The angiogenetic pathway in malignant pleural effusions: Pathogenetic and therapeutic implications (Review)

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Abstract. Increased permeability of the pleural microvasculature is generally attributed to the substances that are released in inflammatory and malignant pleural effusions, although the exact pathogenetic mechanisms of malignant pleural effusions are unclear. Current therapies used to prevent the re-accumulation of pleural fluid and relieve symptoms are of variable efficacy and may cause serious adverse effects. Understanding the mechanisms of fluid accumulation would hopefully permit the development of more specific, effective and safer treatment modalities. Angiogenesis, pleural vascular increased permeability and inflammation are considered central to the pathogenesis of malignant pleural effusions. Vascular endothelial growth factor (VEGF) is a member of the VEGF/platelet-derived factor gene family and consists of at least six isoforms. Since it was shown that VEGF contributes to the formation of malignant pleural effusions, there have been some attempts to implicate, therapeutically, this finding using different molecules (ZD6474, PTK 787 and bevacizumab). However, the role of the biological axis of VEGF and angiopoietins needs further investigation in both the pathogenesis and the treatment of malignant pleural effusion. In both non-small-cell lung carcinoma and breast cancer, it has been shown that the ligand for CXCR4, CXCL12 or SDF-1α, exhibited peak levels of expression in organs that were the preferred destination for their respective metastases. Recent findings imply that new therapeutic strategies aimed at blocking the SDF-1-CXCR4 axis may have significant applications for patients by modulating the trafficking of hemato/lymphopoietic cells and inhibiting the metastatic behavior of tumor cells as well. The purpose of this report is to review novel pathogenetic and therapeutic implications regarding the angiogenetic pathways in malignant pleural effusions.

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Contents

1. Introduction

Malignant pleural effusion is a common condition, observed in patients with lung cancer and is associated with poor survival and quality of life (1-3). At least 25% of patients with lung cancer develop pleural effusion during the course of their disease.

Pleural effusion formation is multifunctional. At least, pleural effusion formation is associated with i) impaired drainage of the pleural space due to obstruction of vessels and lymphatics of the lung and pleura, ii) increased pleural formation and iii) inflammation and associated vascular increased permeability, resulting in plasma leakage; these are fundamental to the development of exudative, protein-rich pleural effusions (4-7). Increased permeability of the pleural microvasculature is generally attributed to factors that are released in inflammatory and malignant pleural diseases (7), although the exact pathogenetic mechanisms of malignant pleural effusion are unclear.

Current therapies used to prevent the re-acumulation of pleural fluid and relieve symptoms include pleurodesis (chemical-induced pleural fibrosis aiming at eliminating the pleura space), indwelling pleural catheters and chemotherapy. All of these are of variable efficacy and may cause serious adverse effects (1-3,8). Understanding the mechanisms of the disease would hopefully permit the development of more specific, effective and safer treatment modalities.

Angiogenesis, pleural vascular increased permeability and inflammation are considered central to the pathogenesis and possible therapeutic approaches in malignant pleural effusion (3,9-12). The purpose of this report is to review the novel pathogenetic mechanisms regarding the angiogenetic

pathway in malignant pleural effusions and the possible new therapeutic implications.

2. Main angiogenetic factors

Vascular endothelial growth factor. Vascular endothelial growth factor (VEGF) is a member of the VEGF/platelet-derived factor gene family and consists of at least six isoforms (VEGF-121, -145, -165, -183, -189 and -206) which are regulated by splicing at the mRNA level. VEGF can bind to two tyrosine kinase receptors, which are VEGFR-1 (flt-1) and VEGFR-2 (FLK-1/KDR) seated on the surface of endothelial cells. Once VEGF binds to these receptors, it induces dimerization, autophosphorylation and signal transduction. Notably, VEGF has potent angiogenic, mitogenic and vascular permeability-enhancing properties (13-18). Studies have shown that VEGF is overexpressed in human tumors (19) and plays an important role in the development of certain types of effusion (17,20-23). In agreement with previous studies (24-27), our group recently showed that VEGF levels are significantly higher in exudates than in transudates. Moreover, we found higher levels of VEGF in malignant than in parapneumonic effusions, and VEGF levels were correlated with markers of increased vascular permeability and pleural inflammation, such as LDH and protein ratios (28) in accord with recent studies (27,29).

Since it was shown that VEGF contributes to the formation of malignant pleural effusions, there have been some attempts to implicate, therapeutically, this finding. ZD6474 is an orally active inhibitor of VEGFR-2 with some additional activity against epidermal growth factor receptor tyrosine kinase (30-35). Matsumori *et al* investigated whether this drug controls experimental metastasis and pleural effusions produced by human non-small-cell lung carcinoma (NSCLC). They found that treatment with ZD6474 inhibits activation of VEGFR-2 and reduces tumor vascularization and tumor cell proliferation (36). Shibuya *et al* combined ZD6474 with radiation therapy using an orthotopic nude mouse model of NSCLC that closely mimics the patterns of tumor growth (37). This combination significantly enhanced the antiangiogenic, antivascular and anti-tumor effects of radiotherapy and was more effective than combined radiotherapy and chemotherapy (37).

PTK 787 is a VEGF receptor tyrosine kinase phosphorylation inhibitor. The efficiency of this agent has been examined in malignant pleural effusion developed in mice (38). It has been noted that oral administration of PTK 787 suppressed pleural effusion formation by inhibiting vascular permeability (38). It has been shown that, given orally, this agent exhibits excellent activity and tolerability, thus it can be used for long-term therapy of malignancies and for other diseases where VEGF mediates angiogenesis and plays an important role in their pathogenesis (39).

Bevacizumab is a humanized anti-VEGF monoclonal neutralizing antibody which blocks the binding of VEGF to its receptor and neutralizes all the isoforms of human VEGF. Li and co-workers used a patient-like severe combined immunodeficient mouse model by the orthotopic implantation of malignant pleural mesothelioma cells in order to examine the therapeutic efficiency of this agent (40). Administration of bevacizumab managed to suppress pleural effusion formation. Moreover, they showed that the combination of bevacizumab with pemetrexed, a new anticancer drug recently approved for the therapy of malignant pleural mesothelioma, augmented this effect (40).

Malignant mesothelioma cells express a number of receptor tyrosine kinase including VEGF receptor, epidermal growth factor receptor, platelet-derived growth factor receptor and Eph receptors (41-43). The Eph transmembrane tyrosine kinases constitute the largest family of receptor tyrosine kinases. EphA2 receptor is overexpressed during various processes such as tumor growth, angiogenesis and metastasis, and is also overexpressed in aggressive malignances (44-47). A recent *in vitro* study showed that silencing the EphA2 receptor using small interfering RNA inhibits the growth and migration of malignant mesothelioma cells. Silencing the EphA2 gene can induce apoptosis in malignant mesothelioma cells through caspase-9 activation (41-43). Conversely, when the receptor is overexpressed, this increases the proliferation and migration of malignant mesothelioma cells. Thus, knocking down the oncogenic protein EphA2 may have therapeutic implications in patients with malignant mesothelioma (48). It has also been noted that activation of the EphA2 receptor by its ligand ephrinA1 downregulates total EphA2 expression via phosphorylation and inhibits mesothelioma cell formation (49).

Endostatin. Endostatin, a 20-kDa C-terminal fragment of collagen XVIII, is released by normal cells and tissues. Endostatin specifically inhibits endothelial proliferation and potently inhibits angiogenesis and tumor growth (50,51). It has been recently observed that thoracoscopic talc insufflation induces pleural mesothelial cells to release endostatin (52). It seems that talc insufflation may alter the angiogenic balance in the pleural space upregulating angiostasis (52). However, further studies are needed to clarify these findings in humans.

Hyaluronan. Hyaluronan (HA) is a non-sulfated glycosaminoglycan that is secreted in significant quantities by pleural mesothelial cells and malignant mesothelioma cells (53). Its receptor, CD44, is expressed by malignant cells with a predilection for the pleura. High molecular weight HA is inactive, while low molecular weight HA produced by hydrolysis of the high molecular weight HA is chemotactic for malignant cells and also has angiogenic properties increasing permeability of the mesothelial monolayer. Low molecular weight HA induces malignant mesothelial cell proliferation and haptotaxis via interaction of the CD44 receptor (54).

Angiopoietin-1 and -2. Angiopoietin (Ang)-1 and -2 are important regulators of angiogenesis and exert their actions through binding a common tyrosine kinase receptor (Tie-2) that is mainly expressed on the surface of endothelial cells (55). Ang-1 and -2 act in conjunction with VEGF in promoting angiogenesis and occur under both normal and disease conditions (55).

In vivo (56,57) and *in vitro* studies (58-60) have demonstrated that Ang-1 has anti-inflammatory and antipermeability properties. It blocks the expression of adhesion molecules on the endothelial cell surface, leukocyte adherence on

endothelial cells and transmigration into tissues and IL-8 production by endothelial cells. In addition, Ang-1 inhibits vascular permeability induced by VEGF and other inflammatory agents. On the other hand, Ang-2 antagonizes the effect of Ang-1 in endothelial cells, suggesting that Ang-2 promotes vascular permeability (61). In agreement with this, Ang-2 destabilizes the endothelial cell monolayer integrity leading to the detachment of endothelial cells *in vitro* (62). More evidence supporting a hyperpermeability and pro-inflammatory function of Ang-2 comes from an *in vivo* study in which Ang-2 was found to induce edema formation and to exert a weak stimulatory effect on leukocyte migration when injected into mouse paw (63).

In malignant diseases, Ang-2 induces the instability of blood vessels and facilitates angiogenesis in the presence of VEGF, and it is one of the poor prognostic factors of NSCLC (64). Moreover, another study revealed that Ang-2 levels but not Ang-1 levels are elevated in exudative pleural effusions, suggesting that Ang-2 along with VEGF participate in pleural inflammation and in the pathogenesis of exudative pleural effusions (27). Tomimoto *et al* confirmed that a high level of VEGF and Ang-2 coexisted in exudative effusion, especially in bloody effusion, and there was a significant correlation between the levels of VEGF and Ang-2 (65). Our group recently demonstrated that, while VEGF is one of the main mediators in exudative malignant pleural effusions, this effect is not mediated through the angiogenetic pathway of angiopoietin receptor Tie-2 (28). In addition, it has been shown that Ang-2 along with VEGF, but not Ang-1, is increased in pleural inflammation (28). However, the role of the above biological axis of VEGF and angiopoietins needs further investigation.

3. CXCR4/stromal cell-derived factor-1 pathway

Recently, attention has been given to one particular member of the chemokine receptor family, CXCR4, because of its key role in HIV infection (66). Stromal cell-derived factor-1α (SDF-1α/CXCL12) is a member of the CXC chemokine family which has been found to recruit $CD34^+$ hematopoietic progenitor cell, megakaryocytes, B cells and T cells. SDF-1α/CXCL12 was expressed on target tissues (67). Although most chemokine receptors bind several chemokines, CXCR4 is a specific chemokine receptor since it only interacts with SDF-1α (68). The involvement of CXCR4 and SDF-1α in these processes makes this chemokine-receptor pair of particular interest for tumor metastasis. Many previous studies have demonstrated that the metastatic propensity of tumors from several different types of cancer including lung, breast, ovarian, renal and prostate is related to the expression of the chemokine receptor CXCR4 (67-70). Furthermore, in both NSCLC and breast cancer, it has been shown that the ligand for CXCR4/CXCL12 exhibited peak levels of expression in organs that were the most common sites of their metastases (69,70).

Our unpublished data revealed a significant increase in SDF-1α expression levels at both the mRNA and protein levels in malignant exudates in comparison to controls (71). We detected a coexpression between post-transcriptional expression protein levels of VEGF and SDF-1α in malignant pleural fluid. These data suggest that SDF-1α along with VEGF and Ang-2 expression may be involved in the dissemination of malignant cells into pleural space. Disseminated cancer cells can block the drainage of pleural space and this eventually leads to pleural effusion.

SDF-1 exerts pleiotropic effects which regulate processes essential to tumor metastasis, such as locomotion of malignant cells, their chemoattraction and adhesion. It also plays an important role in tumor vascularization (72). This implies that new therapeutic strategies aimed at blocking the SDF-1-CXCR4 axis may have important applications in the treatment of pleural effusions by modulating the trafficking of hemato/lymphopoietic cells and inhibiting the metastatic behavior of tumor cells as well (72). In our study, we contributed to the understanding of the pathophysiology of CXCL12/CXCR4 in association with the interaction with VEGF-angiopoietins (71).

The SDF-1α levels in malignant pleural effusions were significantly higher than those in transudate pleural effusions and showed a significant positive correlation with pleural effusion volumes (73). Furthermore, cancer cells in malignant pleural effusions expressed CXCR4, and mesothelial cells of the pleura stained positive for SDF-1α (73). However, there are no studies to support the aforementioned involvement of CXCL12/CXCR4 in malignant pleural effusions of SCLC. Our findings are in agreement with previous studies (69,73), while we found similar data with different-modern methodologies directly in pleural fluid cells of patients with malignancy. This hypothesis raises the possibility that blockade of CXCR4/SDF-1 interaction may lead to the discovery of novel therapeutic molecules.

4. Conclusion

Malignant pleural fluid accumulation is developed mostly as a result of increased production, secondary to mutually dependent biological phenomena including an ongoing angiogenic process and the associated enhanced vascular permeability and pleural inflammation. Many recent studies have demonstrated that angiopoietins/Tie-2 axis blockage impairs new vessel formation in the tumor, attenuates pleural vascular leakage and decreases recruitment of inflammatory cells. In this regard, angiopoietins, along with VEGF, are essential to the regulation of tumor angiogenesis. However, the exact role of individual angiopoietin family members is still unknown. SDF-1α/CXCL12 is expressed on target tissues, and many studies have demonstrated that the metastatic propensity of tumors of several different types of cancer is related to the expression of the chemokine receptor CXCR4. Understanding the mechanisms of fluid accumulation in malignant disease would hopefully lead to development of more specific, effective and safer treatment modalities to prevent the re-accumulation of pleural fluid and relieve symptoms without serious adverse effects.

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