and cyclosporin A inhibit the inducible activity of the interleukin-2 gene in T cells through a noncanonical octamer-binding site. *Mol Cell Biol* 13, 1155-1162 (1993)
179. Swain SL, G. Huston, S. Tonkonogy, & A. Weinberg: Transforming growth factor-beta and IL-4 cause helper T cell precursors to develop into distinct effector helper cells that differ in lymphokine secretion pattern and cell surface phenotype. *J Immunol* 147, 2991-3000 (1991)
180. Hoehn P, S. Goedert, T. Germann, S. Koelsch, S. Jin, N. Palm, E. Ruede, & E. Schmitt: Opposing effects of TGF-beta 2 on the Th1 cell development of naive CD4+ T cells isolated from different mouse strains. *J Immunol* 155, 3788-3793 (1995)
181. Zuber P, M.C. Kuppner, & N. De Tribolet: Transforming growth factor-beta 2 down-regulates HLA-DR antigen expression on human malignant glioma cells. *Eur J Immunol* 18, 1623-1626 (1988)
182. Fakhrai H, O. Dorigo, D.L. Shawler, H. Lin, D. Mercola, K.L. Black, I. Royston, & R.E. Sobol: Eradication of established intracranial rat gliomas by transforming growth factor beta antisense gene therapy. *Proc Natl Acad Sci USA* 93, 2909-2914 (1996)
183. Liau LM, H. Fakhrai, & K.L. Black: Prolonged survival of rats with intracranial C6 gliomas by treatment with TGF-ß antisense gene. *Neurol Res* 20, 742-747 (1998)
184. Jachimczak P, Hessdorfer B, Fabel-Schulte K, Wismeth C, Brysch W, Schlingensiepen KH, Bauer A, Blesch A, & Bogdahn U: Transforming growth factor-beta-mediated autocrine growth regulation of gliomas as detected with phosphorothioate antisense oligonucleotides. *Int J Cancer* 65, 332-337 (1996)
185. Hau P, U. Bogdahn, F. Schulmeyer, A. Brawanski, A. Steinbrecher, A. Zellner, M. Goldbrunner, P. Jachimczak, M. Kunst, G. Stauder, K.-H. Schlingensiepen, & R. Schlingensiepen: TGF-beta-2 antisense oligonucleotide AP12009 administered intratumorally to patients with malignant glioma in a clinical phase I/II dose escalation study: safety and preliminary efficacy data. *Abs Ann Meeting Amer Soc Clin Oncol* 109 (2002)
186. Duman S, A.I. Gunal, S. Sen, G. Asci, M. Ozkahya, E. Terzioglu, F. Akcicek, & G. Atabay: Does enalapril prevent peritoneal fibrosis induced by hypertonic (3.86%) peritoneal dialysis solution? *Perit Dial Int* 21, 219-224 (2001)
187. Agarwal R, S. Siva, S.R. Dunn, & K. Sharma: Add-on angiotensin II receptor blockade lowers urinary transforming growth factor-beta levels. *Am J Kidney Dis* 39, 486-492 (2002)
188. Miyajima A, T. Asano, & M. Hayakawa: Captopril restores transforming growth factor-beta type II receptor and sensitivity to transforming growth factor-beta in murine renal cell cancer cells. *J Urol* 165, 616-620 (2001)
189. Nakagawa T, Kubota T, Kabuto M, & Kodera T: Captopril inhibits glioma cell invasion in vitro: involvement of matrix metalloproteinases. Anticancer Res 15, 1985-1989 (1995)
190. Platten M, C. Wild-Bode, W. Wick, J. Leitlein, J. Dichgans, & M.Weller: N-[3,4-dimethoxycinnamoyl]-anthranilic acid (tranilast) inhibits transforming growth factor-beta release and reduces migration and invasiveness of human malignant glioma cells. *Int J Cancer* 93, 53-61 (2001)
191. Rowland-Goldsmith MA, H. Maruyama, T. Kusama, S. Ralli, & M. Korc: Soluble type II transforming growth factor-beta (TGF-beta) receptor inhibits TGF-beta signaling in COLO-357 pancreatic cancer cells in vitro and attenuates tumor formation. *Clin Cancer Res* 7, 2931-2940 (2001)
192. Bandyopadhyay A, Y. Zhu, S.N. Malik, J. Kreisberg, M.G. Brattain, E.A. Sprague, J. Luo, F. Lopez-Casillas, & L.Z. Sun: Extracellular domain of TGF-beta type III receptor inhibits angiogenesis and tumor growth in human cancer cells. *Oncogene* 21, 3541-3551 (2002)

193. Knudson CB & W. Knudson: Cartilage proteoglycans. *Semin Cell Dev Biol* 12, 69-78 (2001)
194. Kolb M, P.J. Margetts, P.J. Sime, & J. Gauldie: Proteoglycans decorin and biglycan differentially modulate TGF-beta-mediated fibrotic responses in the lung. *Am J Physiol Lung Cell Mol Physiol* 280, L1327-34 (2001)
195. Stander M, U. Naumann, L. Dumitrescu, M. Heneka, P. Loschmann, E. Gulbins, J. Dichgans, & M. Weller: Decorin gene transfer-mediated suppression of TGF-beta synthesis abrogates experimental malignant glioma growth in vivo. *Gene Ther* 5, 1187-1194 (1998)
196. Munz C, U. Naumann, C. Grimmel, H.G. Rammensee, & M. Weller: TGF-beta-independent induction of immunogenicity by decorin gene transfer in human malignant glioma cells. *Eur J Immunol* 29, 1032-1040 (1999)
197. Yakymovych I, U. Engstrom, S. Grimsby, C.H. Heldin, S. Souchelnytskyi: Inhibition of Transforming Growth Factor-beta Signaling by Low Molecular Weight Compounds Interfering with ATP- or Substrate-Binding Sites of the TGF-beta Type I Receptor Kinase. *Biochemistry* 41, 11000-11007 (2002)
198. Inman GJ, F.J. Nicolas, J.F. Callahan, J.D. Harling, L.M. Gaster, A.D. Reith, N.J. Laping, & C.S. Hill: SB-431542 is a potent and specific inhibitor of transforming growth factor-beta superfamily type I activin receptor-like kinase (ALK) receptors ALK4, ALK5, and ALK7. *Mol Pharmacol* 62, 65-74 (2002)

Key words: Transforming Growth Factor-beta, TGF-beta, Glioma, Medulloblastoma, Ependymomas, Meningioma, Proliferation, Invasion, Immunosuppression, Therapy, Review

Send correspondence to: Jeremy N. Rich, MD, Duke University Medical Center, Box 3624, Durham, NC 27710, Tel: 919-684-5301, Fax: 919-684-6674, E-mail: rich0001@mc.duke.edu