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Growth Factors and Malignant Mesothelioma

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Growth factors can act as positive or negative modulators of cell proliferation, differentiation, motility, and angiogenesis. The interaction of these signal molecules with their membrane receptors triggers a number of intracellular signaling pathways, resulting in the activation or repression of various subset of genes. Aberrations in these biochemical signals are linked to developmental abnormalities or to a series of chronic diseases, including cancer. Tumor malignant cells arise as the result of a stepwise progression of genetic events, including deregulated expression of growth factors or of molecules involved in their signaling pathways (1).

The proliferation of normal human and rodent mesothelial cells is regulated by exposure to several growth factors, including epidermal growth factor (EGF) (2,3), tumor necrosis factor-α (TNF-α) (4), platelet-derived growth factor (PDGF) (5), hepatocyte growth factor (HGF) (6), and keratinocyte growth factor (KGF) (7).

This chapter focuses on the several growth factors expressed by mesothelial and malignant mesothelioma cells (MMCs), and discusses how deregulation of their biologic activities is responsible for the onset and progression of this tumor (Table 7.1).

Epidermal Growth Factor and Its Related Molecules

Epidermal growth factor (EGF) has a profound effect on the differentiation of specific cells in vivo and is a potent mitogenic factor for a variety of cultured cells of both ectodermal and mesodermal origin. The EGF precursor exists as a membrane-bound molecule that is proteolytically cleaved to generate the 53-amino acid peptide growth factor that stimulates cells to divide (8).

Epidermal growth factor is a powerful mitogen for human mesothelial cells too. Autotransphosphorylation and activation of the EGF tyrosine kinase receptor (EGFR) occurs after exposure to asbestos triggering the mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) cascade. The MAPK activation by asbestos is

Table 7.1. Overview of growth factors expressed by human mesothelial cells (HMCs) and malignant mesothelioma cells (MMCs)

Growth factor	Receptor	Biologic activity in HMC and MMC
EGF	EGF-R	Growth, differentiation, synthesis of glycosaminoglycans and MMP
TGF-α	EGF-R	Growth
$TGF\beta_1$ $TGF\beta_2$	TGFβ-R	Growth, change of morphology, regulation of pleural inflammation, reduced T-lymphocyte infiltration
TNF-α PDGF-AA	TNFα-R	Proliferation, collagen production, acquisition of fibroblastoid morphology, and upregulation of the synthesis of MMP
PDGF-AB PDGF-BB	PDGFR-α	Growth, motility, hyaluronan, and collagen synthesis
PDGF-AB PDGF-BB	PDGFR-β	Growth, motility, hyaluronan, and collagen synthesis
IGF-I	IGFI-R	Proliferation, proteoglycan synthesis
IGF-II	IGFII-R	Proliferation, proteoglycan synthesis
VEGF	KDR/FIt-1	Proliferation, angiogenesis
FGF-1 FGF-2	FGF-R	Angiogenesis, synthesis of hyaluronan, and proteoglycans
HGF	MET	Proliferation, motility, morphology, invasion, and angiogenesis

EGF, epidermal growth factor; FGF, fibroblast growth factor; HGF, hepatocyte growth factor; IGF, insulin-like growth factor; PDGF, platelet-derived growth factor; TGF, transforming growth factor; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

attenuated by generic inhibitors of growth factor receptor interactions, like suramin, as well as by tyrphostin AG 1478, a specific inhibitor of the EGFR tyrosine kinase activity (3). Although both asbestos-transformed MMCs and spontaneously transformed mesothelial cells express functional EGFRs, only cells transformed by exposure to asbestos fibers express into conditioned medium TGF-α, a growth factor with high affinity for EGFR (9). Interestingly, while TGF-α inhibits the growth of spontaneously transformed mesothelial cells, it stimulates the proliferation of asbestos-transformed cells, as demonstrated by the inhibition of growth observed after incubation with neutralizing antibody raised against TGF-α. Taken together, these data indicate that TGF-α acts as an autocrine growth factor for asbestos-transformed rat mesothelial cells and suggest that differences in mesothelioma etiology may be linked to differences in the molecular alterations present in these tumors (10).

Epidermal growth factor is not only a mitogen but it may also play a role in the process of cell differentiation and the synthesis of glycosaminoglycans in mesothelial cells (11). In addition, it has been recently demonstrated that many different growth factors including EGF, TGF-α, amphiregulin, heparin-binding EGF, beta-cellulin (BTC), stem cell factor, insulin-like growth factors I and II, acidic and basic fibroblast growth factors, and HGF regulate the expression in malignant mesothelioma cells of the extracellular matrix metalloproteinases (MMPs), molecules playing a key role in tumor cell invasion and metastasis (12).

Transforming Growth Factor-β

Transforming growth factor-β (TGF-β) 1 and 2 are dimeric multifunctional polypeptide that control proliferation, differentiation, and other functions in many cell types. Many cells synthesize TGF-β_1 and essentially all of them have specific receptors for this peptide. TGF-β_1 regulates the actions of many other peptide growth factors and determines a positive or negative direction of their effects.

Both TGF-β_1 and -β_2 are secreted by human and murine MMCs through an autocrine mechanism. They may both reduce T-lymphocyte infiltration into tumors and modulate malignant growth of tumor cells, as demonstrated by experiments with antisense oligonucleotides in vitro and in vivo (13). Moreover, TGF-β is responsible of evident morphologic changes in mesothelial cells (14). Both mesothelial cells and cells infiltrating in the pleural space can secrete TGF-α, because high levels of this growth factor were found in pleural effusions and in pleural tissues during disease processes. Also, TGF-β may participate in the regulation of pleural inflammation and enhance both cell proliferation and pleural fluid formation (15), partially due to induction of vascular endothelial growth factor (VEGF) (16). Cell lines derived from MM patients show considerably higher levels of TGF-β messenger RNA (mRNA) expression when compared with normal mesothelial cells. Treatment with exogenous TGF-β has no effects on growth of the MM cells, while the proliferation of the mesothelial cells is slightly induced (17).

Tumor Necrosis Factor-α

Tumor necrosis factor-α (TNF-α) is a homotrimer multifunctional proinflammatory cytokine localized in membrane belonging to the tumor necrosis factor superfamily. It also exists as an extracellular soluble form derived from the membrane form by proteolytic processing. This cytokine is mainly secreted by macrophages that can bind to, and thus functions through, its receptors TNFRSF1A/TNFR1 and TNFRSF1B/TNFBR. It is involved in the regulation of a wide spectrum of biologic processes including cell proliferation, differentiation, apoptosis, lipid metabolism, and coagulation, and has been implicated in a variety of diseases, including autoimmune diseases, insulin resistance, and cancer. Knockout studies in mice also suggested the neuroprotective function of this cytokine.

In human mesothelial cells TNF-α induces the acquisition of fibroblastoid morphology and upregulates the synthesis of matrix metalloproteinase-9 (MMP-9) and type I collagen, which may facilitate peritoneal extracellular matrix remodeling and fibrogenesis (18). Also, TNF-α induces a significant increase in cell proliferation and collagen production of rat pleural mesothelial cells in vitro, suggesting a role for this molecule in healing of the pleura after tissue injury (19).

Platelet-Derived Growth Factors AA, AB, and BB

The proteins AA, BB, and AB are members of the platelet-derived growth factor (PDGF) family and are mitogenic factors for cells of mesenchymal origin characterized by a motif of eight cysteines. They can exist either as homodimers (AA and BB) or as a heterodimer (AB) stabilized by disulfide bonds. The PDGF-α receptor binds all three dimeric forms of PDGF, whereas the PDGF-β receptor binds PDGF-BB with high affinity and PDGF-AB with lower affinity, but not PDGF-AA (20) (Fig. 7.1). They are released by platelets upon wounding and play an important role in stimulating adjacent cells to grow and thereby heal the wound.

Expression of the PDGF receptor (PDGFR) has been detected both in normal mesothelial cells and in MMCs. However, several MMC lines, but not normal mesothelial cells, display constitutively enhanced expression of the c-*sis* (PDGF-BB) and PDGF-AA genes. This PDGF-dependent autocrine circuit has been postulated to play a role in the etiology of this type of malignancy (21). Several independent studies demonstrated that normal mesothelium is responsive to PDGF predominantly via PDGFR-α and at lesser extent via by PDGFR-β receptor, whereas the autocrine stimulation of growth in mesothelioma cells hangs on the PDGF/PDGFR-β interaction (5,22,23). The pattern of PDGF and PDGF receptor expression in mesothelial cells largely corresponds to expression of PDGF and its receptors in vitro (24).

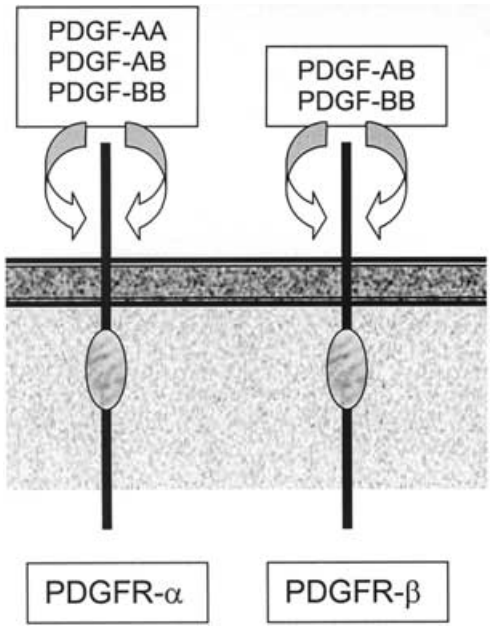

Figure 7.1. Selective binding of platelet-derived growth factor (PDGF) ligands (PDGF-AA, -AB, and -BB) to PDGF receptors.

There are two PDGF-AA transcript isoforms differing in the presence or absence of an alternative exon-derived sequence. However, both normal mesothelial cells and MMCs predominantly express the PDGF-AA transcript lacking the exon-6-derived sequence, which encodes a cell-retention signal. This means that the PDGF-AA protein is most likely secreted by both cell types and may be involved in autocrine growth stimulation via PDGF-α receptors in mesothelial cells. As well, it might also have a paracrine function if it is secreted by malignant mesothelial cells that do not express the receptor. Moreover, the enhancement of transcription seems to be the most likely mechanism for the elevated mRNA levels of PDGF-AA gene in human malignant mesothelioma cells (25). In addition, TGF-β_1, secreted in active form by mesothelial cells, may play a role in the regulation of differential PDGF-R expression, by downregulation of a still lower PDGF-α receptor mRNA level in malignant mesothelioma cells (24).

Overexpression of PDGF-AA is responsible for autocrine downregulation of its receptor. Surprisingly, the PDGF-AA/PDGFR autocrine loop is antiproliferative for mesothelioma cells in vitro, whereas proliferation is stimulated by abrogation of PDGF-α expression. This suggests that PDGF-AA does not contribute to tumorigenicity by the autocrine stimulation of growth. On the other hand, in vivo PDGF-AA overexpression is associated with augmented tumorigenicity, and abrogation of PDGF-AA expression decreases tumor incidence and increases latency period to tumor formation. Thus, the tumorigenic effect of PDGF-AA must act through paracrine mechanisms relevant at early stages of tumor initiation (26). The absence of alterations of PDGF expression in rat mesothelioma, in contrast to what occurs in the human disease, suggests that the production of this growth factor by transformed mesothelial cells may be a species-specific mechanism (27).

Platelet-derived growth factor stimulates mesothelial cell proliferation in vitro and in vivo (28) as well as hyaluronan synthesis in patients with mesothelioma, as demonstrated by partial inhibition by an antiserum raised against PDGF (29). Moreover, PDGF stimulates collagen synthesis that, if combined with increased proliferation, may be important in healing the pleura injured during the progression of the disease (2). Finally, migration of mesothelioma cells on fibronectin, laminin, or collagen-type IV in response to PDGF-BB and inhibition of this effect after pretreatment with blocking antibodies to α3β1 integrin were described, suggesting that cooperation between PDGFR-β and integrin α3β1 is necessary for the motile response of MMCs to PDGF-BB (30).

Insulin-Like Growth Factors

Insulin-like growth factors I and II (IGFs) are polypeptides structurally and functionally related to insulin but having a much higher growth and differentiation-promoting activity.

Cell lines derived from normal rat mesothelium as well as cell lines derived only from spontaneous rat mesotheliomas, but not from

asbestos-induced rat mesotheliomas, showed expression of RNA transcripts for IGF-II. All these cell lines expressed receptors for IGF-I and IGF-II, as well as insulin receptors. Coexpression of IGF-II and its cognate receptor suggests that IGF-II acts as an autocrine growth factor in the spontaneously immortalized cells and in the cells derived from the spontaneous rat tumors. Growth induced by IGF-II secreted into conditioned medium can be inhibited using an IGF-II–specific antibody in a dose-dependent manner. These data suggest that IGF-II expression may be involved in the spontaneous alteration of rat mesothelial cells and may function as an autocrine or paracrine growth factor to modulate the growth of these cells in vitro and in vivo. Ubiquitous expression of IGF-II by cells that have not been exposed to asbestos and the lack of IGF-II expression by asbestos-transformed cells suggest that the mechanisms of changes in growth factor expression differ in mesothelial cells transformed by different mechanisms (31). Similar results were also observed in vitro with IGF-I in human mesothelial cells (32). It was also shown that the existence of stimulatory effects of IGF-I on matrix proteoglycan synthesis was mediated via receptor-growth factor complexes and the protein tyrosine kinase intracellular pathway (33). The inhibitory effect of IGF-1 receptor antisense transcripts on hamster mesothelioma has been demonstrated by decreased growth and tumorigenicity in vitro and in vivo. These results may suggest interesting implications for a therapy of the human mesothelioma (34).

Vascular Endothelial Growth Factor

Vascular endothelial growth factor (VEGF) is a potent angiogenic protein with a selective mitogenic effect on endothelial cells known to be involved in many normal and pathologic processes.

Coexpression of VEGF and its receptors flt-1 and KDR has been reported in samples of mesothelioma, suggesting a potential autocrine loop for malignant pleural mesothelioma cells (35). Malignant mesothelioma cells produce significantly higher VEGF levels than normal mesothelial cells, and this growth factor is found at higher levels in the pleural effusions of MM patients than in the effusions of patients with nonmalignant pleural disease. In addition, VEGF induces increased proliferation of MMCs in a dose-dependent way, via activation of its tyrosine kinase receptor, and can have an impact on patient survival, not only by promoting angiogenesis but also by directly stimulating tumor growth (36).

Simian virus 40 (SV40)–large-tumor antigen (Tag) expression potently increases VEGF protein and mRNA levels in several human mesothelial cell (HMC) lines and concomitant expression of SV40–small-tumor antigen (tag) enhances Tag function, suggesting that VEGF regulation by SV40 transforming proteins can represent a key event in SV40 signaling relevant for tumor progression (37,38). The closely related molecule, VEGF-C, is also implicated in malignant mesothelioma growth; VEGF-C and its cognate receptor VEGFR-3 are coexpressed in mesothelioma cell lines, and a functional VEGF-C

autocrine growth loop was demonstrated in mesothelioma cells (39). Moreover, human MMCs, but not normal mesothelial cells, express a catalytically active lipoxygenases (5-LO), a key regulator of MMC proliferation and survival via a VEGF-related circuit (40).

Angiogenesis is an important part of normal and pathologic processes, including tumor growth, metastasis, inflammation, and wound healing, and VEGF is the best known angiogenic factor, implicated in tumor-associated microvascular hyperpermeability and carcinogenesis. An increased expression of VEGF was found in biphasic and epithelioid mesotheliomas and malignant pleural effusions. Vascular permeability was proportionally increased with VEGF levels in the malignant pleural effusions (41).

Fibroblast Growth Factors 1 and 2

Acidic and basic fibroblast growth factors (FGF-1 and -2) are potent angiogenic cytokines. These proteins are members of the fibroblast growth factor (FGF) family, and FGF family members possess broad mitogenic and cell survival activities and are involved in a variety of biologic processes, including embryonic development, cell growth, morphogenesis, tissue repair, tumor growth, and invasion. These proteins function as modifiers of endothelial cell migration and proliferation, as well as angiogenic factors. They act as mitogens for a variety of mesoderm- and neuroectoderm-derived cells in vitro, and thus are thought to be involved in organogenesis.

Their expression levels correlate significantly with a poor survival of MM patients, supporting the assumption that selective angiogenic cytokines might contribute to the progressive changes of mesothelioma by tumor angiogenesis (42). The expression of angiogenic factors may represent useful markers for diagnosis and prediction of disease outcome. Basic fibroblast growth factor (bFGF) is a potent angiogenic factor that promotes in vitro growth of endothelial cells and in vivo vessel formation. It displays stimulatory effects for the synthesis of hyaluronan and proteoglycans, via protein tyrosine kinase activity elicited by receptor-ligand complexes through an autocrine stimulatory mechanism (11).

Hepatocyte Growth Factor

Hepatocyte growth factor (HGF), also known as scatter factor (SF), is a multifunctional factor involved both in development and tissue repair, as well as pathologic processes such as cancer and metastasis. It is a dimer of an alpha chain and a beta chain linked by a disulfide bond and contains four kringle domains. It is a potent mitogen for mature parenchymal hepatocyte cells, seems to be an hepatotrophic factor, and acts as growth factor for a broad spectrum of tissues and cell types. it has no detectable protease activity. It has been identified

in vivo in many types of tumors together with its tyrosine kinase receptor, c-Met.

Hepatocyte growth factor and its receptor c-Met are often expressed by normal human mesothelial cells and MMCs. Moreover, coexpression of HGF and its receptor was also observed in many samples of mesothelioma, suggesting that the HGF/c-Met signaling system may play a role in the development of this tumor, by either autocrine or paracrine mechanisms. In addition, c-Met expression was found in cells obtained from pleural fluids of patients with mesothelioma (6). In vitro HGF acts as a strong chemoattractant for human MMCs and stimulates motility in all mesothelioma cell lines tested. Furthermore, HGF can stimulate mesothelioma cell migration that can be blocked in the presence of neutralizing anti-HGF monoclonal antibodies. Addition of HGF to mesothelioma cells cultured on collagen type IV is associated with a change of morphology and induction of bipolar shape and protrusion of prominent pseudopodia. Moreover, HGF is mitogenic for mesothelioma cells, suggesting that expression of HGF/c-Met is involved not only in mesothelioma progression but also in its growth (6). In addition, the ability to secrete HGF/SF seems to be correlated with the fibroblast-like morphology, and in general the biologic activity of this growth factor is dependent on the cell phenotype, because HGF induces both cell-spreading and proliferation in epithelioid cells but only stimulation of cell motility in fibroblastoid cells (43). This growth factor also enhances cell adhesion and invasion, as demonstrated by the HGF-induced synthesis of many matrix metalloproteinases and serine proteases critical for tumor progression (44). On the basis of the significantly higher microvessel density values of malignant mesotheliomas overexpressing HGF/SF, it is absolutely possible that HGF/SF also may be an additional relevant factor in tumor angiogenesis in malignant pleural mesotheliomas (45).

Interestingly, the urokinase-type plasminogen activator receptor (uPAR) expression is induced by exposure to asbestos at the surface of rabbit and human mesothelial cells, suggesting that altered expression of this receptor could be involved in asbestos-induced remodeling of the pleural mesothelium, partially due to the uPAR-dependent HGF activation (46). Finally, other findings suggest that when SV40 infects HMCs, it causes Met activation via an autocrine loop, replicates in HMCs, and infects other adjacent HMCs, inducing an HGF-dependent Met activation, change of morphology, and increase of S-phase entry (Fig. 7.2). This mechanism may explain how a limited number of SV40-positive cells may be sufficient to direct noninfected HMCs toward malignant transformation (47).

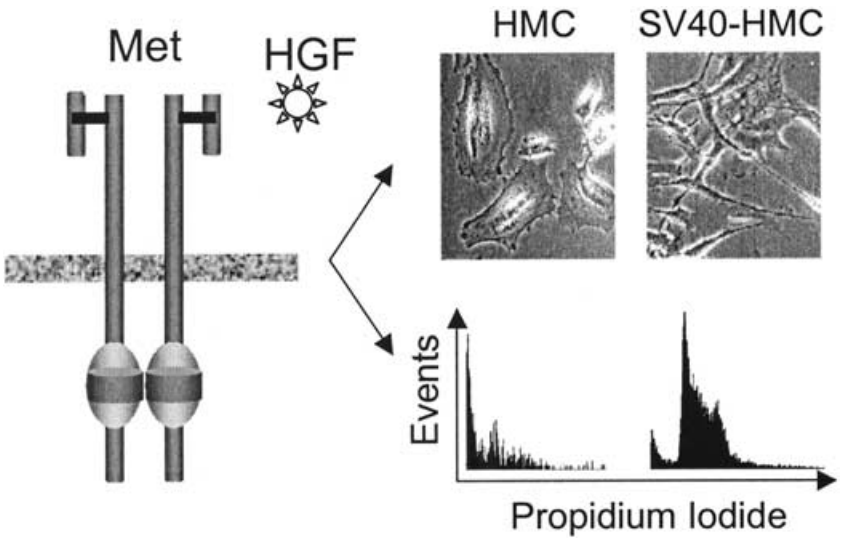

Figure 7.2. Hepatocyte growth factor (HGF)/Met autocrine loop induces change of morphology (upper panel) and S-phase entry (lower panel) in SV40 human mesothelial cells.

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