J. Toxicol. Pub. Health
Vol. 23, No. 3, pp. 197-205 (2007)

Official Journal of
Korean Society of Toxicology
Available online at http://www.toxmut.or.kr

Perspectives - Minireview

Role of Transforming Growth Factor-β in Tumor Invasion and Metastasis

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Received August 2, 2007; Accepted September 5, 2007

Cancer metastasis is a major determinant of cancer patient mortality. Mounting evidence favors a strong positive role for TGF-β in human cancer progression. The complex pattern on cross-talk of TGF-β and the related other signaling pathways is an important area of investigation that will ultimately contribute to understanding of the bifunctional role of TGF-β in cancer progression. This review summarizes some of the current understanding of TGF-β signaling with a major focus in its contribution to the tumor cell invasion and metastasis. Five issues are addressed in this review: (1) TGF-β signaling, (2) TGF-β and EMT, (3) TGF-β and MMP, (4) TGF-β and Ras, and (5) Role of TGF-β in invasion and metastasis. Due to the bifunctional cellular effects of TGF-β, as a tumor promoter and a tumor suppressor, more precisely defined TGF-β signaling pathways need to be elucidated. According to the current literature, TGF-β is clearly a major factor stimulating tumor progression through a complex spectrum of the interplay and cross-talk between various signaling molecules. Understanding the role of TGF-β in invasion and metastasis will provide valuable information on establishing strategies to manipulate TGF-β signaling which should be a high priority for the development of anti-metastatic therapeutics.

Key words: TGF-β, EMT, MMP, Invasion.

INTRODUCTION

Tumor cell invasion and metastasis are complex processes in which extracellular matrix (ECM)-degrading proteinase activity and migration through the ECMs are involved (Fidler, 1990). Undoubtedly, multiple gene products are required for the invasive activity. An essential part of the metastatic process includes degradation of the basement membrane and the stromal extracellular matrix, which allows cells to migrate into neighboring tissues. Members of matrix metalloproteinase (MMP) family, especially, MMP-2 and MMP-9, have been shown to be deeply involved in tumor invasion and metastasis formation since they can degrade type IV collagen, the major structural collagen of the basement membrane and thus play a critical role in tumor invasion and metastasis formation (Ura *et al.*, 1989; Stetler-Stevenson, 1990; Liotta and Stetler-Stevenson, 1991; Tryggvason *et al.*, 1993; Sato *et al.*, 1994).

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Transforming growth factor (TGF)-β family is a multifunctional cytokine, which include TGF-βs, activins, and bone morphogenetic protein (BMPs) (Piek *et al.*, 1999). TGF-β exerts diverse effects on cellular processes including proliferation, differentiation and apoptosis (Massague, 1998; Whitman, 1998). TGF-β was originally isolated as one of two components (TGF-α and TGF-β) that could induce a transformed phenotype in normal rat kidney fibroblasts (De Larco and Todaro, 1978; Roberts and Sporn, 1990). In mammals, there are three different TGF-βs (β1, β2 and β3) which are encoded by different genes but all function through the same receptor signaling system (Roberts and Sporn, 1990; Massague, 1998).

TGF-β signaling has been mainly implicated in cancer (reviewed in de Caestecker *et al.*, 2000; Akhurst and Derynck, 2001; Elliott and Blobe, 2005; Bierie and Moses, 2006). TGF-β plays multiple roles in epithelial cell types, from which a majority of human cancer arise, including cell growth arrest and enhancing migration (Tucker *et al.*, 1984; Moses *et al.*, 1985; Dumont *et al.*, 2003). The role of TGF-β in carcinogenesis is complex. TGF-β can act both as a tumor suppressor and as a

significant stimulator of tumor progression, invasion and metastasis (Cui *et al.*, 1996). TGF-β has been identified as a potent inhibitor of the progression of normal epithelial cells and endothelial cells by growth arrest in the cell cycle (Alexandrow and Moses, 1995; Taipale *et al.*, 1998). In early tumor stages, transformed epithelial cells are still sensitive to TGF-β-mediated growth arrest (Markowitz and Roberts, 1996). In later stage of tumorigenesis, however, it contributes to tumor progression (Reiss and Barcellos-Hoff, 1997; Akhurst and Balmain, 1999) by inducing an epithelial-mesenchymal transition (EMT), cell invasion and migration of epithelial tumor cells (Portella *et al.*, 1998; McEarchern *et al.*, 2001).

The present review aims to highlight the current understanding of the role of TGF-β in tumor invasion and metastasis. The following themes are addressed in this review: **(1) TGF-β signaling.** Different signaling modules are responsible for a unique function of the cytokine in epithelial cells. An intact TGF-β signaling pathway is necessary for cancer cell invasion and metastasis. This review summarizes the signaling molecules activated by TGF-β and their roles in TGF-β-induced cellular responses, especially cell invasion. **(2) TGF-β and EMT.** One aspect of tumor cell biology that is thought to contribute to metastasis is EMT since it causes increased cellular plasticity, allowing cells to move out of the primary tumor into the circulation (Thiery and Chopin, 1999). TGF-β is able to induce EMT in a number of cancer models either by itself or in cooperation with other signaling pathways. **(3) TGF-β and MMP.** Many recent studies have documented a direct correlation between high levels of expression of MMP and an increased invasive capacity of tumor cell lines. TGF-β has been demonstrated to regulate expression of MMP-2 and/or MMP-9 (Lin *et al.*, 2000) in a cell type-specific manner. As the enzymatic degradation of ECM barriers is a key step in tumor cell invasion, it is probable that TGF-β mediates tumor cell invasion by regulation of ECM-degrading MMPs. **(4) TGF-β and Ras.** Elevated levels of Ras expression and activated mutation of Ras have been suggested as a marker for tumor aggressiveness of cancer. TGF-β collaborates with oncogenic Ras and brings about metastatic and invasive phenotypic changes in Ras-transformed cells. Studies on the contribution of TGF-β to tumor progression through the collaboration with oncogenic Ras are summarized in this review. **(5) Role of TGF-β in invasion and metastasis.** TGF-β is widely overexpressed in many human cancers and this alteration is associated with tumor cell invasion and metastasis. This review covers current understanding of TGF-β with a major focus on the contribution of TGF-β signaling network to tumor invasion and metastasis.

TGF-β signaling. The TGF-β signaling pathway has been summarized in the several recent reviews (Attisano and Wrana, 2002; Shi and Massague, 2003; Derynck and Zhang, 2003). Signaling of TGF-β is mediated by a heteromeric complex of two types of transmembrane serine/threonine kinase receptors, called TGF-β type I receptor and TGF-β type II receptor (Shi and Massague, 2003; Ten Dijke and Hill, 2004). When TGF-β binds to the receptor complex, the TGF-β type II receptor kinase phosphorylates and activates the TGF-β type I receptor kinase. The activated TGF-β type I receptor then phosphorylates receptor-activated Smads (R-Smads) which consist of Smad2 and Smad3 (Massague, 1998). After phosphorylation by the TGF-β type I receptor kinase, the R-Smads bind to Co-Smad (Smad4), a common partner for all the R-Smads, and move into the nucleus. Up to date, seven TGF-β type I receptor, commonly called activin receptor-like kinases (ALK)1 to 7, and five TGF-β type II receptor have been identified (Manning *et al.*, 2002). Smad6 and Smad7 are inhibitory Smads (I-Smads) that block phosphorylation of Smad2 or Smad3, thus inhibiting TGF-β signaling (Elliott and Blobe, 2005). Since the Smad proteins do not have enzymatic activity, this signaling pathway is not amplified, and cellular responses are sensitive to small changes in the level of Smad protein expression which may result from the transcriptional regulation of Smad expression (de Caestecker *et al.*, 2000). Treatment with TGF-β caused the decrease in mRNA of Smad3 (Yanagisawa *et al.*, 1998).

Following TGF-β receptor-induced phosphorylation, R-Smads partner with the Co-Smad and translocate to the nucleus. In the nucleus, this Smad complex associates with other transcription factors to activate transcription of target genes (Zhang and Derynck, 1999; Massague, 2000). TGF-β target genes involved in cell proliferation and transcriptional interactors of the Smad complex were summarized in de Caestecker *et al.* (2000). Identification of the target genes of TGF-β and understanding their roles in both normal and malignant cells will provide insight into the molecular mechanisms of TGF-β-induced cellular responses.

In addition to Smads, TGF-β-mediated signaling investigated in cancer involves activation of a number of direct downstream targets including mitogen-activated protein kinase kinase 1 (MAPKK1), TGF-β-activated kinase 1 (TAK1), phosphoinositide-3 kinase (PI3K), Ras, RhoA, protein phosphatase 2A, and mitogen-activated protein kinases (MAPKs) (Bakin *et al.*, 2000; Derynck and Zhang, 2003). The common pathways of TGF-β

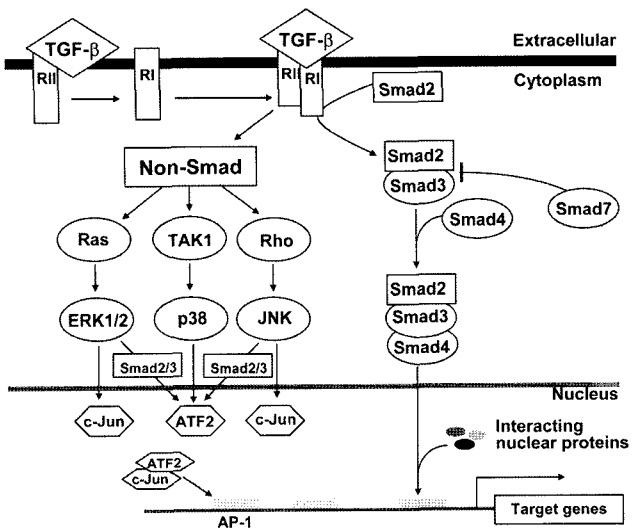

Fig. 1. Smad-dependent and Smad-independent TGF-β signaling pathways. TGF-β binds TGF-β type II receptor and activates the TGF-β type I receptor kinase. The activated TGF-β type I receptor phosphorylates Smads at C-terminal serines, and these R-Smads (Smad2 and Smad3) then form a complex with a Co-Smad (Smad4). Activated Smad complexes translocate into the nucleus, where they regulate transcription of target genes. In addition to Smads, TGF-β-mediated signaling involves activation of a number of downstream targets including Ras, Rho, TAK1 and MAPKs.

and the signaling pathways *via* a MAPK/Smad-interdependent interaction (de Caestecker *et al.*, 2000) are depicted in Fig. 1. TGF-β activates a variety of MAPK pathways including c-Jun-N-terminal protein kinase (JNK), extracellular signal-regulated kinase (ERK) and p38 MAPK, that may be required for both Smad-dependent and Smad-independent transcriptional responses to TGF-β (Mulder, 2000; Atfi *et al.*, 1997; Hartsough and Mulder, 1995; Hanafusa *et al.*, 1999). Activation of the MAPK pathway by TGF-β may also affect transcriptional response through direct effects on Smad-interacting transcription factor, for example, the JNK substrate c-Jun or the p38 MAPK substrate activating transcription factor (ATF) 2, allowing convergence of TGF-β-induced Smad and MAPK pathway (Massague, 2000; Itoh *et al.*, 2000).

The dual ability of TGF-β to activate Smads and MAPK signaling has an important role in TGF-β-induced EMT transdifferentiation, which depends in part on the ERK and/or p38 MAPK pathway (Yu *et al.*, 2002; Bakin *et al.*, 2002). In addition, several intercellular proteins that mediate signaling by receptor tyrosine kinase, G-protein-coupled receptor or cytokine receptors also participate in the TGF-β signaling network (Derynck and Zhang, 2003). Elucidation of the interplay between these signaling pathways as well as the complex patterns of cross-talk with other signaling pathways will contribute to understanding of the bifunctional role of TGF-β in cancer.

TGF-β and EMT. EMT, a process in which epithelial cells acquire mesenchymal characteristic, is believed to be a crucial step during tumor expansion. EMT is a complex process in which epithelial cells undergo dramatic changes, such as acquisition of a fibroblastoid phenotype, loss of expression of epithelial-specific protein and induction of various mesenchymal markers, and finally digestion and migration through ECM (Fig. 2). Acquisition of EMT properties during tumor progression is associated with dissolution of epithelial integrity, increased migration, local invasion and metastasis. The ability of mature epithelial cells to acquire a mesenchymal phenotype can increase their ability to invade the

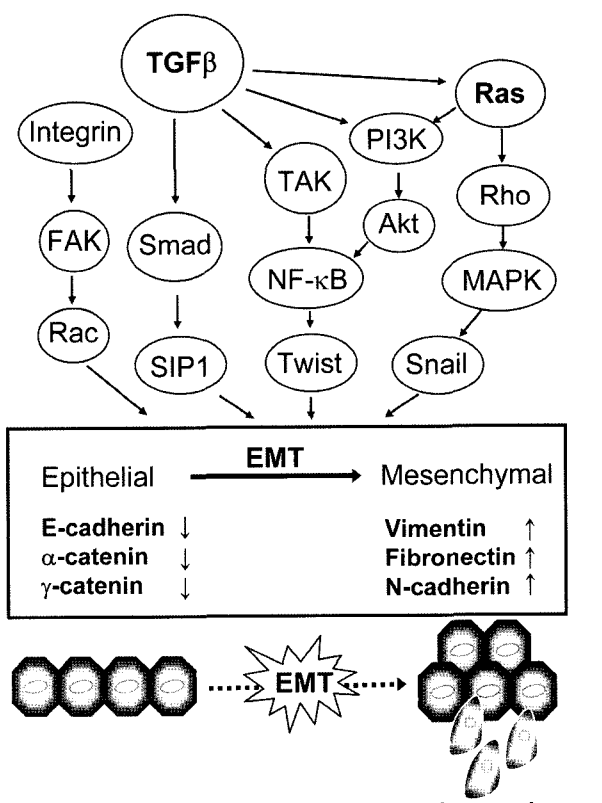

Fig. 2. Signaling pathways mediating EMT. Activation of signaling pathways such as TGF-β signaling leads to dramatic changes in cell shape, gene expression and cellular behavior, resulting EMT. The morphological transition of epithelial to a mesenchymal appearance is accompanied by a gain of mesenchymal cell markers such as vimentin, fibronectin and N-cadherin, and a loss of epithelial markers such as E-cadherin, α-catenin and γ-catenin.

extracellular matrix and metastasize (Frixen *et al.*, 1991; Thiery and Chopin, 1999). EMT is a well-established process during development and has been documented to occur in 24% to 45% of human breast cancers (Oft *et al.*, 1998), 39% to 60% of gastric cancers (Rosivatz *et al.*, 2002), and 74% of renel cell cancers (Oft *et al.*, 1998). During tumorigenesis, EMT may increase the motility and invasiveness of cancer cells, and malignant transformation may be associated with signaling pathways promoting EMT (Boyer *et al.*, 2000).

The cells undergoing EMT must counteract TGF-β-mediated growth control, alter shape and down-regulate epithelial markers (Thiery, 2002). The calcium-dependent cell-cell adhesion receptor, E-cadherin, is one of the crucial targets for inactivation or transcriptional repression during EMT. E-cadherin is commonly downregulated in many cancers, and its overexpression can suppress invasion by tumor cells (Thiery, 2002). TGF-β-induced EMT coincides with loss of E-cadherin expression (Oft *et al.*, 1996; Miettinen *et al.*, 1994). Phenotypic markers for an EMT include an increase capacity for migration and invasion, as well as resistance to anoikis/apoptosis (Lee *et al.*, 2006). TGF-β-induced EMT has been shown in various carcinomas *in vitro* and *in vivo* such as ovarian adenosarcoma cells (Kitagawa *et al.*, 1996) and Ras-transformed mammary carcinoma cells (Oft *et al.*, 1996) and squamous skin carcinomas (Portella *et al.*, 1998).

The mechanism of TGF-β-induced EMT is complex: TGF-β-induced Smads and RhoA activation and Ras/PI3K signaling may play distinct roles (Piek *et al.*, 1999; Santibanez *et al.*, 2002; Iglesias *et al.*, 2000; Bhowmick *et al.*, 2001). Although TGF-β-activated Smad proteins are important in EMT-induction, Smads alone are not sufficient to trigger EMT and RhoA, ERK and/or p38 MAPK pathway in cooperation with integrin signaling are required (Bhowmick *et al.*, 2001; Bakin *et al.*, 2002; Yu *et al.*, 2002). The Smads, especially Smad3 and Smad4, are critical for the EMT response *in vitro* (Valcourt *et al.*, 2005) and *in vivo* (Oft *et al.*, 2002; Li *et al.*, 2003; Tian *et al.*, 2003, 2004; Saika *et al.*, 2004). Mutant TGF-β type I receptors that lack the Smad-docking site fail to induce EMT but can activate endogenous p38 MAPK or JNK signaling (Yu *et al.*, 2002; Itoh *et al.*, 2003). In a related study, TGF-β and the extracellular matrix protein laminin-5 induced EMT and hepatocellular carcinoma cell invasion by upregulating Snail and Slug, down-regulating E-cadherin, translocating β-catenin into nuclei, and inducing dramatic spreading and morphological changes in the cancer cells (Giannelli *et al.*, 2005).

A study on the epithelial cell plasticity in tumor progression demonstrated that local concentration of factors such as TGF-β, TGF-α and fibroblast growth factor at the primary tumor site might initially be responsible for the EMT (Thiery and Chopin, 1999), resulting in invasion and intravasation, but the ultimate histological appearance of the tumor would depend on reduced concentrations of these factors at the site of metastasis. The transient switch to an invasive fibroblastic phenotype would be followed by reconversion to an epithelial morphology depending on the local microenvironment (Akhurst and Derynck, 2001). Exploring the mechanisms of EMT and their regulation by TGF-β may be of great importance in seeking new therapeutic targets for cancer metastasis.

TGF-β and MMP. MMPs can trigger the morphological transformation of epithelial cells (Lochter *et al.*, 1997; Sternlicht *et al.*, 1999). Increased enzymatic degradation of extracellular matrix proteins by MMPs facilitates tumor spread (Platten *et al.*, 2001; Wick *et al.*, 2001). Production of active MMP-2 and MMP-9 protease is important in tumor invasion, migration and intravasation. The enhanced invasion *in vitro* and metstasis *in vivo* was thought to be partially attributed to TGF-β-dependent up-regulation of MMP-2 and MMP-9 activities (Welch *et al.*, 1990). Exogenous TGF-β directly increases the motility of glioma cells by enhancing expression of collagen and subunit of $\alpha_{2,5}$, β_3 integrin, as well as by up-regulating the activity of MMP-2 and MMP-9 at the cell surface of glioma cells (Wick *et al.*, 2001).

TGF-β up-regulates expressions of MMP-2 and MMP-9 in normal and cancer cells (Wahl *et al.*, 1993; Kim *et al.*, 2004, 2005). Enhanced expression of MMP-2 but not MMP-9 by TGF-β was reported in pancreatic cancer cells (Ellenrieder *et al.*, 2001) whereas TGF-β was shown to induce MMP-9 in transformed keratinocytes (Johansson *et al.*, 2000; Santibanez *et al.*, 2002). We have previously reported that TGF-β induces upregulation of MMP-2 and MMP-9 through transcriptional activation in human breast epithelial cells (Kim *et al.*, 2004). For the regulation of MMP-9 expression by TGF-β, there might be other mechanism(s) including increased mRNA stability as previously shown in human prostate cancer cells (Sehgal and Thompson, 1999).

Activation of p38 MAPK pathway, but not that of ERKs pathway, is required for TGF-β-induced up-regulation of MMP-2 and MMP-9 in human breast epithelial cells (Kim *et al.*, 2004). On the contrary, it has been shown that blocking ERK pathway potently suppressed TGF-β-induced MMP-9 expression in transformed keratinocytes (Santibanez *et al.*, 2002). Critical role of p38

MAPK- and MMP-dependent pathway in TGF-β-induced osteoblast elongation was demonstrated (Karsdal *et al.*, 2001). Expression of MMP-13, but not that of MMP-2, was induced by TGF-β in this osteoblast cell system (Karsdal *et al.*, 2001). These data demonstrate that the effects of TGF-β on the expressions and matrix-degrading activities of MMPs is cell type-specific, indicating that the effects of TGF-β on cells are not a function of the peptide itself, but rather of the total set of growth factors and their receptors that is operant in the cell at a given time (Roberts *et al.*, 1985).

Studies on the transcriptional regulation by TGF-β have shown that the promoter of TGF-β-regulated genes such as TIMP-1 (Campbell *et al.*, 1991) and MMP-1 (Mauviel *et al.*, 1996) contain AP-1 sites. ATF2, a member of CREB/ATF family of transcription factors, has been shown to be a nuclear target of Smad and TAK1/p38 MAPK in TGF-β signaling (Sano *et al.*, 1999). It is phosphorylated and activated upon TGF-β treatment, mediating the cellular responses exerted by TGF-β (Ionescu *et al.*, 2003). A recent study revealed ATF2 as a potential transcription factor responsible for TGF-β-induced MMP-2 up-regulation leading to malignant progression of human breast epithelial cells (Kim *et al.*, 2007).

TGF-β and Ras. Ras proteins are activated by multiple extra cellular stimuli and are involved in regulatory biological processes from the outside of the cell to its interior through a complex array of downstream effectors, thereby controlling a variety of cellular response such as proliferation and cytokine/matrix production (Bourne *et al.*, 1991; Marshall, 1995; Tanaka *et al.*, 2002). H-Ras-specific induction of invasive and migratory phenotypes was observed and the molecular mechanisms underlying were elucidated in human breast epithelial cells (Kim *et al.*, 2003; Song *et al.*, 2006).

Ras-transformed cells exhibit a limited growth inhibitory response to TGF-β (Schwarz *et al.*, 1988) but may respond to TGF-β with invasive activity and metastatic behavior (Oft *et al.*, 1996; Yin *et al.*, 1999). In cooperation with activated Ras, TGF-β can induce a complete EMT in both mammary and keratinocyte-derived tumors (Akhurst and Derynck, 2001; Oft *et al.*, 2002), and it can drive metastasis of epitheloid tumors (Muraoka *et al.*, 2002). TGF-β has been shown to activate Ras in TGF-β-sensitive intestinal and epithelial cells (Mulder and Morris, 1992; Yan *et al.*, 1994). TGF-β contributes to tumor progression by enhancing cell invasiveness and migration through the collaboration with oncogenic Ras in Ras-transformed human breast epithelial cells (Clair *et al.*, 1987; Kim *et al.*, 2005). This process requires cooperation of Ras-MAPKs and TGF-β signaling pathways contributing to tumor invasion (Watson *et al.*, 1991; Clark and Der, 1995). TGF-β stimulates H-Ras-mediated cell migration and invasive phenotypes in human breast epithelial cells, which involves activation of p38 MAPK and ERKs pathways (Kim *et al.*, 2005). Investigating how H-Ras and TGF-β signal transduction pathways interact with each other in human breast epithelial cells may provide insight into a better understanding of the molecular mechanisms for the contribution of TGF-β to breast cancer progression in collaboration with activated H-Ras.

Role of TGF-β in invasion and metastasis. The process by which cancer cells invade and metastasize involves complex interactions between the cancer cells and their extracellular environment (Blobe *et al.*, 2000). Many studies demonstrated the role of TGF-β signaling in tumor metastasis. Increased levels of TGF-β result in more invasive cancer cells, which may represent one of the tumor-promoting activities of TGF-β, by decreasing the adhesiveness and increasing the motility and proteolytic activity of cancer cells (Maehara *et al.*, 1999).

TGF-β was shown to be a major effector of breast tumor metastasis *in vivo* (Yin *et al.*, 1999). When TGF-β signaling was blocked in MDA-MB-231 breast cancer cells, immunodeficient mice injected with these cells developed fewer tumors and less bone metastases. Exogenous TGF-β increases the invasiveness and metastatic behavior of breast cancer cells *in vivo* (Tobin *et al.*, 2002; Elliott and Blobe, 2005). In some colon cancer models, TGF-β increases tumor invasion and metastasis to increase tumorigenicity (Oft *et al.*, 1998). TGF-β regulates the blastic bone metastases characteristic of prostate cancer by mediating the ability of prostate cancer cells to migrate and invade into the bone (Festuccia *et al.*, 1999).

Overexpression of a dominant active TGF-β type I receptor resulted in a delay of tumorigenesis with an increase in metastasis (Siegel *et al.*, 2003). In another study, epithelial cell-specific ablation of TGF-β type II receptor in tumors resulted in a decrease of tumor latency with an increase in pulmonary metastases (Forrester *et al.*, 2005). These studies indicate that modification of epithelial cell autonomous TGF-β signaling can specifically influence tumor progression and metastasis (Bierie and Moses, 2006).

Although TGF-β is clearly a major factor in stimulating tumor invasion and metastasis, strategies for drug development will require detailed studies aimed at unraveling the complexities of TGF-β signaling, due to the biphasic effects of TGF-β during tumor tumorigenesis.

Specific strategies are needed for inhibition of only those components of TGF-β activation that have therapeutic potential for anti-metastatic therapeutics.

CONCLUSIONS

Cancer metastasis, the dissemination of the primary tumor to distant body sites, is a major determinant of cancer patient mortality. Mounting evidence favors a strong positive role for TGF-β in human cancer progression. TGF-β is widely overexpressed in many human cancers and this alteration in tumors is associated with tumor metastasis. Since TGF-β is clearly a major factor stimulating tumor invasion and metastasis, establishing strategies to manipulate TGF-β signaling should be a high priority for the development of anti-metastatic therapeutics. This review summarizes some of the current understanding of TGF-β signaling with a major focus in its contribution to the tumor cell invasion and metastasis.

TGF-β is involved in a large number of human cancers, and changes in signaling through this pathway often correlate with tumor progression. Since TGF-β has bifunctional cellular effects, as a tumor promoter and a tumor suppressor, more precisely defined TGF-β signaling pathways remain to be elucidated. Specific pathways would be involved in mediating the specific and context-dependent effects of TGF-β. Although Smad-mediated signaling is well established as the predominant TGF-β signaling pathway, the significance of other signaling pathways such as MAPK, Rho, PI3K pathways, remains to be further elucidated (Elliott and Blobe, 2005). Once these pathways and other potential signaling pathways downstream of TGF-β are defined and the contributions of these pathways to TGF-β-mediated tumor cell invasion and metastasis are established (Elliott and Blobe, 2005), more specific targeting of TGF-β signaling pathway will be possible, allowing targeted therapies to the cancer metastasis. TGF-β-mediated metastatic potential appear to be dependent on factors that may be unique to each type of tissues, cell types, mutation and promoter present in an individual type, stage, microenvironment or experimental model of cancer (Bierie and Moses, 2006). Although much has been known on the functions of TGF-β in the tumor progression, much more remains to be elucidated to provide implications on detailed understanding of molecular events for malignant phenotypic conversion of cells mediated by TGF-β.

ACKNOWLEDGEMENTS

This work was supported by a 2006 Research Grant from Duksung Women's University, Seoul, Korea.

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