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A Delicate Balance: TGF-β and the Tumor Microenvironment

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Abstract The activated form of TGF-β is a known regulator of epithelial cell autonomous tumor initiation, progression, and metastasis. Recent studies have also indicated that TGF-β mediates interactions between cancer cells and their local tumor microenvironment. Specifically, the loss of TGF-β signaling in stromal components including fibroblasts and T-cells can result in an ''activated'' microenvironment that supports and even initiates transformation of adjacent epithelial cells. TGF-β signaling in cancer can be regulated through mechanisms involving ligand activation and expression of essential components within the pathway including the receptors and downstream effectors. TGF-β signaling in the tumor microenvironment significantly impacts carcinoma initiation, progression, and metastasis via epithelial cell autonomous and interdependent stromal–epithelial interactions in vivo. J. Cell. Biochem. 101: 851–861, 2007.

Key words: TGF-β; transforming growth factor beta; tumor microenvironment; cancer; carcinoma; stromal–epithelial interactions

The tumor microenvironment is defined as the non-epithelial components of the area immediately surrounding tumor cells including supportive fibroblasts, immune cells, extracellular matrix (ECM), and blood vessels. Many studies have demonstrated that in cancer, the microenvironment does not play a purely benign, supportive role. Rather, transformed stroma has the capacity to initiate and promote malignant change in adjacent epithelium. In addition, non-transformed stroma can be altered by signals derived from adjacent carcinoma thereby leading to a tumor reactive stroma that can further contribute to tumor progression. A critical role for the tumor microenvironment in cancer development and progression is not surprising: epithelial and mesenchymal cell interactions with each other and their local microenvironment are critical to the organized, sequential maturation of tissues within a developing organism. Further, many of the pathways that regulate these interactions during embryogenesis are also misregulated in cancer.

The transforming growth factor beta (TGF-β) signaling pathway is implicated as a critical regulator of development, cancer initiation and progression through tumor cell autonomous signaling, and interactions within tumor microenvironment. The complex role that TGF-β plays in cancer via cell autonomous mechanisms has been previously investigated and described [Roberts and Wakefield, 2003]. However, recent work has implicated TGF-β as a critical regulator of the tumor microenvironment. Among its roles, TGF-β appears to orchestrate fibroblast chemotaxis and activation, resulting in a "cancer associated fibroblast"-like state, activation of immune cells and stromal–epithelial cross-talk, all of which can be linked to increased progression and invasion in cancer. While tumor-cell autonomous signaling continues to play an important role in cancer initiation and progression, epithelial–microenvironment-associated interactions represent a large number of

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mechanisms that require further investigation. The study of signals and interactions present within the tumor microenvironment is critical to enhance our understanding of carcinoma initiation, progression, and metastasis. Further, the stromal–epithelial interactions within the tumor microenvironment may provide additional targets for the design and application of therapeutic intervention strategies in cancer.

INITIAL OBSERVATIONS: TGF-β SUPPRESSES AND PROMOTES TUMORIGENESIS IN VIVO

Cell autonomous regulation mediated by TGF-β signaling was first described over 25 years ago when isolated polypeptides, produced by fibroblasts that had been transformed by the Maloney Sarcoma Virus, were shown to induce growth of normal fibroblasts in soft agar colony forming assays [de Larco and Todaro, 1978]. In the original description, it was reported that the sarcoma growth factor (SGF) components responsible for this activity were able to compete with the epidermal growth factor (EGF) for binding to the epidermal growth factor receptor (EGFR). Subsequent experiments by two independent groups demonstrated that the EGFR binding activity produced by the transformed cells could be separated from the fraction that supported anchorage independent soft agar colony formation [Moses et al., 1981; Roberts et al., 1981]. The EGFR binding activity is now known to be attributed to transforming growth factor alpha (TGF-α) expression whereas the activity in the remaining fraction was TGF-β. The complexity associated with TGF-β regulation of cell behavior was evident through early experiments that demonstrated TGF-β-mediated growth inhibition, in both two dimensional cultures and three dimensional culture conditions that previously stimulated progressive anchorage independent growth [Tucker et al., 1984; Roberts et al., 1985]. These results suggested that TGF-β could induce growth inhibition and support anchorage independent growth, however the responses were cell type and context dependent. It was also clear from early observations that normal epithelial cells, demonstrated using primary keratinocytes, responded to TGF-β stimulation with arrest of the cell cycle in G1 while a number of cancer cell lines were able to evade this response [Shipley et al., 1986]. TGF-β stimulation was also found to induce reversible epithelial to mesenchymal transition (EMT) and squamous to spindle epithelial cell transition, both of which were associated with enhanced invasiveness of tumor cells [Miettinen et al., 1994; Cui et al., 1996; Brown et al., 2004]. Together these observations suggested that TGF-β could function as a cell autonomous tumor suppressor or tumor promoter depending on the cell type and context of stimulation. Subsequently, TGF-β signaling in the tumor microenvironment has been shown to regulate cancer through many mechanisms involving both tumor cell autonomous and host–tumor interactions [Roberts and Wakefield, 2003]. A diverse repertoire of clear mechanistic roles for TGF-β signaling has been identified that can contribute to the regulation of tumor initiation and progression within the carcinoma-associated tumor microenvironment. However, many aspects of signaling through this pathway have not been thoroughly investigated including the influence of TGF-β-mediated stromal–epithelial cross-talk that can significantly contribute to the regulation of normal development and tumorigenesis in vivo.

THE TGF-β SIGNALING CASCADE

TGF-β ligands are secreted in a latent form as part of a three-component complex. Once activated in the extracellular matrix, the TGF-β ligands TGF-β1, TGF-β2, and TGF-β3 can interact with the transforming growth factor receptor types I, II, and III (TβRI, TβRII, TβRIII) [Derynck and Zhang, 2003]. Specifically, the three isoforms interact with TβRII in a high affinity manner either alone (TGF-β1 and TGF-β3), or in the presence of TβRIII (TGF-β2) [Derynck and Zhang, 2003]. TβRII is a serine/threonine kinase receptor that is constitutively active via autophosphorylation. TGF-β isoforms bind to the TβRII, which is then capable of recruiting and trans-phosphorylating TβRI, resulting in downstream signaling. TβRI and TβRII predominantly homodimerize at the cell surface in the absence of TGF-β, but TβRI and TβRII also have the capacity to heterooligomerize [Chen and Derynck, 1994]. Studies using chimeric receptors suggest that the TβRI-TβRII complex is also capable of basal ligand-independent signaling [Feng and Derynck, 1996].

TβRI phosphorylation initiates several downstream cascades that can be Smad dependent

and independent. In the Smad-dependent cascade, activation of TβRI recruits receptor Smads (R-Smads, Smad2 and Smad3), and a number of associated proteins including Smad anchor for activation (SARA), which ultimately targets the complex for endocytosis. Smads2 and 3 are subsequently phosphorylated, facilitating an interaction with Smad4, which accompanies the activated R-Smads to the nucleus for transcriptional regulation. Smad-independent networks are complex and involve cross-talk with multiple signaling pathways, including RhoA, PI3K, Cdc42, Rac1, Ras, PP2A, MEKK1, TAB1/TAK1, Daxx, and Par6 [Derynck and Zhang, 2003; Bierie and Moses, 2006b]. The response to TGF-β in any cell type, depends on the level of expression and net activation for each Smad dependent and independent pathway present at the time of stimulation.

TGF-β SIGNALING IN HUMAN CANCER

TGF-β signaling components, in human cancer, are often misregulated during tumor progression. It is clear that aberrant TGF-β expression, activation and mutation of signaling components are critically important in human cancer: alterations of the TGF-β signaling pathway have prognostic significance in patients with prostate, breast, and colorectal carcinoma [Bierie and Moses, 2006a]. The gene encoding the type II TGF-β receptor, *TGFBR2*, is commonly mutated in association with human cancer. The type II receptor is critical for initiation of the TGF-β signaling cascade for all three TGF-β isoforms, therefore loss of this protein abrogates TGF-β signaling. *TGFBR2* mutations have been detected in 28% of colon cancers, 25% of ovarian cancers, 21% of head and neck squamous cell carcinomas (HNSCC), and 12% of breast cancers [Levy and Hill, 2006]. When the gene is not mutated, *TGFBR2* often shows significant downregulation, ranging from 12 to 44% in non-small cell lung cancers, bladder cancers, HNSCCs of the esophagus, ovarian carcinomas, and prostate cancers [Levy and Hill, 2006]. *TGFBR2* is rarely subject to loss of heterozygosity (LOH), a process that can result in the inactivation of tumor suppressor genes. However, tumors that have associated microsatellite instability (MIN+) often lose TβRII function [Kim et al., 2000]. Microsatellite repeats are stretches of short, repetitive sequences that tend to accumulate mutations after loss of mismatch repair (MMR) machinery. The target of mutation in the *TGFBR2* gene associated with MIN+ cancer appears to be a 10 bp poly-adenine stretch which facilitates insertion or deletion of adenine nucleotides in the absence of MMR. However, the breadth of *TGFBR2* mutation is not limited to MIN+ tumors: it is also mutated in a number, albeit less commonly, of microsatellite stable tumors [Kim et al., 2000]. The genes *TGFBR1*, *SMAD2*, and *SMAD4* are also commonly altered in human cancer [Levy and Hill, 2006]. In addition, antagonists for this pathway have been reported that can contribute to the regulation of TGF-β signaling in the tumor microenvironment. Together, inactivation of individual TGF-β pathway components through multiple mechanisms, can contribute to carcinoma initiation and progression in vivo.

TGF-β EXPRESSION AND ACTIVATION

The Latent TGF-β Complex

TGF-β undergoes significant intracellular processing prior to its secretion and regulation of cellular signaling pathways (Fig. 1). It is synthesized as a protein homodimer, which is cleaved intracellularly to release the mature, disulfide-linked TGF-β dimer, and a dimeric propeptide (latency-associated protein or LAP). Prior to secretion, the LAP then interacts with TGF-β in a non-covalent, but high affinity manner to form the small latent complex (SLC). The final step in processing is the disulfide link between the SLC and the latent TGF-β binding protein (LTBP1-4, a family of fibrillin-like proteins). This 240 kDa, heterotrimeric complex—TGF-β, LAP, and LTBP—is known as the large latent complex (LLC) and is secreted into the ECM where it remains biologically inert until subsequent activation [Rifkin, 2005].

The LTBP family is widely expressed, and it is structurally similar to the ECM protein, fibrillin. Fibrillin and LTBPs are composed of multiple EGF-like repeats and both can function as part of the structural matrix [Rifkin, 2005]. LTBPs have been implicated as a contributing or initiating factor in several cancers: LTBP4 homozygous null mice developed colorectal cancer, LTBP1 was identified as part of a signature that mediates metastasis to the lung and LTBP3 was identified as part of the cohort of genes upregulated in myoepithelial cells

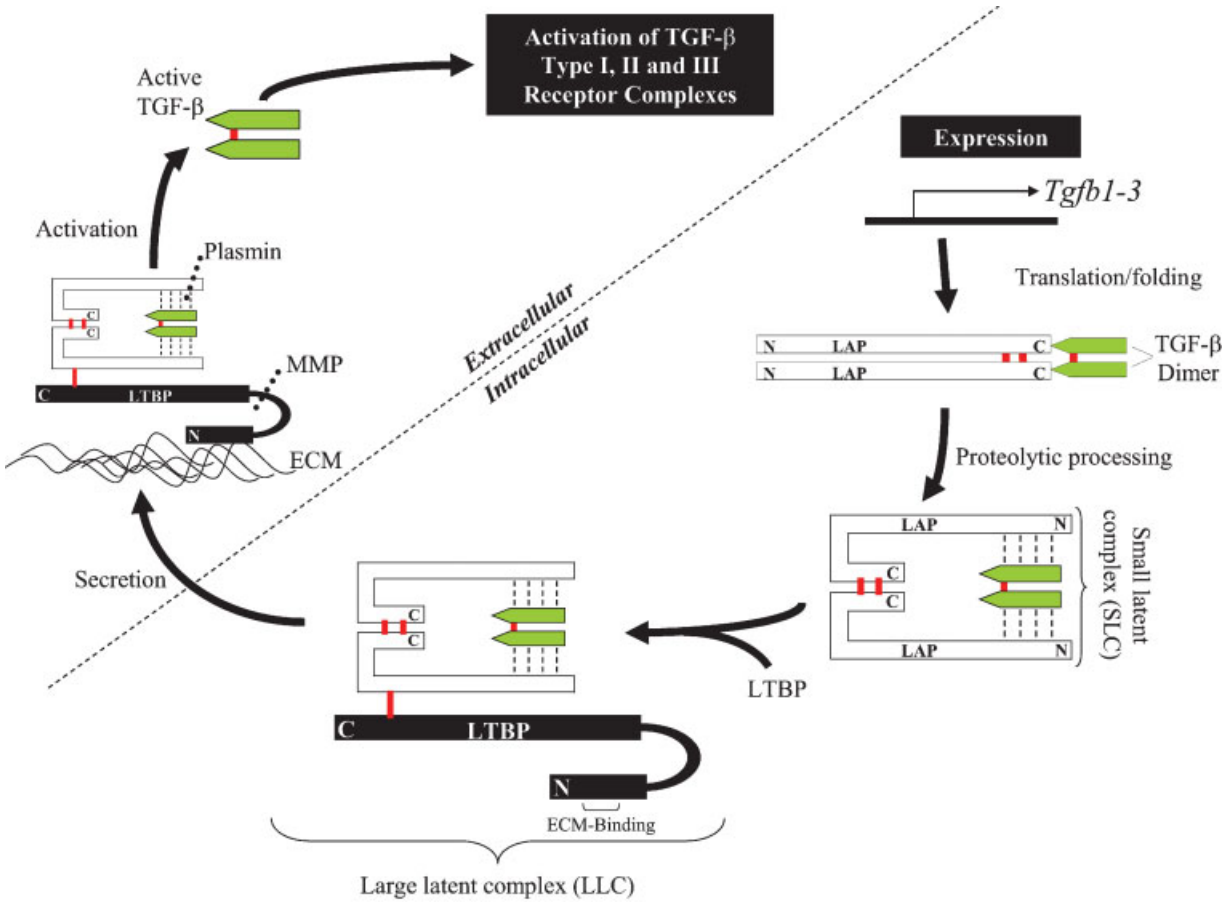

Fig. 1. TGF-β is secreted as part of a latent complex, then activated through proteolysis or conformational changes. TGF-β is expressed as a proprotein dimer—dimerized by disulfide bonds (red bar)—consisting of the latency-associated protein (LAP) and mature TGF-β. Proteolytic processing results in formation of the small latent complex (SLC), consisting of dimerized LAP and dimerized TGF-β interacting via non-covalent N-linked carbohydrate interactions (dashed lines). The SLC then interacts with the latent TGF-β binding protein (LTBP) and is secreted as the large latent complex (LLC). The amino terminus of the LTBP interacts with the ECM. Mature, active TGF-β may be released through proteolytic cleavage (dashed line) of the LTBP (MMP) or LAP (plasmin) or conformational change (induced by TSP-1).

associated with mammary ductal carcinoma in situ (DCIS) [Sterner-Kock et al., 2002; Allinen et al., 2004; Minn et al., 2005]. Loss of LTBP was shown to have profound effects both on the ECM and the availability of active TGF-β, but the precise role for these two mechanisms in tumor initiation and progression remains to be elucidated [Sterner-Kock et al., 2002].

TGF-β Expression and Activation

Latent TGF-β is expressed by many cell types and it is present in significant quantities within the extracellular matrix [Derynck et al., 1985]. However, the biologically active form of TGF-β appears to be present in relatively low concentrations, stimulating maximum TGF-β responses with a small amount of TGF-β activation [Annes et al., 2003]. Once activated, the half-life of TGF-β is significantly decreased when compared to latent TGF-β [Coffey et al., 1987]. Immunohistochemical studies have suggested that expression and activation of TGF-β may not be as promiscuous as once thought, but potentially more focal within the tumor microenvironment. In some tumor types, including those that occur in the breast, latent TGF-β has been detected at the leading edge of the tumor mass [Dalal et al., 1993]. Early studies using antibodies specific to activated TGF-β also suggested localized activation. In situ studies in breast tissue have been able to demonstrate focal activation in the epithelia, specifically associated with luminal rather than cap or myoepithelial cells [Ewan et al., 2002].

The activation of TGF-β is a critical and often overlooked step in TGF-β signaling pathways,

however it can effectively regulate the TGF-β pathway within the tumor microenvironment. Release of the active growth factor from its latent complex, is achieved through mechanisms now known to include the activity of thrombospondin-1 (TSP-1), MMP-2, MMP-9, MT1-MMP, plasmin, integrin $\alpha_v\beta_8$, and integrin $\alpha_v\beta_6$ [Hyytiainen et al., 2004].

TSP-1 appears to be responsible for a large proportion of the TGF-β activation in vivo and TSP-1 deficient mice share some phenotypic similarities, particularly in the lung, with TGF-β knock-out mice [Crawford et al., 1998]. TSP-1 is predominantly released by fibroblasts, endothelial cells, and immune cells resulting in activation of TGF-β [Schultz-Cherry et al., 1995]. While the anti-angiogenic role for TSP-1 in cancer has been described, it also appears to play a role in tumor initiation and progression through other mechanisms, including TGF-β activation [Lawler and Detmar, 2004]. In the breast, TGF-β activation may be a hormone-dependent, TSP-1-mediated effect, during proliferative periods of mammary development including puberty, estrus, and pregnancy [Harpel et al., 2001; Ewan et al., 2002].

Until recently, it was not known if TGF-β was activated within the extracellular matrix or at the cell surface, however MMP-9 has now been implicated as a TGF-β activator at the surface of mouse mammary carcinoma cells in complex with the hyaluronan (HA) receptor, CD44 [Yu and Stamenkovic, 2000]. This complex can have a significant impact on cancer progression, as previously illustrated when disruption of the MMP-9:CD44 complex was shown to reduce tumor invasion and cell survival in lung parenchyma [Yu and Stamenkovic, 2000]. HA is a glycosaminoglycan that is present mainly in the ECM, and the CD44:HA cell–ECM interaction has been shown to result in signal transduction that can regulate tumor cell motility and metastasis. Interestingly, TβRI also contains a CD44 binding domain. In MDA-MB-231 human breast cancer cells, HA binding to CD44 activates the TβRI kinase domain resulting in Smad2/3 and CD44 phosphorylation, which in turn correlated with the increased motility of tumor cells [Yu and Stamenkovic, 2000]. These results suggest a multimodal role for HA and CD44 in the cell surface activation of latent TGF-β, TGF-β receptor-mediated pathways, and cell motility in cancer.

STROMAL–EPITHELIAL INTERACTIONS INFLUENCE TUMOR PROGRESSION

Early in cancer research, it was noted that the stroma often surrounding tumors differed histologically from normal stroma. This type of reactive stroma was associated with many types of solid tumors and some considered it to be a host response that could limit growth of the tumor and perhaps prevent angiogenesis [Seemayer et al., 1979]. While much of the research in the field suggests that reactive stroma supports tumor development and progression, normal stroma can also limit the growth of transformed epithelia in some contexts. For example, transplanting preneoplastic human mammary carcinoma cells with fibroblasts derived by reduction mammoplasty from normal donors retarded growth and differentiation of mammary epithelial and derivative tumor cells while fibroblasts from tumor-bearing donors enhanced growth and tumor progression [Shekhar et al., 2001].

Cancer-Associated Fibroblasts Support Prostate Tumor Progression

To investigate the role for tumor reactive or transformed stroma in adjacent carcinoma progression, Olumi et al. performed an informative set of tissue recombination experiments in vivo. Co-cultures of prostate epithelial organoids and prostate fibroblasts were engrafted under rodent kidney capsules, an area natively lacking fibroblasts [Olumi et al., 1999]. Using initiated prostate epithelial cells derived from a benign prostatic hyperplasia (BPH-1), which were immortalized and karyotypically abnormal yet unable to form tumors alone, recombination with cancer-associated fibroblasts (CAFs) resulted in significant tumor formation. However, when CAFs were recombined with normal prostate epithelium, no tumors developed, suggesting that the CAFs in this context had an incomplete capacity for initiation of carcinoma in vivo. Later work from the same group demonstrated that epithelial cell lines established from the above-describe tumors were capable of forming tumors in vivo without co-engrafted fibroblasts [Hayward et al., 2001]. This suggested that co-culture with CAFs not only provided transient growth-related signals, but resulted in a permanent malignant transformation of the previously initiated epithelial cells.

Although the mechanisms involved in the stromal–epithelial interactions regulating tumor progression are likely complex, one potential mechanism for the CAF derived contribution to adjacent carcinoma progression was later proposed. Using microarray analysis, secreted frizzled related protein 1 (SFRP1) was identified due to its upregulation in developing prostate and CAFs, and more specifically, in prostate epithelial cells upon co-culture with CAFs [Joesting et al., 2005]. SFRP1 is a Wnt signaling inhibitor that shares homology with the canonical Wnt receptor, *frizzled*. Treatment with SFRP1 increased proliferation and decreased apoptosis in a human prostatic epithelial cell line, suggesting that the observed upregulation of SFRP1 could provide an important growth advantage within the tumor microenvironment.

Cancer-Associated Fibroblasts Support Initiation and Progression of Breast Cancer

A number of studies have investigated stromal–epithelial interactions in the mammary gland. In one study, similar in design to those previously conducted in the prostate, human mammary-derived CAFs were shown to promote tumor progression associated with MCF-7 human breast cancer cells harboring an activated *ras* oncogene (MCF-r-ras) when compared with paired normal fibroblast cell lines derived from identical patients, using a subcutaneous tissue recombination xenograph model [Orimo et al., 2005]. These studies demonstrated increased angiogenesis of the CAF-grafts through the mobilization and recruitment of endothelial progenitors, an effect putatively mediated by SDF-1/CXCL12. Treatment of MCF-7-ras cells in vitro with exogenous SDF-1 increased proliferation, an effect that could be abrogated through siRNA-mediated attenuation of the SDF-1 receptor, CXCR4. Functionally, injection of an SDF-1 neutralizing antibody reduced tumor growth and microvascular density in vivo, and reduced the CAF endothelial progenitor recruitment capacity in vitro. Thus, it is evident that in both prostate and breast cancer models, CAFs have the capacity to support growth of transformed cells through paracrine mechanisms and have the potential to transiently or permanently modify adjacent carcinoma cells in vivo.

Oncogenic potential can be demonstrated through the study of initiated or transformed cells, however oncogenic capacity is more stringently studied through the transformation of normal epithelium. A recent study has now shown that human mammary epithelium can be reconstituted and recombined with experimental stromal fibroblasts in cleared mouse mammary fat pads [Kuperwasser et al., 2004]. Using this technique the cleared mouse mammary fat pad can be engineered to contain human fibroblasts, producing a pre-engrafted cleared fat pad (PECFP), that is suitable for grafting of human mammary organoids (clusters of luminal epithelial and myoepithelial cells). In this study, the authors demonstrated that recombining primary fibroblasts with mammary epithelial organoids in the PECFP produced relatively normal duct and acinar structures. However, in 3 of 10 epithelial organoid preparations that were grafted without normal fibroblasts ductal hyperplasia similar to benign human breast proliferations were observed. Interestingly, xenograph preparations of organoids delivered without normal fibroblasts into fat pads engineered to overexpress HGF, TGF-β, or HGF + TGF-β resulted in structures bearing similarity to ductal carcinoma in 1 of the 10 organoids preparations. When normal fibroblasts were recombined with this epithelial cell organoid preparation, normal mammary structures were observed. The approach in this study was novel and further validated previous results, demonstrating that factors produced by fibroblasts could influence malignant transformation of adjacent mammary epithelial cells in vivo [Barcellos-Hoff and Ravani, 2000]. These two informative studies demonstrated that tumorigenesis in non-transformed epithelial cells can be regulated by fibroblast-derived stromal–epithelial interactions in vivo.

TGF-β REGULATES CARCINOGENESIS VIA STROMAL–EPITHELIAL INTERACTIONS

We are beginning to understand the role for an activated microenvironment, that in the absence of TGF-β signaling, can initiate and contribute to carcinoma progression (Fig. 2). An early study investigating the loss of TGF-β signaling in the fibroblast compartment associated with mammary gland development, demonstrated that fibroblasts can regulate adjacent epithelial cell morphogenesis. Zinc-inducible overexpression of a dominant negative type II TGF-β receptor transgene resulted

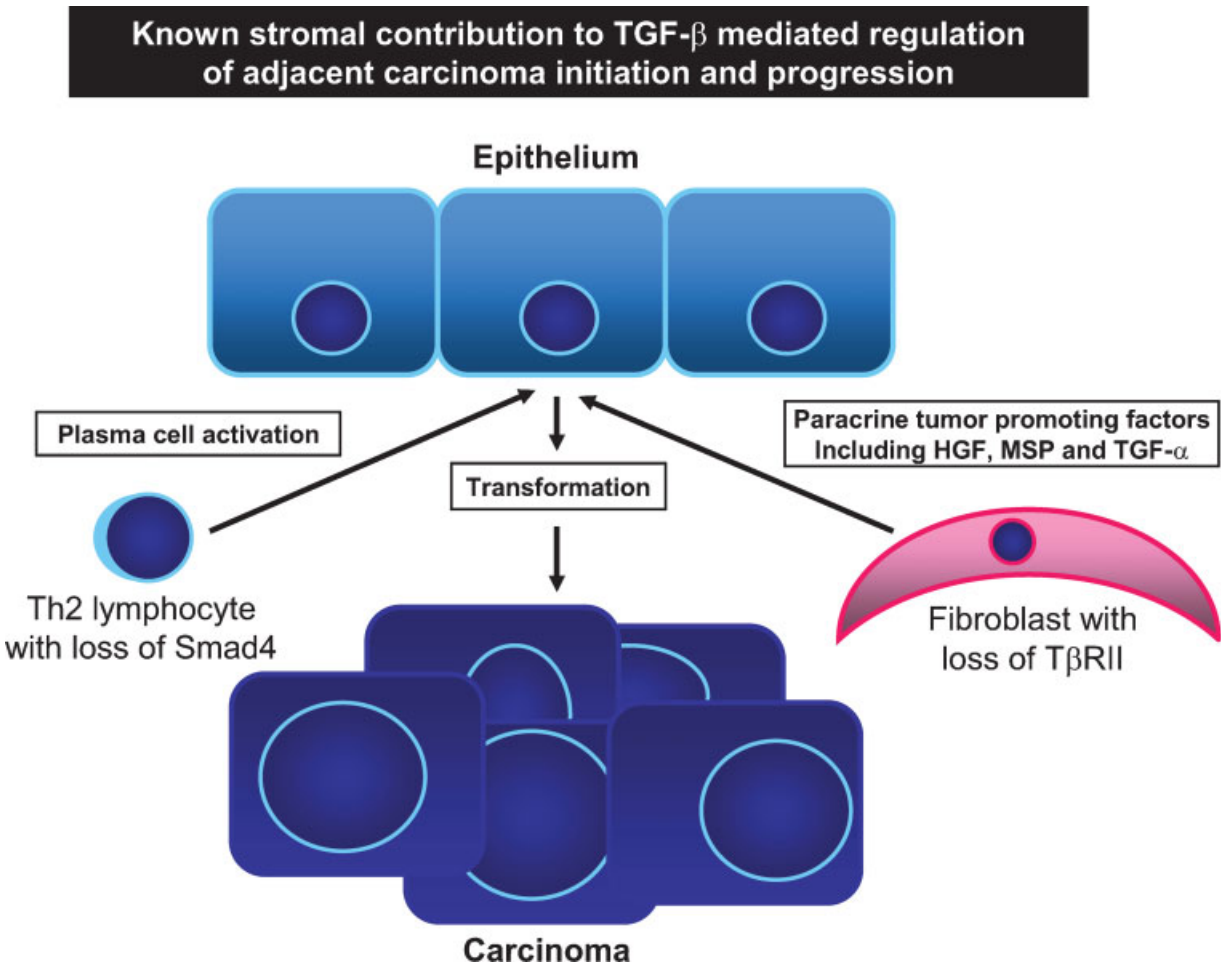

Fig. 2. TGF-β mediates fibroblast- and immune-derived stromal–epithelial interactions during carcinoma initiation and progression in the tumor microenvironment. Loss of TGF-β signaling via loss of TβRII (fibroblasts) or Smad4 (T-cells) in the stromal compartment can initiate tumorigenesis. Loss of TβRII in fibroblasts results in the loss of TGF-β growth inhibition and is associated with secretion of HGF, MSP, and TGF-α. These factors induce cell cycle dysregulation, transformation, and increased motility and invasion of nearby epithelial cells in the prostate, forestomach, and breast. Loss of Smad4 in T-cells results in a shift to a Th2 phenotype, with upregulation of cytokines that activate large numbers of plasma cells and induce fibroblast proliferation. This modified stromal microenvironment induces epithelial hyperplasia and ultimately carcinogenesis in the colon.

in attenuation of TGF-β signaling in the stroma adjacent to mammary epithelium [Joseph et al., 1999]. The loss of TGF-β signaling in fibroblasts increased lateral ductal branching and correlated with an increased level of HGF mRNA expression. The results from this study suggested that TGF-β signaling could contribute to the regulation of stromal–epithelial interactions in vivo.

To further investigate TGF-β signaling associated with fibroblast-derived stromal–epithelial interactions that could contribute to cancer initiation and progression, TβRII was knocked-out in specific tissues from mice with Cre-LoxP technology. Specifically TβRII signaling was ablated using the FSP1 (S100A4) promoter to drive expression of Cre recombinase in vivo [Bhowmick et al., 2004]. The endogenous FSP1 promoter is expressed in a sub-set of the stromal fibroblasts that reside in tissues including prostate, forestomach, and skin. In the prostate, FSP1-Cre driven TβRII knock-out mice demonstrated stromal hyperplasia with an accompanying nuclear atypia and hyperplasia of adjacent epithelial cells resulting in prostatic intraepithelial neoplasia (PIN). The forestomach in this mouse model, also demonstrated an increased abundance of fibroblasts, but in this case the stromal expansion was accompanied by invasive squamous cell carcinoma. Both the prostate and forestomach fibroblasts demonstrated

- TGF-ß is secreted as a latent, ECM-associated molecule that is activated through proteolysis or conformational changes.
- TGF-ß signaling can result in tumor suppression or tumor progression depending on the cell type and context of stimulation.
- Cancer-associated fibroblasts are capable of promoting tumorigenesis while normal fibroblasts suppress tumor progression. In addition, cancer-associated fibroblasts have been shown to cause permanent changes in initiated epithelial cells that can subsequently contribute to carcinoma progression.
- TGF-ß can result in fibroblasts activation thereby contributing to tumor progression and invasion.
- Loss of TGF-ß signaling in the stromal compartment, either by loss of the type II TGF-ß receptor in fibroblasts or Smad4 in T-cells, can contribute to carcinoma initiation and progression.
- TGF-ß is a critical regulator of the carcinoma associated tumor microenvironment through cell autonomous responses in the epithelial cell compartment and cross-talk derived from TGF-ß responses within the stromal compartment.

Fig. 3. Summary: The role of TGF-β in the tumor microenvironment.

increased levels of activated HGF with parallel increased levels of phosphorylated c-Met, the HGF receptor, in whole tissue extracts from the prostate and forestomach. Expression of c-myc was also demonstrated with phosphorylated c-Met in both the prostate and forestomach, suggesting that HGF contributed to cell cycle dysregulation via cyclin-dependent kinase inhibitors [Bhowmick et al., 2004]. This was the first study to clearly demonstrate that TGF-β signaling (or lack thereof) could regulate the oncogenic potential of adjacent epithelium through cross-talk derived from the stromal compartment in vivo.

Disruption of TGF-β signaling through loss of the type II TGF-β receptor appears to result in a form of fibroblast activation, with increased proliferation and upregulation of growth factor expression. To investigate the impact of this system in breast cancer, TGF-β type II receptor signaling was conditionally knocked out in mouse mammary fibroblasts using the FSP1-Cre transgene to mediate recombination, as previously described. Similar to observations in the forestomach and prostate [Bhowmick et al., 2004], ablation of TGF-β signaling in mammary fibroblasts resulted in an increased rate of fibroblast proliferation [Cheng et al., 2005]. When engrafted under the kidney capsule with mammary carcinoma cells, TβRII signaling deficient fibroblasts were able to promote adjacent carcinoma growth and invasion through upregulation of paracrine factors including TGF-α, macrophage-stimulating protein (MSP), and HGF [Cheng et al., 2005]. These secreted factors induced phosphorylation and activation of their cognate receptors ErbB1 and ErbB2, RON, and c-Met, respectively. Inhibition of downstream signaling from the cognate receptors through administration of pharmacologic inhibitors (TGF-α) or neutralizing antibodies (MSP, HGF, c-MET) limited the increased proliferation and invasion observed in the controls [Cheng et al., 2005]. Together, these two studies demonstrated an upregulation of paracrine HGF signaling, and in the breast TGF-α and MSP, by fibroblasts subsequent to the loss of the type II TGF-β receptor in vitro and in vivo.

TGF-β Signaling Deficient T-Cell Populations Can Induce Carcinoma in Adjacent Epithelium

It has long been known that chronic inflammation can promote tumor progression [Coussens and Werb, 2002]. Until recently, however, it was unclear whether aberrant inflammatory responses only provide a supportive environment for tumor progression or actively contribute to tumor initiation and progression. Kim and colleagues used Cre-LoxP technology driven by two T-cell specific promoters (Lck and CD4) to knock-out the key TGF-β downstream transcriptional effector *Smad4* [Kim et al., 2006]. The knock-out mice exhibited an expansion of the gastrointestinal stromal compartment with a significant plasma cell infiltrate that was associated with increased levels of IgA locally and in the serum. The loss of *Smad4* expression resulted in skewed maturation toward a Th2 phenotype, with increased levels of cytokines including IL-4, -5, -6, and -13 in vivo and in vitro. Knock-out mice produced through expression of Cre under control of either promoter went on to spontaneously develop

carcinoma in the gastrointestinal tract (94 and 100%, respectively). In addition, these mice also exhibited a high rate of oral squamous cell carcinoma. Interestingly, germline mutations of *SMAD4* are present in human Familial Juvenile Polyposis, the symptoms of which this model closely recapitulates [Kim et al., 2006]. Further, this study illustrates a concept and mechanism wherein the loss of TGF-β signaling from a stromal component, independent of an epithelial cell autonomous defect, can initiate and promote carcinoma in vivo.

GENETIC AND EPIGENETIC CHANGES IN THE FIBROBLAST COMPARTMENT

How do fibroblasts associated with cancer undergo the change from normal, supportive neighbors to activated, potentially carcinogenic aggressors? It has been suggested that wide-scale LOH detected in whole breast tissue may be at least partly attributable to contributions from the stromal compartment. Specifically, PTEN, a tumor suppressor with homology to protein tyrosine phosphatases and tensin, and TP53, the tumor suppressor gene p53, has been shown to be mutated only in the stroma or the epithelia, but rarely both compartments associated with human breast cancer [Kurose et al., 2002]. Furthermore, Kurose and colleagues propose a multi-stage, stepwise mechanism of carcinogenesis of the breast similar to the Vogelstein model for progression of colon cancer [Fearon and Vogelstein, 1990; Kurose et al., 2001]. In this model for disease progression, both the epithelium and stromal compartments undergo LOH mutations with some regions lost earlier in the epithelial compartment and others lost earlier in the stromal compartment.

In addition to genetic alterations in the tumor microenvironment, distinct epigenetic changes have also been observed in association cancer progression. Using a novel technique, termed methylation-specific digital karyotyping, epigenetic changes in stromal components from normal breast tissue, in situ and invasive breast carcinoma have been detected [Hu et al., 2005]. The epigenetic changes observed in this study were correlated with cell type and stage of tumor progression. The use of this technology resulted in identification of an unknown gene, *CXorf12*, that was shown to exhibit hypermethylation in tumor stroma when compared with stroma from normal breast tissue. Further, it was also shown that *CXorf12* expression was attenuated in the tumor stroma compared with stroma from normal breast tissue. These results suggest, that methylation can be differentially regulated between the compartments within the tumor microenvironment, and this epigenetic regulation can impact corresponding gene expression in vivo. Together, the presence of genetic and epigenetic changes in the stromal cell compartment independent of epithelial transformation could be initiating events that in turn, via multiple cross-talk mechanisms, results in escalating aberrant gene expression in both epithelial cells and fibroblasts that can contribute to initiation and progression of a carcinoma-associated tumor microenvironment.

CONCLUSIONS

It is evident that TGF-β signaling requires a delicate balance of interactions within the tumor microenvironment. Epithelial cell autonomous TGF-β signaling regulates cancer initiation and progression in mouse and human carcinomas. TGF-β likewise, potently regulates cancer progression through interactions within the tumor microenvironment including fibroblast recruitment/activation, epithelial-fibroblast-associated cross-talk, and modification of the ECM. Currently, TGF-β appears to be a critical regulator of the tumor microenvironment through at least two distinct mechanisms: (1) cell autonomous TGF-β responses in the epithelial cell compartment and (2) cross-talk derived from TGF-β responses in cells associated with the stromal compartment.

Experimental inactivation of TGF-β signaling in fibroblasts and immune cells has recently shown, that mutation of stromal components within a microenvironment associated with specific epithelial populations, can subsequently initiate carcinoma through distinct stroma to epithelial cross-talk mechanisms. Previous studies, investigating LOH in the stromal compartment indicated that the fibroblast population does exhibit mutation independent of epithelial defect in human cancer. These mutations can impact important regulatory pathways, resulting in activation of the stromal component that can contribute to carcinoma initiation and enhance progression. Three recent papers demonstrate that loss of

TGF-β signaling—via loss of TβRII in fibroblast cells or Smad4 in T-cells—can both initiate and support tumor growth [Bhowmick et al., 2004; Cheng et al., 2005; Kim et al., 2006]. Once these cells lose TGF-β signaling, upregulation of HGF, MSP, TGF-α, and other secreted factors such as cytokines can have a significant influence on adjacent epithelial and stromal cell populations in vivo.

While this review focuses on the extracellular milieu, it is evident that the two compartments, carcinoma cells and the local tumor microenvironment, can act synergistically to enhance carcinoma progression. Together, these initiating signals may set in motion a sequence of events that result in a reactive, supportive, and transiently or permanently altered tumor microenvironment. Elucidating the complex role for TGF-β in the tumor microenvironment will not only be critical to our understanding of the systems that regulate initiation, progression, and metastasis associated with cancer, but will also likely uncover many potential targets for treatment in human disease.

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