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Review

TGF-β1 Interactome: Metastasis and Beyond

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Abstract. *The ubiquitous cytokine transforming growth factor-β1 (TGF-β1) is one of the most potent metastatic inducers. Functional interactomic mapping using high-throughput proteomic and genomic data provides valuable insights into the regulation of tumor suppressive and metastatic attributes of TGF-β1. Polarity changes of the TGF-β1 interactome at a given time contributes to these contrasting effects. Differential expression profiles of pivotal interactomic nodes contribute to these polarity changes. These insights are of immense value in the development of effective cancer therapeutics. Moreover, TGF-β1 interactomic nodes are useful in discovering novel cancer biomarkers. This review describes an initial version of the TGF-β1 interactome in relation to tumor progression and metastasis. Thus, this review embodies an important step towards the mapping of comprehensive and individualized TGF-β1 interactomes that will assist in the development of personalized cancer therapeutics.*

The majority of deaths associated with various cancer types are due to the process of cancer cells entering the circulation and disseminating to specific distal sites (1). The advent of newer molecular platforms and system biology tools has given an in-depth insight into the hallmarks of metastasis, increasing the understanding of cellular and sub-cellular molecular events. Cancer progression and metastasis have shown a clear link with cytokine changes in the tumor microenvironment (2). TGF-β1, a ubiquitous cytokine, induces cancer cells to proliferate and promotes them to form an invasive phenotype. This is characterized by an epithelial to mesenchymal transition (EMT) (3). Cells undergoing EMT demonstrate cellular morphological changes and increased expression in mesenchymal markers such as vimentin and decreased expression of E-cadherin (4, 5), which aid in invasion and metastasis. TGF-β1 additionally promotes metastasis by regulating the composition of extracellular matrix, proteolysis and inflammatory responses (6, 7). Proteomic studies have shown different proteins to be involved in different stages of metastasis (8, 9). All this evidence suggests an interatomic nature of the TGF-β1 induced metastatic process.

The complete set of protein–protein and protein–DNA interactions surrounding TGF-β1 defines TGF-β1 interactome, and surrounding connections appear to show a polarity towards pro-metastatic or anti-metastatic effects depending on individualistic protein expression levels. The constitutive production and meticulous regulation of growth factors, cytokines and ligand independent activation of survival pathways are among the many important factors that help direct the interactomic polarity towards carcinogenic effects. Interactomic connections can alter the cell polarity and cell matrix by inducing changes in the actomyosin cytoskeleton of the tumor cell and subsequent cross talk with the stroma of a distant site helps to seed these cells away from a primary site. This cumulative interactomic polarity facilitates the final life-threatening metastatic process. However, *TGF-β1* is a potent inhibitor of epithelial cell growth and functions as tumor suppressor in early stages of cancer, and in late stages, promotes metastasis by enhancing invasion and angiogenesis (10). Thus, the interactomic polarity appears to be reversible for opposing effects depending on the tumor development stages. Understanding the pro-metastiatic and anti-metastatic polarity changes within *TGF-β1* interactome will assist in the development of effective targeted therapies. This review focuses on analyzing and understanding the possible cellular proteomic interactions that govern the *TGF-β1* interactome in regulating malignant dissemination.

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TGF-β1 Interactome and Carcinogenesis

TGF-β1 belongs to a family of polypeptide growth factors that include activins, inhibins, and bone morphogenic proteins (BMP). *TGF-β* ligands are secreted from cells in three isoforms (TGF-β1, TGF-β2, TGF-β3) with the latency associated protein (LAP), which make these isoforms inactive (11). This latent TGF-β complex contains another protein called the latent binding protein, which helps in extracellular localization of the latent complex (12). TGF-β1 is activated *in vivo* by proteolytic cleavage of LAP at low pH or *via* interactions with other proteins such as thrombospondins and *αVβ6* integrin (12, 13). Active TGF-β1 is released as a dimer and is involved in numerous regulatory activities that influence development, tissue repair, immune defense, inflammation and tumorigenesis (14). In order to initiate these effects, *TGF-β1* has to first bind with the TGF-β type II (TβRII) and Alk5/TGF-β type I (TβRI) heterotetrameric receptor complex (15). Both TβRI and TβRII are receptor serine-threonine kinases. The type II receptor is a constitutive kinase, while the type I receptor kinase is only activated after complex formation and trans-phosphorylation by the type II receptor. Inactivation of the kinase domain of TβRI abrogates signaling, emphasizing the importance of the type 1 receptor for cell responsiveness to TGF-β1 (16). Once bound to TGF-β1, the constitutively active TβRII receptor comes into close proximity with the type I receptor. This phosphorylates the regulatory segment, a 30-amino-acid region of the type I receptor upstream of the kinase domain (17), causing the signaling inhibitor FKBP12 to be translocated, exposing the active site (18). The Smad transcription factors are then able to from a complex with the receptor. Phosphorylation of TβRI activates a downstream signaling cascade through phosphorylation of receptor associated Smads. Eight Smad proteins are involved in the pathway. Smads 2/3 are phosphorylated by active TβR1, which then goes on to bind with Smad4. Smads 1/2/3/5/8 can be directly phosphorylated by TGF-β1, activin and bone morphogenic protein (BMP) receptors. Smad 6/7 have inhibitory effects on TGFβ and BMP signaling (Figure 1).

In many cancer types, up-regulated proteases are strongly associated with cancer metastasis and poor outcome. The proteases such as matrix metalloproteinases, serine proteases and cathepsins couple with TGF-β1 to form interactomic connections in protein processing and cell-cell communication during metastatic process (19, 20). The many interacting proteins of TGF-β1 pathway have been investigated by co-precipitation assays and by binary screens of protein arrays such as yeast two-hybrid screens (21, 22). These studies show that TβRI to form complexes with many cellular proteins such as STRAP (23), TRAP-1 (24), FKBP12 (25), PI3 kinase (26) apart from Smads (16). Smad proteins primarily act as transcription factors or repressors, which promote the regulation of TGF-β1 associated gene transcription (27). All these studies indicate that TGF-β1 signaling involves a complex interactome of integrated pathways such as Smad, Her2/neu, MAP kinase, phosphoinositol-3-kinase, α-farnesyltransferase and PP2ABα (28). These interactions with other proteins significantly modulate the intensity of signaling and the type of response. Further propagation of TGF-β1 signaling includes phosphorylation of TGF-β1 receptor substrates and translocation of various proteins, involving a number of protein–protein and protein–DNA interactions. Thus, it is clear that biological processes related to TGF-β1 are not driven by the effects of few genes or proteins, but by the polarity of their networks at a given time. Consequently, the responsiveness of cancerous cells is defined by the polarity of fundamental components of TGF-β1 signaling pathway (Figure 2).

TGF-β1 Interactome and Metastasis

In early studies, immunohistochemical detection demonstrated a significant correlation between intense TGF-β1 staining and metastasis (29). A general relationship can be deduced where more intense TGF-β1 staining correlates with a higher grade of malignancy and metastasis. It not clear whether the TGF-β1 is produced by the tumor or as a response to the malignant condition by the host or by both mechanisms, but what is clear is that increased serum levels of TGF-β1 significantly correlate with metastasis (30). Supporting evidence with tumor cell lines transfected with *TGF-β1* cDNA illustrate an increased tendency towards the metastatic process (31). This emphasizes that in early stages of cancer TGF-β1 interactome has suppressive effects, but in later stages the interactome is polarized towards tumor progression. Consequently, these tumor cells were fully resistant to growth inhibitory effects of TGF-β1 (32). Similar results were obtained when a transgenic mice model was used to study metastasis in spindal cell carcinomas (33). A study by Weeks *et al*. using the gene-switch-TGF-beta1 mice model demonstrated similar findings that overexpression of *TGF-β1* in papillomas caused significant tumor regression in early stages, but induced metastatic squamous cell carcinoma transformation in late stages (34). These tumors demonstrated increased metalloprotease expressions and reduced E-cadherin expressions, indicating that these cellular proteins to play a significant role in the transition between pro-metastatic and anti-metastatic polarity events within the network. However, further evidence is needed to come to a definitive conclusion.

Important functional proteins in the TGF-β1 interactome are subjected to mutations in certain cancer types. Mutations are the most common cause of malignant transformation, invasion and metastatic dissemination. Mutations in TβRI node can be clearly identified in head, neck and breast cancer

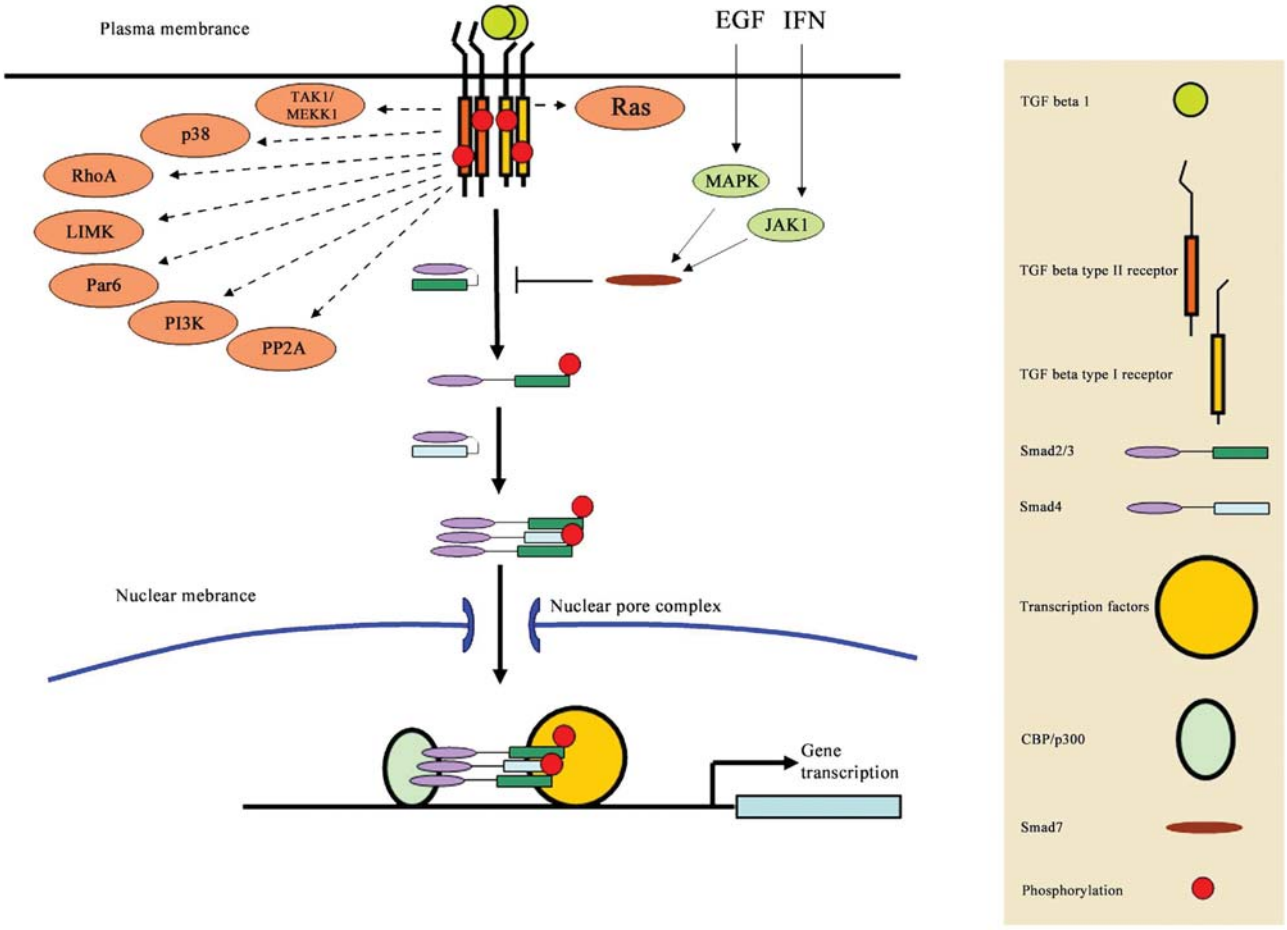

Figure 1. *Central TGF-β1 signaling pathway. Basic TGF-β1 signaling cascade is preceded by TGF-β1 dimer binding to the dimeric type II receptor, type I receptor phosphorylation and subsequent Smad2/3/4 phosphorylation. Other lateral interactions such as Ras/ERK pathway can decrease TGF-β receptor levels, RhoA/LIMK induce actin polymerization and TAK1 activates apoptosis when activated by p38 MAPK pathway.*

metastasis and expression of TβRII is decreased during the oncogenesis process in head and neck squamous cell carcinoma and laryngeal carcinoma (35). However, according to Abe *et al*., the *TβRII* gene can stay in a non-mutated state in certain cancer types such as breast, liver and pancreatic carcinoma (36), indicating a tumor specific regulation of the interactomic receptor proteins. Mutations of *SMAD4* are common in human colorectal carcinoma, especially in patients with late stage, metastatic disease (37). Therefore, mutations of various proteins of the TGF-β1 interactome contribute to the induction of metastatic events.

TGF-β1 Interactomic Influence on EMT

The cell–cell and cell–microenvironment interactions in the extracellular matrix can actively restrain cancer progression. Polarization towards pro-metastatic features of the TGF-β1 interactome can manipulate and regulate components of the extracellular matrix for successful invasion and metastasis (38). EMT is characterized by reduced expression of cell adhesion molecules, acquisition of a fibroblast like cellular morphology, disruption of tight and adherence junctions and a change from cytokeratin to a vimentin based cytoskeleton (39). Abrupt regulation of cytoskeleton molecules such as E-cadherin, ZO-1, keratin and vimentin is a common feature seen with EMT (40, 41). These changes help tumor cells to undo their cell-cell connections and behave freely acquiring a more invasive spindle shaped phenotype. TGF-β has been known to activate EMT using Smad-dependant and independent transcriptional events in heart and palate formation in mouse models (42). Pathological sections of cancerous tissues, which show higher levels of TGF-β1 and other cytokines in the invasive front, exhibit EMT. Dominant negative TGF-β receptors transfected into tumor cells inhibit spindle cell transformation and transfection of the same receptors into spindle cell carcinoma cell lines tends to

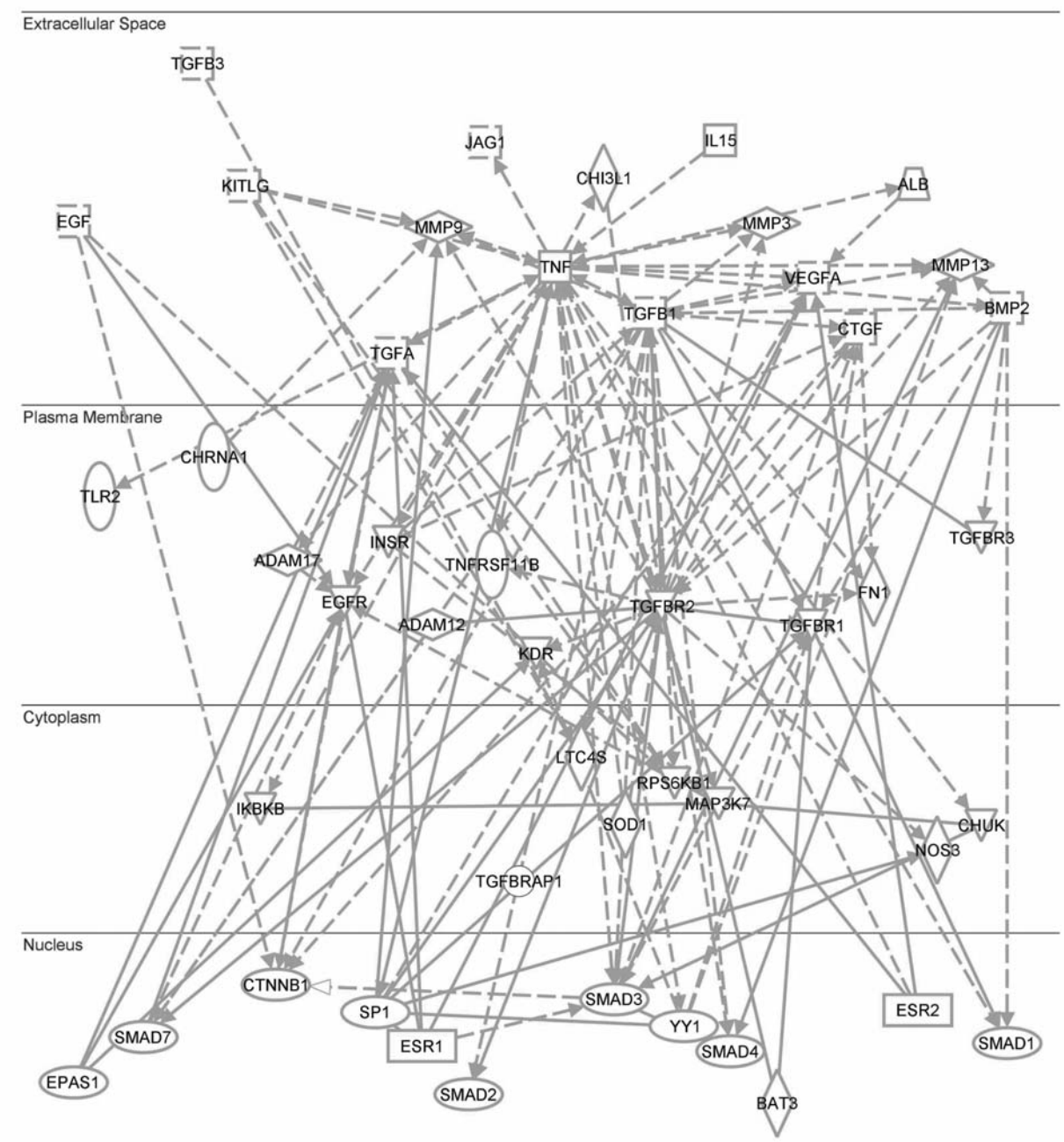

Figure 2. *TGF-β1 interactome profiling in mammalian system. TGF-β1 metastasis regulatory interaction network mapping in the mammalian system was carried out with Ingenuity Pathway analysis (IPA, ingenuity systems, www.ingenuity.com). A list of all the metastasis-related molecules were created using IPA. Another list of molecules of TGF-β1 pathway were created using IPA grow out function. Using the compare data tool, common molecules were connected to obtain the interactome. This workflow will give an interactome for metastasis related molecules in the neighborhood of TGF-β1. Then this network was mapped to known biological pathways algorithmically based on their connectivity, which showed the most significant biological relationships between each related molecule. Regulator elements represented as nodes and their interactions as edges. The edges are directed to link the causal interactions. The links that have been observed most frequently are given the highest weight. All edges and lines are supported by references from data sources such as Ingenuity curated findings, BIND, BIOGRID, Cognia, DIP, INTACT, Interactome studies, MINT, IMPS and additional sources incorporated to Ingenuity. All the direct and indirect pathways were mapped using a stringent filter for human, mouse and rat. Different shapes in nodes represent functional classes of the gene product. Solid and dashed lines represent direct interactions and indirect interactions respectively. The extracellular cytokine interactome shows the involvement of cytokines such as TNF, MMP9, MMP3 and IL-15. The final interactomic map demonstrates an array of transcription factors (except Smads) that are possibly involved in the metastatic process. Thus, these transcription factors and other interactomic interactions can be utilized for the development of novel therapeutics and for the discovery of biomarkers. Furthermore, these interactomic maps demonstrate an immense potential in the toxicology analysis of the identified potential thereapeutic targets.*

reverse the EMT transformation (43). Moreover, transfection of dominant negative *TβRII* into highly metastatic carcinomas tends to inhibit the metastatic process (44). Furthermore, the introduction of TβRII has resulted in a more invasive phenotype in HNPCC cells in vitro (44). Thus, the mechanism of EMT induction by TGF-β1 interactome appears to be driven by a receptor-mediated mechanism. TGF-β1-induced EMT commonly down-regulates E-caderin expression in many tumor cells (40, 41) by a mechanism through Smad-dependent expression of *HMGA2* (high mobility group A2 gene)(45). Par6 (a regulator of tight-junctions) phosphorylation is mandatory for TGF-β1-dependent EMT. Par6 in conjunction with Smurf1 (E3 ubiquitin ligase), degrades guanosine triphosphatase RhoA, leading to a loss of tight junctions (46). Snail (a transcription factor) causes direct suppression of E-cadherin and is over-expressed in breast cancers (47). Thus, these different functional groups within the interactome appear to have significant implications in the EMT process.

TGF-β1 application to normal murine mammary gland (NMuMG) epithelial cells shows increased expression of Ras, Raf, MEK1/2, and Erk1/2, suggesting the merging of different pathways to bring out a polarity towards TGF-β1 induced EMT (48, 49). The notch signaling pathway plays an important role in TGF-β1-induced EMT in epithelial cells (50). TGF-β1 can also induce a reversible EMT in different types of normal and transformed epithelial cell lines (51). Cell lines that have a higher invasive potential reveal diminished epithelial keratins and increased mesenchymal vimentin. The suppression of keratins and expression of vimentin by TGF-β1 is significantly correlated with EMT and short survival (52). Sato et al. has shown that Smad3 is directly involved in TGF-β1-induced EMT (53), and several other studies suggest that Smad-independent signaling pathways such as PI3-kinase-AKT-Rho signaling are involved in TGF-β1-induced EMT (54-56). Thus, an interactomic polarity towards TGF-β1-dependent EMT causes a down regulation of cell adhesion molecules and disruption of cellular junctions through the interaction of different pathways.

Induction of Angiogenesis in Invasion and Metastasis

The ability to form new blood vessels represents a critical step for growth and metastasis as blood vessels transport nutrients and oxygen to sustain tumor cell survival. Additionally, blood vessels allow for intravasation and hematogenous spread. This process is accomplished by the release of pro-angiogenic signals, such as vascular endothelial growth factor (VEGF), fibroblastic growth factor (FGF) and connective tissue growth factor (CTGF) from tumor cells (57, 58). Angiogenesis is controlled through the balance of pro- and anti-angiogenic factors within the interactome. Thus, the understanding of intractomic parameters regulating the tumor vascular compartments will facilitate cancer therapeutics that will aid in preventing tumor spread and recurrences.

TGF-β1 can induce the formation of tube-like structures in collagen gels. TGF-β1-treated cells develop pseudopodia, junctional complexes between adjoining cells, and organized basal lamina-like material (59). According to Yang *et. al*., dose-dependant angiogenic capillary sprouts are seen in chick chorioallantoic membrane as a result of TGF-β1 treatment (60). In this study, the formation of large blood vessels was induced by TGF-β1 and small blood vessels were primarily induced by basic fibroblast growth factor (bFGF), suggesting these molecules to act synergistically within the interactomic nodes. Studies using mouse models that are defective in TβRII and TβRI demonstrate obvious defects in the angiogenesis process (61, 62). Hypoxic conditions in tumor interior stimulate VEGF levels through the activation of hypoxia-inducible factor 1 and Smad proteins (63). Thus, the hypoxia-dependent activation of interactomic players appears to induce angiogenesis. Similarly, Hasegawa et al. has found a correlation between TGF-β1 expression and vessel density in non small cell lung carcinoma tissue samples (64). Introduction of the *TGF-β1* gene into human prostate tumor cells stimulated angiogenesis and metastasis after orthotopic implantation in severe combined immunodeficiency mice. Metastatic lesions expressed higher levels of TGF-β1 and matrix metalloproteinases (MMP) 2/9, but the expression levels of IL-10 or TIMP-1 were low (65), suggesting interactomic connections of IL-10 and TIMP-1 to be tumor suppressive and MMP-2 and MMP-9 to be angiongenic (66) and prometastatic (67). Thus, these inteactomic nodes show potential as intervention sites for future thereapeutic discovery. Recent studies support this hypothesis and their results show TGF-β1 to be a key regulator of angiogenesis brought about by MMP-2 and MMP-9 during metastatic spread (68, 69). Hence, TGF-β1 interactomic connections with MMP-2 and MMP-9 have the potential to be utilized to halt the metastatic process by controlling angiogenesis.

Immunosuppressive Properties of TGF-β1 Interactome

Immune surveillance is one of the major mechanisms that prevent metastasis (70). However, tumor cells have developed their own strategies to overcome this barrier. One such strategy is to use TGF-β1, a potent immune suppressor, to dampen immune surveillance. Transfection of *TGF-β1* cDNA into a highly immunogenic C3H-derived tumor resulted in reduction of cytotoxic T-cell mediated response (71). The investigations of Arteaga and co-workers

demonstrated that TGF-β works in an autocrine fashion to induce metastasis by suppressing natural killer cells (72). Chinese hamster ovary (CHO) cells transfected with an expression vector encoding for *TGF-β1* suppressed natural killer cells (73). Fas ligand (*CD95L*) and *TGF-β1* combination promote depletion of T-lymphocytes and neutrophils, allowing tumor cells to evade immune surveillance. Therefore *CD95L* combinations with the interactome may help avoid immune surveillance. Furthermore, neoplastic cell surface expression of major histocompatibility complex (MHC) class I and II molecules was observed to be down-regulated by TGF-β1 (74). Moreover, TGF-β1 inactivates lymphokine activated killer cells and blocks immunoglobulin synthesis that are important for recognition of neoplastic cells by CTL (75). Thus, immune therapies for prevention of metastasis require approaches to reverse the polarity of these interactomic nodes of immune depletion.

Role of TGF-β1 Interactome in Suppression of Metastasis

TGF-β1 was initially considered to be one of the most potent growth inhibitors isolated and was thought to primarily act as an inhibitor of tumor progression (76). This was purely due to the observation of TGF-β1 acting as an autocrine negative growth regulator in keratinocytes and breast cancer cells (77). Studies carried out using xenograft mouse models have demonstrated that inhibition of TGF-β1 helps trigger the formation of human colon adenocarcinomas (78). The exact mechanism of these contradictory effects remains elusive. However, distinct polarity changes in different tumor types and other individualistic variations may help explain this phenomenon. The gene for TβRII is frequently mutated in patients with hereditary non-polyposis colorectal cancer. These patients show a truncated receptor incapable of signal transduction, suggesting a tumor suppressive function in the receptor proteins (79). Hence, overexpression of the same receptor in carcinoma cells can prevent malignant transformation process. Keratinocytes that lack Smad3 or TβRII expression show rapid proliferation and migration causing squamous cell carcinoma (80), suggestive of the metastatic suppressive nodes of the TGF-β interactome. The altered transcription factor production with these tumors or the CpG island methylation of the promoters of *TβRI* and *TβRII* can contribute to low expression levels of these receptors. Metastatic suppressor activities such as growth inhibition have been observed when TβRII is over-expressed in thyroid carcinoma cells and breast cancer cells in vitro and in nude mice (81, 82). However, most tumors do not have inactivated *TβRI* and *TβRII*, indicating the presence of other significant contributory proteins in controlling the polarizations towards malignant transformation.

TGF-β1 controls the expression of a number of fundamental proteins that play key roles in the control of cell cycle progression from the G_1 phase to the DNA synthesis S phase. The cell cycle is regulated by protein kinases composed of regulatory cyclin subunits and their catalytic cyclin-dependent kinase (CDK) partners. Cellular proliferation and differentiation is controlled by CDKs in the G_1/S phase. TGF-β1 exerts mitogenic effects during the G_1 phase by modulating CDK activities. Stimulation of $p15^{Ink4b}$ by TGF-β can lead to the inhibition of both cyclinD/CDK4/6 and cyclin A/E/CDK2 inhibiting cell division (83, 84). Over-expression of Smad7 eliminates TGF-β-mediated decrease in cyclin A and B levels, CDK2 inactivation and up-regulation of $p27^{Kip1}$, inducing cell proliferation (85). Therefore, TGF-β interactomic nodes can influence growth inhibition by simultaneously inhibiting CDK functions and eliminating proliferative functions.

Genes encoding Smad2 and Smad3 are relatively less mutated compared to receptor proteins (86-88). Allelic loss of *Smad3* with homozygous inactivation of $p27^{Kip1}$ that is responsible for the regulation of actin cytoskeleton induces leukemogenesis (89). *Smad4* mutations are detected principally in pancreatic carcinomas and in colon carcinomas. Inactivation of both alleles of *Smad4* contributes to the metastatic progression of these cancers (90, 91). *Smad 2* and *4* mutations commonly occur in the MH2 domain, which is important in the formation of heteromeric complexes and transcriptional activation (3). Smad4 mutations have also been observed simultaneously with mutations in *TβRI* and *TβRII*, suggesting tumor suppressor activities of Smad4 to be more complex than once thought. Smad7 overexpression can decrease Smad responsiveness and increase tumorigenesis, overriding TGF-β induced growth arrest (92). Changes in Smad signaling are associated with poor prognosis in head and neck cancer (93) and are frequently associated with advanced disease and the presence of lymph node metasatasis (94). This indicates the importance of Smads as crucial players in deciding the inteactomic polarizations towards metastasis.

The TGF-β1 interactome can suppress the tumor growth by other means such as through the process of programmed cellular death (95). Apart from regulating the cell cycle, TGF-β can limit unwanted cellular proliferations through its effects on apoptotic pathways. Apoptosis is important to eliminate damaged or abnormal cells from normal tissues. Alterations in this process often assist malignant transformation and metastasis. Apoptosis-related proteins in the TGF-β signaling pathway have been shown to enhance cell death through activation of caspase 3 (95). Production of reactive oxygen species links with the TGF-β interactome in inducing apoptosis while antioxidents participate in blocking this process (95). These proteins appear govern TGF-β inteactions towards apoptosis. Furthermore, activation

of p38 MAP kinase pathway through activation of TGF-β activated kinase 1 (TAK1) and mitogen-activated protein kinase kinase 3 (MKK3) is also responsible for TGF-β-induced apoptosis (96). Studies carried out using hepatoma cells demonstrate that mixed lineage kinase 3 (MLK3) participate in mediating TGF-β induced apoptosis (97). A study carried out by Kamaraju et al. indicates that p38 MAP kinase and p160/Rho/ROCK pathways have a contributory role in the Smad-dependant growth inhibition of breast cancer cells (98). TGF-β receptor-activated p38 MAP kinase pathway can induce Smad-independent apoptosis responses in breast cancer cells (99). Non-Smad signaling transducers have show to regulate apoptosis induced by the TGF-β interactome by providing cross links with Notch, tyrosine kinase pathways and cytokine receptors (100).

Proto-oncogene *c-myc* controls cell cycle entry into the S phase by regulating the transcription of various cell cycle related genes. Thus, down-regulation of *c-myc* is critical for inhibition of cell proliferation by TGF-β. In fact, a Smad-responsive element has been identified in the *c-myc* promoter (101). According to Pietenpol *et al*., TGF-β can rapidly inhibit transcription of *c-myc* in epithelial cells (102). These data demonstrate the capability of TGF-β interactome in constraining cancerous growths through *c-myc* down-regulation. Repression of Smad-mediated transcription is seen with increased expression of *c-Ski* and *SnoN* proto-oncogenes, resulting in abrogating tumor suppressor function of TGF-β interactome (103). TGF-β can down-regulate proto-oncogenes such as *c-myc*. However this inherent ability seems to be lost in many cancer types. All these evidence suggest a fundamental principle for developing future therapeutics that is based on controlling the polarity of TGF-β interactome towards inhibiting growth and metastasis.

Utilization of Interactomic Nodes to Prevent Tumor Metastasis

As explained previously, TGF-β1 interactome demonstrates a dual polarity, as a tumor suppressor in early oncogenesis and as a tumor promoter in late stages. As mentioned earlier, it would be highly beneficial, if TGF-β1 interactomic nodes could be manipulated to change the polarity from tumour promoter to tumor suppressor. However, this approach poses multiple problems, since the tumor suppressive properties and tumor promoter properties seem to merge at times. A feasible approach would be to start treatment before the tumor cells becomes resistant to growth inhibition by TGF-β1. Although identifying agents that augment the growth arrest seems promising, identification of specific stage without growth inhibition can be very demanding in a clinical setting. One effective approach employed is to isolate the interactomic nodes that boost the growth inhibition arms of the interactome and to implement these therapeutics very early in disease. Another approach is to antagonize the tumor promoting nodes of the interactome, using large molecule and small molecule inhibitors such as anti-TGF-β1 antibodies, anti-sense RNA or overexpression of receptors (104). For instance, the expression of anti-sense TGF-β1 in EMT6 cells reduces the tumorigenicity, indicating the usefulness of these in enhancing anti-tumor responses (105). Yang *et al*. in their study claim that the transgenic expression of TGF-β RII:fc fusion protein can help prevent metastasis (101). Administration of anti-endoglin, which targets endothelial cells, assists in suppressing angiogenic properties (106). Combinations of IL-2 with anti-TGF-β antibodies can be utilized to hamper the carcinogenesis in highly metastatic melanoma (107). Translit is an anti-allergic compound that can antagonize interactome-induced immune suppression and is effective in the treatment of gliomas (108). However, when TGF-β1 receptor blockers are used, there is a risk of side-effects due to the blocking of TGF-β1 signaling in normal cells. Thus, TGF-β binding proteins may be good candidates when administered in various dosages, so that they can neutralize only the nodes that act to promote tumor formation, growth, and metastasis (Table I).

Small molecular inhibitors are a very attractive avenue in regulating the TGF-β1 pathway. These molecules can be used easily to target interactomic nodes such as TGF-β-activated kinase 1 (TAK-1) and TβRI. A number of search methods have been used to identify the potential small molecular targets. One such approach is to use high-throughput virtual screening using computational methods. These methods utilize all the chemical, biological and structural information available to make calculated predictions of target molecules with higher accuracy and reproducibility. These methods are useful in narrowing down the huge number of potential targets to manageable levels. Using these methods, Callahan et al. have identified a number of novel agents such as SB-203580 that were able to inhibit ALK5/ TβRI kinase and p38 significantly (109). They have successfully isolated inhibitors of ALK5 that do not demonstrate any inhibition of p38 kinase. Thus, these novel small molecules can be used to activate or inhibit a specific arm within the interactome. SB-505124, a more potent member of imidazole inhibitors of p38, can inhibit activin receptor-like kinase (ALK) 5. This can inhibit ALK4, ALK5, and ALK7 dependent activation of Smad2 and Smad3 without affecting ALK1, ALK2, ALK3 or ALK6-induced Smad signaling. Tumor angiogenesis and metastasis in human glioma and osteosarcoma have been inhibited using the small molecule inhibitor, SB-431542. Other inhibitors such as pyrazole inhibitors (LY550410 and LY580276), which interact with the ATP-binding site of the TβRI kinase domain, have been isolated by Lilly research laboratories (110). Using shape-based virtual screening of nearly 200,000 compounds Biogen Inc has discovered HTS-466284. This

Table I. *Therapeutic potential of TGF-β interactomic nodes.*

Strategy	Tumor type	Tested model	Reference
TGF-β antisense molecules			
TGF-β antisense modified MOT	Ovarian teratoma	Animal model C3H mice	(115)
IFN-gamma and antisense *TGF-β* gene expression	Metastatic breast cancer	4T1 model	(116)
Antisense *TGF-β* plasmid vector (pCEP-4/*TGF-β* antisense)	Glioma	Rat 9L Glioma tumor model	(117)
Antisense *TGF-β* transgene	Mammary tumor	BALB/c and C57BL/6 mice	(118)
Electrophoration of simian TGF-β expression vector pCEP-4	Glioma	Wistar rats	(119)
Antisense *TGF-β2* in pCEP-4 vector	Hepatocellular carcinoma	Buffalo rats	(120)
Anti-TGFβ2 oligonucleotide (AP-12009)	Glioma, adenocarcinoma and melanoma	Phase I/II-studies in human	(121)
TGF-β antibody			
2G7 IgG2b *TGF-β* neutralizing antibody	Breast cancer	Athymic mice	(72)
Monoclonal antibodies prepared against conjugated transforming growth factor beta 1	Metastatic cancer	Nude mice	(122)
Anti-pan *TGF-β* antibody (GC-1008)	Pulmonary fibrosis	Phase I	(123)
Inhibition of *TGF-β* receptors			
Expression of dominant-negative mutant TGF-beta type II receptor (dnTGF-RII)	Breast cancer	Nude mice	(124)
Expression of dnTGF-RII in $CD4^+$ and $CD8^+$ T-cells	Melanoma	CD4dnTGF-RII transgenic mice	(125)
dnTGF-RII-expressing bone marrow cells	Prostate cancer	C57BL/6 mice	(126)
dnTGF-RII expressed using retroviral vectors	Colon carcinoma cells	Balb/C mice	(44)
Small molecular TGF-βR1 kinase inhibitor LY-2157299, LY-550410, LY-580276, LY-364947	Glioma	VM/Dk mice	(127)
Selective inhibitor for *ALK4, ALK5*, and *ALK7*	Various cancer types	HaCaT, NIH 3T3, C2C12, and T47D cells	(128)

compound can inhibit autophosphorylation of TβRI kinase and is an effective, non-toxic inhibitor of TβRI (111). SD-093 and LY580276 can inhibit the *TGF-β*-induced EMT and these compounds are effective metastatic inhibitors (112, 113). SD-093 is special in this case as it can inhibit the autocrine TGF-β-induced invasion and metastasis without affecting any morphological characteristics (114). This indicates that virtual screening can be a powerful tool to narrow down and discover inhibitory nodes in the TGF-β1 interactome. These approaches are both efficient and inexpensive means of discovering novel drug targets. Thus an effective therapeutic strategy would be to evaluate different combinations of these target molecules depending on the individualistic nature of the problem. Pre-clinical approaches employing these strategies will direct the development of personalized medicine for future cancer therapy.

Conclusion

Past years have yielded significant advances in understanding of inherent anti-metastatic and prometastatic characteristics of the TGF-β1 interactome. These advances have assisted the discovery of novel biomarkers, diagnostic tools and potential therapeutic targets for the prevention of cancer. However, tumor suppressor and pro-metastatic interactions of this ubiquitous cytokine still needs comprehensive scientific exploration in order to effectively utilize them in the clinical setting. The stage or the switch, which triggers the polarity effects of tumor suppression and tumor promotion, still seems elusive to scientific probing. Nevertheless, promising pre-clinical evidence of the metastatic deterrent power is an attractive foundation for novel therapeutic discovery. As revealed by interactomic maps all TGF-β-related molecules may not match the anti-metastatic potential. However, large molecular and small molecular inhibitors that utilize a variety of nodes of the interactome are being developed by various laboratories hold great promise. Further scientific probing of potential TGF-β1 tumor suppressor nodes would supplement the existing knowledge regarding the prevention the metastatic process. The complexity of the TGF-β1 metastatic interactome is such that it tends to have different responses depending on tumor type, stage of the tumor and the individualistic characteristics. Thus, future research should be directed towards generating personalized interactomic maps, using high throughput screening methods such as micro arrays, protein profilers, mass spectrometry and related computational models. Investigations using appropriate cell culture and animal models should be utilized to further validate and optimize these high throughput screening models. Personalizing different links

in TGF-β1 interactome, including autocrine, paracrine, feed-forward, feed-backward loops and other molecular links of various proteins and genes, offer powerful avenues for the designing of competent combination therapeutic tools. The discovery of effective therapeutics based on these novel strategies will immensely facilitate the development of personalized cancer therapeutics in the future.

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