Am J Cancer Res 2022;12(11):4879-4891
www.ajcr.us /ISSN:2156-6976/ajcr0146082

Review Article

The role of angiogenesis in malignant pleural effusion: from basic research to clinical application

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Received August 22, 2022; Accepted October 26, 2022; Epub November 15, 2022; Published November 30, 2022

Abstract: Malignant pleural effusion (MPE) is associated with advanced stages of various malignant diseases, especially lung cancer, and is a poor prognostic indicator in these patients. However, the management of MPE remains palliative. A better understanding of the pathogenesis of MPE may lead to the development of new and more effective therapeutic options. Here, we shed light on recent advances in the mechanisms of MPE formation and provide an overview of current targeted therapies for the vascular endothelial growth factor pathway. We also retrospectively enrolled 19 patients with lung adenocarcinoma from the West China Hospital to analyze the efficacy of bevacizumab for MPE using different routes of administration.

Keywords: Malignant pleural effusion (MPE), vascular endothelial growth factor, angiogenesis, treatment, cancer, non-small cell lung cancer

Introduction

Patients with advanced tumors frequently develop malignant pleural effusion (MPE). MPE is defined as the presence of tumor cells in the pleural effusion [1]. MPE, almost all of which are exudative, frequently occurs in patients with advanced tumors and is often predominantly infiltrated by lymphocytes, especially $CD4^+$ T cells [2-4]. Lung cancer is the most common malignancy associated with MPE; approximately one-third of all MPEs occur in patients with lung cancer [5]. Metastatic breast cancer and lymphoma are the second and third most commonly associated cancers, respectively. Malignant pleural mesothelioma is the most common primary pleural tumor associated with MPE, with approximately 90% of patients with malignant pleural mesothelioma also having MPE [6]. Approximately 15% of cancer patients die from MPE [7].

MPE treatment mainly aims to shrink tumors and absorb pleural fluid to relieve dyspnea, cough, and other symptoms. Dyspnea is the most common symptom in patients with MPE and requires palliative intervention [8]. Current options for treatment include repeated thoracentesis, pleurodesis, tube thoracostomy, pleurectomy, and internal or external drainage catheters [9-11]. However, these therapeutic options are not without limitations. Pleurodesis is accompanied by fever, chest pain, and coughing. Indwelling pleural catheters are costly and can only be used in patients who have failed pleurodesis or are unsuitable for pleurodesis [12]. Therefore, these palliative methods are unsatisfactory.

Pleural fluid accumulates when production is in excess of clearance, and drainage is impaired when tumors metastasize. Previous studies have identified that MPE occurs when tumor cells initially invade the visceral pleura mainly through the hyperpermeable pleural vasculature networks [7, 13, 14], after which inflammatory, mesothelial, and endothelial cells interact with invading tumor cells and promote MPE for-

mation. MPE is also associated with immune dysfunction; however, there is very little information about the underlying mechanism [15-18]. This review provides new insights into MPE pathophysiology and examines the current state of MPE treatment.

Pathophysiology of MPE

Fluid drainage obstruction: blockade of the drainage system

Pleural effusion accumulates when production outweighs removal. Necropsy studies have reported that pleural fluid clearance via the lymphatic system originates from the parietal pleura stomata and drains through the mediastinal nodes. Mediastinal lymph node invasion has been defined as a predictor of effusion. Therefore, impaired pleural fluid drainage caused by tumor invasion of the drainage system is believed to be one of the mechanisms of MPE formation [19]. However, patients without parietal pleural invasion have also been reported to develop MPE [20]. In addition, the protein level in MPE is higher than that in normal pleural fluid, indicating that there may be plasma leakage in MPE [21, 22].

Increased fluid filtration: abnormal permeability of vasculature networks

In the past few decades, related studies have discovered that redundant plasma leakage through hyperpermeable vasculature networks is involved in MPE formation [23]. Vasoactive mediators, such as vascular endothelial growth factor (VEGF), tumor necrosis factor-α (TNF-α), and angiopoietin (ANG)-1, are vital to this process [13, 14, 24]. VEGF is a family of proteins, including VEGF-A (hereafter referred to as VEGF), VEGF-B, VEGF-C, VEGF-D, VEGF-E, and placental growth factor, that are essential for angiogenesis during homeostasis and disease states by regulating vascular permeability and neoangiogenesis [25]. Clinical work has discovered that VEGF levels are much higher in MPE than in parapneumonic effusion, indicating that VEGF plays a central role in MPE formation and is a potential therapeutic target [13, 23, 26-30]. In addition, antiangiogenic treatment can temporarily “normalize” tumor vasculature [31]. Bevacizumab, a humanized anti-VEGF monoclonal neutralizing antibody that blocks VEGF from binding to its receptor, has been shown to suppress pleural effusion formation in preclinical and clinical studies [32, 33]. VEGF has two tyrosine kinase receptors, VEGFR-1 (FLT-1) and VEGFR-2 (FLK-1/KDR), which are predominantly expressed in endothelial cells [25]. Some studies have also examined the role of VEGFR-2 in MPE formation. ZD6474, a novel anti-VEGFR-2 inhibitor with additional activity against epidermal growth factor receptor (EGFR), can control established lung metastases and pleural effusions in a mouse model of lung cancer produced by human lung cancer cells. These results indicate that ZD6474 could be used to control MPE by inhibiting the activation of VEGFR-2 and reducing tumor vascularization and tumor cell proliferation [34]. Another VEGFR/platelet-derived growth factor receptor tyrosine kinase phosphorylation inhibitor, PTK787, inhibits MPE formation in a xenograft model using human lung adenocarcinoma (PC14PE6) cells [35]. Because the VEGF-VEGFR signaling pathway regulates vascular permeability, these results suggest that neoangiogenesis is the primary mechanism of MPE formation.

Factors indirectly involved in MPE formation by regulating VEGF accumulation in the pleural space have also been studied. The transforming growth factor-beta (TGF-β) signaling pathway is crucial during normal development and carcinogenesis [36]. Recently, TGF-β was found to participate in MPE formation by stimulating mesothelial cells to produce more VEGF both in vivo and in vitro [37]. Interleukin-6 (IL-6), a cytokine with pro- and anti-inflammatory properties, can be produced by almost all stromal and immune cells. It regulates both innate and adaptive immunities [38]. Yeh et al. found that IL-6-induced activation of STAT3 in lung cancer may be involved in MPE formation by upregulating VEGF [39]. Additionally, osteopontin (OPN), a multifunctional cytokine that participates in lung cancer development, metastasis, and angiogenesis, is involved in the formation of MPE by promoting VEGF secretion [40]. However, Psallidas et al. proposed that OPNs of different origins can promote MPE formation in different ways. Host-originated OPN recruits macrophages to cancer cells to participate in pleural fluid accumulation and promote tumor angiogenesis. By contrast, tumor-derived OPN induces MPE formation by blocking apoptosis in cancer cells. Additionally, OPN can directly

cause vascular hyperpermeability in a VEGF-independent manner [41]. TNF-α is also involved in MPE formation. TNF-α functions via nuclear factor-kappa B and neutral sphingomyelinase-dependent pathways to induce TNF-α and VEGF, respectively [42]. These factors may be effective target molecules for reducing MPE in patients with cancer.

Ang-1 and Ang-2 may also contribute to MPE formation. Ang-1 and Ang-2 are essential regulators of angiogenesis and bind to a tyrosine kinase receptor (Tie-2) that is mainly expressed on endothelial cells. Ang-2 acts as a natural antagonist of Ang1/Tie-2 signaling [43]. Kalomenidis et al. discovered that Ang-2 levels are elevated in exudative pleural fluid and correlate with VEGF levels in the fluid [44]. Furthermore, Economidou et al. found that VEGF regulates exudative pleural fluid formation in an Ang-1/Tie-2 pathway-independent manner [24]. These results indicate that, in addition to VEGF, the Ang-2/Tie-2 pathway might participate in MPE formation; however, this needs to be investigated further.

VEGF-independent mediators of vascular hyperpermeability have also recently been identified. Myo9b is a multidomain motor protein expressed in immune tissues, such as the spleen, and various immune cells, such as macrophages and dendritic cells [45, 46]. Myo9b expression is positively correlated with MPE development, and Myo9b deficiency inhibits its MPE formation in a mouse model, in addition to prolonging survival by decreasing vascular permeability and inhibiting tumor angiogenesis and tumor cell proliferation [47]. Furthermore, high levels of monocyte chemoattractant protein (MCP-1), also known as chemokine ligand 2, have been detected in mouse and human MPE samples [48, 49]. MCP-1 regulates MPE formation by affecting vascular permeability and recruiting macrophages into the pleural fluid. Marazioti et al. determined that C-C motif chemokine ligand 2 (CCL2) promotes MPE formation: in a mouse model, CCL2 blockade reduced MPE induced by murine and human adenocarcinoma cells [50]. In summary, during MPE production, tumor cells promote vascular permeability by directly affecting the VEGF/VEGFR pathway or increasing VEGF production. Immune cells and tumor cells can also directly increase vascular permeability by producing Ang-1/2, Myo9b, and MCP-1 (**Figure 1**).

The MPE microenvironment as a contributor to tumorigenesis

Studies on the MPE microenvironment have shown that a variety of immune and non-immune cells accumulate in the pleural fluid. The MPE microenvironment contains cancer cells and various immune cells (>10^8/L), such as diverse lymphoid cells and myeloid subsets [51, 52].

Lymphocytes, especially $CD4^+$ helper T cells, are frequently present in MPE. $CD4^+$ T cells can be divided into Th1 and Th2 subsets according to the type of cytokine production, and these subsets have distinct functions. Th1 cells predominantly produce IL-2, TNF-α, and IFN-γ, which stimulate the development of $CD8^+$ effector T cells. Th2 helper cells primarily produce IL-4, IL-5, IL-10, and IL-13. The balance of Th1 and Th2 cells in pleural effusion is controversial; some believe that Th2 cells are dominant in MPE and secrete soluble ST2 protein [4, 53], whereas others have demonstrated that T cells in MPE are mainly naïve or not definitely polarized to Th1 cells [54]. However, the Th1/Th2 cell balance in MPE inevitably influences the pathophysiological processes of pleural diseases.

Th17 cells are also involved in MPE formation. Th17 cells differentiate from naïve $CD4^+$ T cells with the addition of IL-1β, IL-6, IL-23, and TGF-β and are characterized by the production of IL-17 [55, 56]. Patients with lung cancer have more Th17 cells in the pleural fluid than in the blood, and Th17 cells in the pleural fluid predict better survival. Th17 cells in MPE can be chemoattracted from the blood by the CCR4-CCL22 and CCR6-CCL20 pathways and can differentiate from naïve $CD4^+$ T cells [56]. However, the role of IL-17 in MPE remains controversial. Lin et al. demonstrated that IL-17 inhibits MPE formation and improves survival xenograft mouse models of Lewis lung cancer and colon adenocarcinoma [16]. However, Nieto et al. suggested that the concentrations of IL-17 in MPE in lung cancer patients are higher than those in patients with heart failure-related effusion, and IL-17 levels are negatively

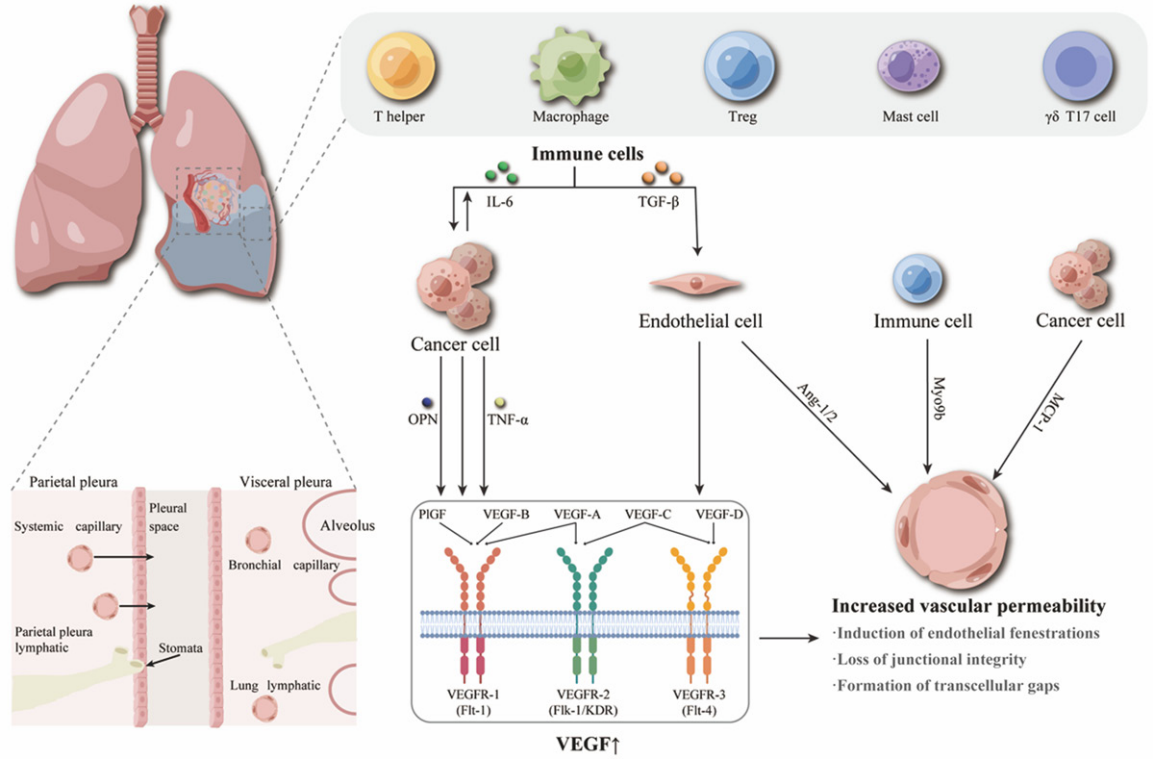

Figure 1. Mechanisms of MPE pathogenesis. Fluid drainage obstruction and increased filtration are the main causes of pleural effusion. During MPE production, tumor cells promote vascular permeability by directly affecting the VEGF/VEGFR pathway or increasing VEGF production. Immune cells and tumor cells can also directly increase vascular permeability by producing Ang-1/2, Myo9b, and MCP-1. The immune component of malignant pleural effusion mainly includes T helper cells, macrophages, Tregs, mast cells, and γδT17 cells. Abbreviations: MPE, malignant pleural effusion; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor; IL-6, interleukin 6; TGF-β, transforming growth factor-β; OPN, osteopontin; TNF-α, tumor necrosis factor α; PlGF, placental growth factor; Ang, Angiopoietin; Myo9b, myosin IXB; MCP-1, monocyte chemoattractant protein-1; Treg, regulatory T cell.

correlated with survival [57]. Heart failure-related effusion is generally transudative pleural effusion with low cell and protein content [58]. Other immune cells, such as NK cells, γδT cells, and myeloid cells, also secrete IL-17 [59]. Therefore, further efforts toward understanding the function of IL-17 and Th17 cells in MPE formation are needed.

In addition to Th17 cells, γδT17 cells also suppress MPE development. Increased γδT17 cells in the pleura predict improved survival in murine and human MPE. However, Wei et al. found that IL-10 suppresses secretion of IL-17A from γδT cells in MPE via RORγt [60]. Moreover, IL-10 was also reported to participate in MPE formation by suppressing the differentiation of Th1 cells from T cells and inhibiting the CXCR3-CXCL10 signaling pathway, which recruits Th1 and Th17 cells into MPE [61]. Considering the critical roles of γδT17 cells and IL-10 in MPE, they may be promising targets for controlling MPE.

Regulatory T cells (Tregs) have attracted much attention for their role in MPE formation. MPE has more Tregs than blood and benign pleural effusions. Tregs are considered immunosuppressive cells [62, 63] and can not only differentiate from naïve $CD4^+$ T cells in the presence of TGF-β but also chemoattract into the MPE via the CXCL1-CXCR2 pathway, CCL17, and CCL22 [18, 63, 64]. In addition, natural $CD4^+CD25^-$ T cells in MPE can transform into $CD4^+CD25^+FOXP3^+$ T cells, which have suppressive functions [65]. Tregs suppress immunity in MPE in several ways. They upregulate immune checkpoints, such as CTLA-4 and PD-L1. They also highly express TNFR2, which binds to TNF-α to promote its immunosuppres-

sive function; blockade of TNFR2 increases IFN-γ-expressing $CD8^+$ T cells in MPE [66]. A higher Treg/Th17 cell ratio is partially related to poor survival in lung cancer patients with MPE [17, 63]. Ye et al. demonstrated that $CD39^+$ Tregs in MPE inhibit the differentiation of Th17 cells in a latency-associated peptide-dependent manner [56]. These findings lay the foundation for developing novel immunotherapy strategies based on Treg clearance in patients with MPE.

Macrophages and mast cells have significant effects on MPE formation. Macrophages are significantly increased in MPE, and $CD206^+CD14^+$ macrophages can be used as biomarkers of MPE [67]. Further, stimulated macrophages in the pleura can chemoattract lymphocytes by producing IL-8 [68]. They also impair T cell cytotoxicity by inducing TGF-β [69]. Tumor-associated macrophage (TAM)-derived TGF-β upregulates the expression of CCL22 in TAMs via c-Fos. Subsequently, CCL22 chemoattracts Tregs to the pleural space to further stimulate TGF-β production by TAMs in an IL-8-dependent manner [70]. These findings indicate that macrophages participate in building an immunosuppressive tumor microenvironment in MPE. Anastasios et al. found that CCL2 and OPN in the pleural space attract mast cells, which induce pleural vasculature leakiness and trigger NF-κB activation by producing tryptase AB1 and IL-1β [71]. These data suggest that immune treatments based on TAMs or mast cells may be effective strategies for controlling MPE.

With the development of molecular techniques, activating mutations in EGFR and KRAS have also been found to affect MPE pathogenesis [72, 73]. The EGFR L858R mutant promotes cancer cell invasion and MPE formation by activating the CXCL12-CXCR4 pathway. Mutant KRAS upregulates CCL2 in the blood to mobilize myeloid cells from the bone marrow to the pleural space [73]. These studies indicate that patients with MPE may benefit from targeted therapies based on EGFR or KRAS mutations.

Novel therapies for MPE based on the VEGF/VEGFR pathway

Currently, the management of MPE is palliative, including repeated thoracentesis, pleurodesis, tube thoracostomy, pleurectomy, and internal or external drainage catheters. The advantages and disadvantages of these treatments have been thoroughly reviewed by Neragi-Miandoab [8]. Several preclinical studies have assessed novel therapeutic interventions against MPE, including monoclonal neutralizing antibodies, soluble receptors, and small-molecule inhibitors. Blockade of VEGF, VEGFR, TNFR2, IL-5, IL-10, CCL2, and Ang signaling improves MPE in preclinical models [34, 35, 50, 61, 66, 74]. Among these, the VEGF/VEGFR pathway is the most well-studied in MPE formation because of its prominent role in blood vessel formation. In this section, we shed new light on the strategies of targeting the VEGF/VEGFR pathway in MPE.

Many studies have explored the efficacy of VEGF/VEGFR blockade in patients with MPE and mouse models of MPE. In an early study, treatment of mice implanted with human lung adenocarcinoma with PTK 787, a VEGF/VPE receptor tyrosine kinase phosphorylation inhibitor, significantly reduced MPE formation (Table S1) [35]. ZD6474, a novel orally active inhibitor of VEGFR-2, can also control MPE in a mouse model of non-small cell lung cancer (NSCLC). ZD6474 inhibits the production of pleural fluid by inhibiting the activation of VEGFR-2 and reducing tumor vascularization (Table S1) [34]. Related clinical trials and retrospective studies have also been recently conducted (**Tables 1** and S2). The earliest clinical analysis was performed by Kitamura et al. [75]. They treated 13 patients with MPE secondary to NSCLC with conventional chemotherapy plus bevacizumab (15 mg/kg) and examined the pleural effusion control rate (PECR), defined as the proportion of patients without reaccumulation of MPE for 8 weeks from the initiation of treatment. Twelve patients (92.3%) achieved pleural effusion control. In addition, prospective studies have examined the efficacy of bevacizumab in controlling MPE. Tamiya et al. enrolled 23 patients with lung adenocarcinoma accompanied by MPE. The patients were treated with platinum-paclitaxel plus bevacizumab, and PECR was defined as the percentage of patients without re-accumulation of MPE on chest radiography or computed tomography (CT) during treatment. In that study, the PECR was 91.3% (21/23) [76]. Usui et al. also performed a single-arm, open-label phase II trial of bevacizumab plus chemotherapy [77], in

Table 1. Clinical Studies of VEGF/VEGFR blockade in MPE

Type of clinical research	Primary disease	Drug	Intervention	Patients number	*Control rate of MPE (PECR) or OR	Ref.
Retrospective study	NSCLC	Conventional chemotherapy plus bevacizumab (15 mg/kg)	Intravenous	13	PECR: 92.3%	[75]
Prospective study	Lung adenocarcinoma	Platinum-paclitaxel plus bevacizumab (15 mg/kg)	Intravenous	23	PECR: 91.3%	[76]
Prospective study	NSCLC	Carboplatin-pemetrexed and bevacizumab (15 mg/kg)	Intravenous	28	PECR: 92.9%	[77]
Prospective study	NSCLC	Chemotherapy plus bevacizumab (15 mg/kg)	Intravenous	20	PECR: 80%	[78]
Prospective study	NSCLC	Cisplatin (30 mg) plus bevacizumab (300 mg)	Intrapleural injection	72	OR: 83.33%	[80]
Retrospective study	NSCLC	Pemetrexed (100-600 mg) plus bevacizumab (200 mg)	Intrapleural injection	45	OR: 86.36%	[81]

*The pleural effusion control rate (PECR) was described as the proportion of patients without reaccumulation of MPE for eight weeks from the initiation of treatment. *Complete remission (CR) was defined as when the pleural fluid had disappeared and lasted for at least four weeks; partial remission (PR) was defined as when >50% of the pleural fluid had disappeared, symptoms had improved, and the fluid did not increase for at least four weeks; OR, overall response was the sum of CR and PR.

which PECR was defined as the proportion of patients without pleurodesis at 9 weeks. They enrolled 28 patients with NSCLC, and the PECR was 92.9% (26/28). A more recent study performed by Rintaro et al. also demonstrated that bevacizumab (15 mg/kg) was effective in controlling MPE, with a PECR of 80% [78].

Intrapleural therapies have been adopted to treat a vast array of pleural diseases, and intrapleural injection has become an effective route for the administration of traditional chemotherapeutics and targeted agents [79]. Intracavitary injection of bevacizumab has been shown to control MPE. In 2013, Du et al. prospectively enrolled 72 patients with NSCLC accompanied by MPE to receive thoracentesis followed by either intrapleural cisplatin (30 mg) plus bevacizumab (300 mg) or cisplatin (30 mg) alone [80]. The curative efficacy of the combination therapy was significantly superior to that of cisplatin alone (overall response 83.33% vs. 50.00%). Additionally, Song et al. found that intrapleural injection of bevacizumab (200 mg) plus pemetrexed could effectively control MPE in patients with NSCLC [81]. These studies indicate that intrapleural injection of bevacizumab can effectively control MPE caused by NSCLC. However, the optimal dose of bevacizumab has not yet been established. Chen et al. recently attempted to optimize intrapleural bevacizumab dosing in MPE secondary to NSCLC [82]. They retrospectively enrolled 71 patients with MPE secondary to NSCLC who received a low dose of bevacizumab (100 mg/week, 200 mg/2 weeks, or 200 mg/3 weeks) or a high dose of bevacizumab (200 mg/week, 400 mg/2 weeks, or 400 mg/3 weeks). Complete response was defined as complete disappearance of pleural effusion within 4 weeks. In that study, patients who received a low dose of bevacizumab had better overall survival (OS) and less toxicity than those who received a high dose, whereas those who received a high dose of bevacizumab had better progression-free survival (PFS).

We also retrospectively enrolled 19 patients with lung adenocarcinoma accompanied by MPE from the West China Hospital, Sichuan University, between October 2017 and May 2018 (the study was approved by the Medical Ethics Committee of West China Hospital, Sichuan University). After draining the pleural fluid by thoracentesis, patients were administered either a combination of 100 mg bevacizumab plus 30-50 mg cisplatin, 30 mg cisplatin alone, or 100 mg bevacizumab alone by intrapleural injection every 2 weeks. One patient was administered intravenous bevacizumab at 7.5 mg/kg. CT was performed at the end of the first treatment cycle. Complete remission (CR) was defined as disappearance of the pleural fluid for at least 4 weeks; partial remission (PR) was defined as disappearance of >50% of the pleural fluid with improved symptoms and no increase in the remaining fluid for at least 4 weeks; remission not obvious was defined as disappearance of <50% of the accumulated fluid; and progressive disease was defined as an increase in fluid accumulation. The overall response rate (ORR) was calculated as the proportion of patients achieving CR and PR. The median PFS and OS were assessed. Adverse reactions were evaluated using the Common Toxicity Evaluation

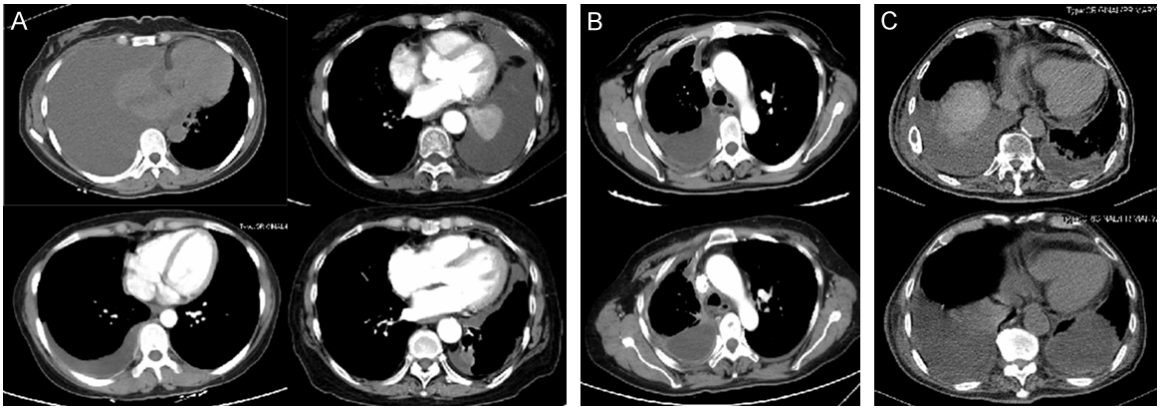

Figure 2. Chest computed tomography scans showed the outcomes of four weeks of treatment. A. Two patients with MPE achieved partial remission (PR) of pleural effusion. B. One MPE patient with stable disease (SD). C. One MPE patient obtained progressive disease (PD) of pleural effusion.

Table 2. Short-term efficacy of bevacizumab-containing treatment on MPE

Clinical outcomes	Bev + Cis via IP n = 11	Bev via IP n = 4	Cis via IP n = 3	Bev via I.V n = 1
CR	0	0	0	0
PR	3	2	2	1
SD	6	0	0	0
PD	2	2	1	0
ORR (%)	81.82	50.00	66.67	100.00

MPE, malignant pleural effusion; Bev, bevacizumab; Cis, cisplatin; IP, intrapleural perfusion; I.V, intravenous injection; complete remission (CR) was considered when the pleural fluid had disappeared and was stable for at least four weeks; partial remission (PR) was considered when >50% of the pleural fluid had disappeared, symptoms had improved, and the remaining fluid had failed to increase for at least four weeks; remission not obvious (NC) was considered when <50% of the accumulated fluid had disappeared; progression disease (PD) was considered when the accumulated fluid had increased. The overall response rate (ORR) was calculated by taking the sum of CR and PR.

Criteria according to the National Cancer Institute. Quality of life was assessed using the Karnofsky performance score (KPS). Data analysis was performed using SPSS version 19.0 (SPSS Inc., Chicago, IL, USA). Kaplan-Meier plots were used to evaluate PPFS, PFS, and OS. Median PPFS and PFS were compared by log-rank test. The median values and 95% confidence intervals (CIs) are reported. Differences with a two-sided *P* value of <0.05 were considered statistically significant.

The patient characteristics are summarized in Table S3. In combination therapy with bevacizumab, cisplatin chemotherapy was implemented in 11 patients (57.89%). In monotherapy, 4 patients (21.05%) received intrapleural bevacizumab therapy, 3 patients (15.79%) received intrapleural cisplatin therapy, and 1 patient (5.26%) received intravenous bevacizumab therapy. Pleural effusion was discovered in fifteen patients before treatment commenced, while 4 patients developed new fluid effusion during the treatment process. After 4 weeks of treatment, 42.10% (8/19) of patients exhibited a noticeable effusion decrease, 26.32% (5/19) of patients experienced an effusion increase, and 31.58% of patients (6/19) experienced no apparent changes in the pleural effusion volume. Radiological changes after 4 weeks of combination therapy are shown in **Figure 2**. The MPE responses are listed in **Table 2**. The ORRs of MPE treated with intrapleural perfusion of bevacizumab plus cisplatin, bevacizumab monotherapy, and cisplatin monotherapy were 81.82%, 50.00%, and 66.67%, respectively. The ORR of MPE treated with intravenous bevacizumab injection was 100%. No significant differences (P>0.05) were observed between the groups.

The median OS were 22 months among patients who received intrapleural perfusion of bevacizumab plus cisplatin (n = 9), 15 months for those who received intrapleural perfusion of bevacizumab alone (n = 3), and 22 months for those who received intrapleural perfusion of cisplatin alone (n = 2) (**Figure 3A**). However, there was no significant difference in the medi-

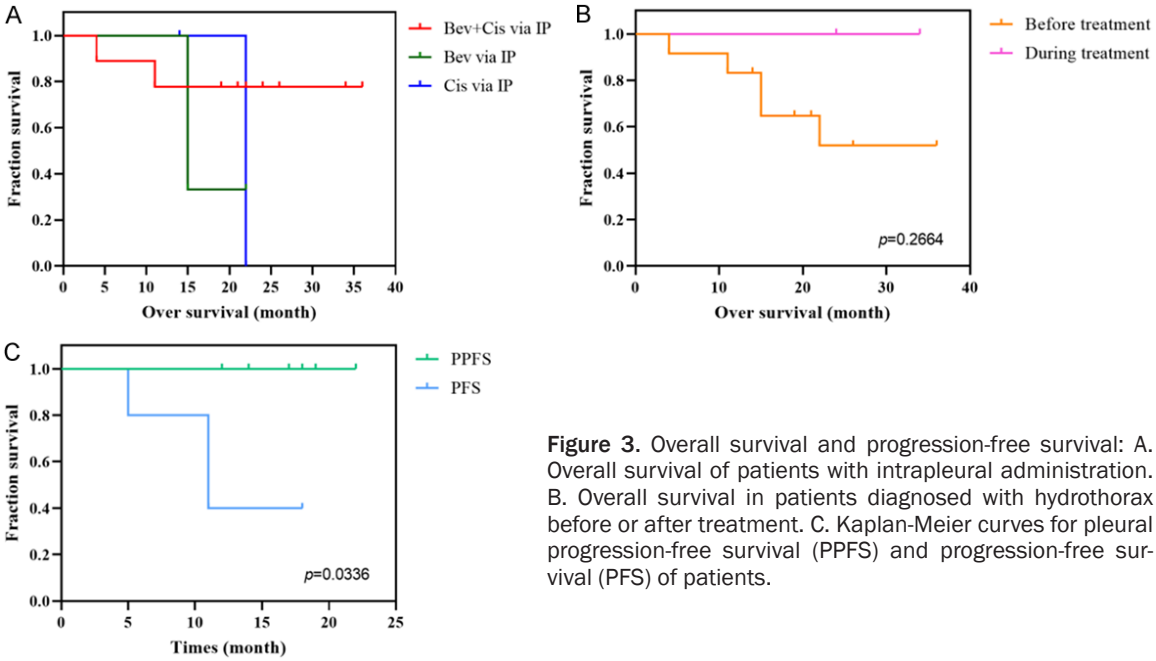

Figure 3. Overall survival and progression-free survival: A. Overall survival of patients with intrapleural administration. B. Overall survival in patients diagnosed with hydrothorax before or after treatment. C. Kaplan-Meier curves for pleural progression-free survival (PPFS) and progression-free survival (PFS) of patients.

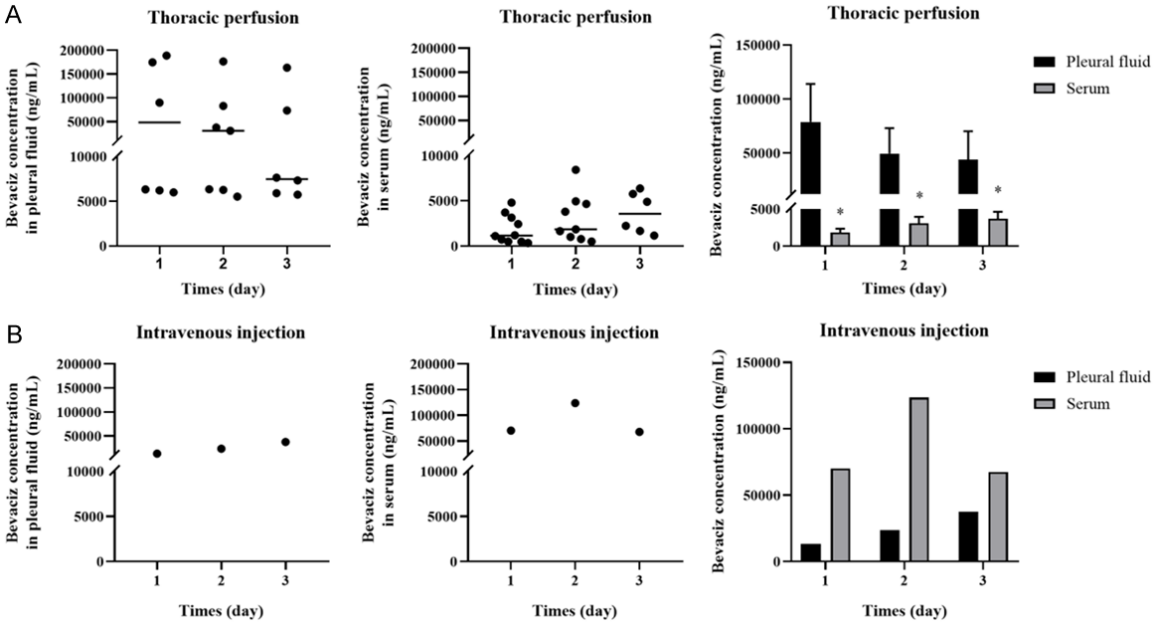

Figure 4. Bevacizumab concentrations in pleural fluid and serum of patients with intrapleural administration (A) and one patient with intravenous administration (B). The serum bevacizumab level was significantly lower than that in the pleural fluid of patients with intrapleural administration (P<0.05).

an OS among the three groups (P>0.05). The median OS of patients diagnosed with hydrothorax before or after treatment were also not significantly different (P = 0.2664) (**Figure 3B**) in all patients who received treatment. However, Kaplan-Meier curves showed a significant difference in PPFS and PFS (17.5 months vs. 11 months; P = 0.0336) (**Figure 3C**).

To test the levels of bevacizumab and VEGF in the pleural fluid and serum, the pleural fluid and serum were centrifuged at 4,000 rpm for 10 min at 4°C, after which the supernatant was collected and assessed by ELISA using the BEVACIZUMAB ELISA kit and VEGF-A ELISA kit (USCN, Wuhan, China) according to the manufacturer's instructions. The assay plates were

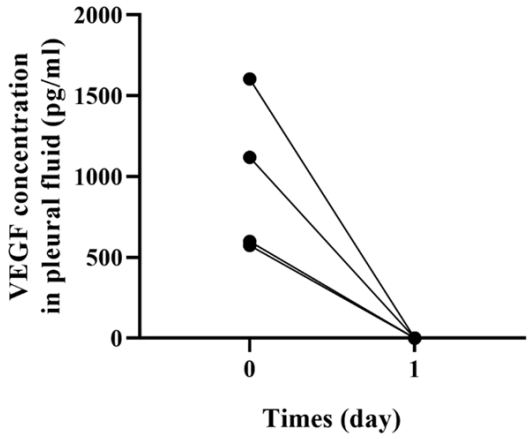

Figure 5. VEGF concentrations in the pleural fluid of patients with intrapleural administration.

read on a microplate reader (Bio-Rad, model 550, USA). Bevacizumab in the pleural fluid and serum were measured on days 1, 2, and 3 after intrathoracic perfusion (**Figure 4A**) and intravenous injection (**Figure 4B**). In patients with intrathoracic perfusion of bevacizumab, the mean ± standard error levels of bevacizumab in the pleural fluid on days 1, 2, and 3 were 78,697.83 ± 35,248.67 ng/mL, 49,388.34 ± 23,610.83 ng/mL, and 43,859.71 ± 26,241.54 ng/mL, respectively. The mean ± standard error levels of bevacizumab in the serum on days 1, 2, and 3 were 1844.84 ± 500.11 ng/mL, 3070.95 ± 872.65 ng/mL, and 3688.27 ± 924.71 ng/mL, respectively. The concentration of bevacizumab in the pleural effusion decreased in a time-dependent manner, whereas that in the serum increased in a time-dependent manner. In patients receiving bevacizumab intravenously, the bevacizumab levels in the pleural fluid on days 1, 2, and 3 were 13,171.87 ng/mL, 23,623.04 ng/mL, and 37,662.23 ng/mL, respectively. The serum levels on days 1, 2, and 3 were 70,138.23 ng/mL, 123,681.70 ng/mL, and 67,565.68 ng/mL, respectively. At the individual level, the concentration of bevacizumab in the serum among patients who received intravenous bevacizumab reached a maximum on day 2. Nevertheless, the concentration of bevacizumab in the pleural effusion was lower than that in serum. However, intrathoracic perfusion administration significantly improved the overall level of bevacizumab in pleural effusion. Furthermore, the VEGF concentration in the pleural fluid quickly reduced 1 day after intrapleural bevacizumab administration (**Figure 5**).

However, these studies were small-sample studies, and more effort is needed to explore optimized bevacizumab dosing in controlling MPE.

Discussion and future perspectives

The pathogenesis of MPE is complex. In addition to tumor cells, various somatic and immune cells and related signaling pathways, such as mast cells, T cells, myeloid cells, and the NF-κB pathway, are involved in MPE formation. A detailed understanding of the pathogenesis of MPE will allow us to develop more effective prevention and treatment strategies and prolong the survival of cancer patients. The VEGF/VEGFR signaling pathway plays a vital role in the development of MPE, and therapeutic strategies based on the VEGF pathway provide hope for the treatment of MPE. MPE secondary to NSCLC or lung adenocarcinoma can be effectively controlled by either intravenous chemotherapy plus bevacizumab or intrapleural chemotherapy plus bevacizumab. However, the studies investigating the efficacy of bevacizumab in controlling MPE are all small-sample studies, and there is a lack of uniform standards for intrapleural doses of bevacizumab. High-quality randomized controlled trials are necessary to compare the efficacy and safety of intravenous and intrapleural administration of bevacizumab in MPE.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (Grant No. 81872489, 82073369).

Disclosure of conflict of interest

None.

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Table S1. Preclinical studies of VEGF/VEGFR blockade in MPE

Model	Treatment	Outcome	Ref.
Mice bearing lung adenocarcinoma	VEGF/VPF receptor tyrosine kinase phosphorylation inhibitor, PTK 787	remarkably inhibited MPE formation	[35]
Mice bearing human NSCLC	Anti-VEGFR2 (ZD6474)	Significantly inhibited the MPE formation	[34]

Table S2. Summary of clinical trials targeting VEGF/VEGFR in MPE

NCT number	Drug	Intervention	Primary disease	Phase	Results
NCT00402896	ZD6474	Oral 300 mg/day	Lung cancer	II	The median time to pleurodesis was 35 days
NCT02054052	Bevacizumab	Intrapleural injection 100 mg	Non-small-Cell Lung Cancer (NSCLC)	II	No Results Posted
NCT02250118	Bevacizumab	Intrapleural injection range 0.5-5 mg/kg	Breast Cancer	I	No Results Posted
NCT02005120	Bevacizumab vurses recombinant human endostatin	Intrapleural injection Bevacizumab or recombinant human endostatin	NSCLC	II	No Results Posted
NCT00533585	BAY 43-9006 (Sorafenib) + Bevacizumab + Paclitaxel + Carboplatin	Intravenous	Lung Cancer	I	No Results Posted
NCT01661790	Bevacizumab and Cisplatin	Bevacizumab 300 mg & Cispltin 30 mg by intrapleural administration of each 2 weeks	NSCLC	III	*CR: 47% (17/36)

*Complete remission (CR) was defined as when the accumulated fluid had disappeared and was stable for at least four weeks.

Table S3. Patient characteristics

Characteristic	N = 19	
Age (years)	Median (Range)	63 (42-84)
Gender	Male	11 (57.89%)
	Female	8 (42.10%)
Smoking history	Yes	12 (63.16%)
	No	7 (36.84%)
EGFR mutation	Wild type	13 (68.42%)
	Mutated	1 (5.26%)
	Others	2 (10.53%)
	Unknown	3 (15.79%)
Histology	Adenocarcinoma	19 (100%)
diagnosis times of hydrothorax	First visit	15 (78.95%)
	Course of disease	4 (21.05%)
Systematic treatment history for pleural effusion	Yes	2 (10.53%)
	No	17 (89.47%)
Chemotherapy regimens	Intrapleural perfusion with Bevacizumab + Cisplatin	11 (57.89%)
	Intrapleural perfusion with Bevacizumab	4 (21.05%)
	Intrapleural perfusion with Cisplatin	3 (15.79%)
	Intravenous injection with Bevacizumab	1 (5.26%)