Pulmonary Vascular Destabilization in the Premetastatic Phase Facilitates Lung Metastasis

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Abstract

Before metastasis, certain organs have already been influenced by primary tumors. However, the exact alterations and regulatory mechanisms of the premetastatic organs remain poorly understood. Here, we report that, in the premetastatic stage, angiopoietin 2 (Angpt2), matrix metalloproteinase (MMP) 3, and MMP10 are up-regulated in the lung by primary B16/F10 tumor, which leads to the increased permeability of pulmonary vasculatures and extravasation of circulating tumor cells. Subsequent studies show that Angpt2, MMP3, and MMP10 have a synergistic effect on disrupting vascular integrity in both in vitro and in vivo models. Lentivirus-based in vivo RNA interference of Angpt2, MMP3, and MMP10 attenuates the pulmonary vascular permeability and suppresses the infiltration of myeloid cells in the premetastatic lung. Moreover, knocking down these factors significantly inhibits the spontaneous lung metastasis in the model by orthotopic implantation of MDA-MB-231-Luc-D3H1 cells in nude mice. Further investigations reveal that the malignancy of tumor cells is positively correlated with their capabilities to induce the expression of Angpt2, MMP3, and MMP10. Luciferase reporter assay and chromatin immunoprecipitation assay also suggest that transforming growth factor-β1 and tumor necrosis factor-α signaling are involved in the regulation of these premetastatic factors. Our study shows that pulmonary vascular destabilization in the premetastatic phase promotes the extravasation of tumor cells and facilitates lung metastasis, which may provide potential targets for clinical prevention of metastasis. [Cancer Res 2009;69(19):7529–37]

Introduction

Metastasis, a fatal step in the progression of solid malignancies, consists of a series of sequential procedures, including tumor cell extravasation, survival in the circulation, arrest in a new organ, extravasation into the surrounding tissue, initiation and maintenance of growth, and vascularization of the metastatic tumor (1–3). Clinical observations on cancer patients and studies in rodent models have revealed that various types of cancer can form metastases in specific organs. For example, breast cancer frequently metastasizes to lung and bone; in murine models, both orthotopically and i.v. implanted cancer cells preferentially colonize in the lung and lymph node (1, 4). The lung, as a preferential organ for metastasis, has the highest efficiency at arresting the circulating cancer cells by the capillary size restriction (1). However, recent studies showed that besides such physical factors, genetic regulation, and activation of specific chemokines, cytokines and proteases in cancer cells or premetastatic niche also play an important role in directing metastasis to a designated organ (3, 5).

Although certain molecular alterations in cancer cells lead to the acquisition of metastatic property, the sites of metastasis are determined not only by the characteristics of these cancer cells but also by the local microenvironment of the host tissues (5, 6). Emerging lines of evidence revealed that the hematopoietic progenitor cells and myeloid cells can be recruited to the lung by primary tumors and participate in the formation of premetastatic niche (4, 7). Passing through the vascular barrier is critical and essential for extravasation of tumor cells and subsequent metastasis. Several studies showed that certain factors, such as angiopoietin (Angpt) 4 and matrix metalloproteinase (MMP) 1, secreted by metastatic tumor cells, can disrupt vascular endothelial tight junctions and perturb the integrity of capillary walls to facilitate metastasis (8, 9). However, the mechanisms underlying vascular remodeling and metastatic tumor cell survival in the premetastatic phase remain largely unknown.

In the current study, we found that in the premetastatic phase, primary tumors influenced the local microenvironment and altered the gene expression in the lung. Angpt2, MMP3, and MMP10 were induced to disrupt the vascular integrity. Knocking down these factors significantly attenuated tumor cell extravasation and myeloid cell infiltration in the premetastatic phase and then inhibited subsequent lung metastasis.

Materials and Methods

Cells and reagents. MRC-5, B16/F10, B16/F0, B16/F1, and MDA-MB-231 were obtained from the American Type Culture Collection. Human primary mammary epithelial cells (HMEpiC) and human hepatocytes are from ScienCell. Human dermal microvascular endothelial cells (HMEC-1) are from our lab stock. MDA-MB-231-Luc-D3H1 is obtained from Xenogen Corp. FITC-lectin is from Sigma. GFR-Matrigel and antibodies of CD31, CD11b, and CD14 are from BD Pharmingen. The proteins of Angpt2, MMP3, and MMP10; Angpt2 antibody; and MMP3 antibody are from R&D Systems. MMP10 antibody is from Santa Cruz Biotechnology. Rhodamine-conjugated lectin and dextran (70 kDa) are from Molecular Probes.

Primary tumor inoculation. All animal studies were approved by the Institutional Animal Care and Use Committee of Tsinghua University. For each animal experiment in this study, the results were further verified by at least one more reproduction. B16/F10 cells (5 × 10⁶ per mouse) suspended in GFR-Matrigel were i.d. injected into 6- to 8-wk-old female C57/BL6 mice (for pulmonary vascular analysis, gene screening assays) or nude mice (for cell invasion assay also suggest that transforming growth factor-β1 and tumor necrosis factor-α signaling are involved in the regulation of these premetastatic factors. Our study shows that pulmonary vascular destabilization in the premetastatic phase promotes the extravasation of tumor cells and facilitates lung metastasis, which may provide potential targets for clinical prevention of metastasis. [Cancer Res 2009;69(19):7529–37]

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extravasation, survival, metastasis assays). Mice were anesthetized and an injection site on the back of the mice was shaved to remove the hair (for C57/BL6 only). The site was swabbed with 70% ethanol. The needle, which was held nearly parallel to the plane of the skin, was inserted into the skin and beveled up. Tumor cells were injected, and the injection volume was limited to 60 μL per site to avoid tissue trauma. The mice inoculated with B16/F10 cells for the indicated days were used for further experiments.

**Extravasation of circulating tumor cells.** The extravasation assay was performed as previously described (8) with slight modifications. Enhanced green fluorescent protein (eGFP)-labeled B16/F10 cells (1 × 10⁷ per mouse) were i.v. injected to normal (control) mice or tumor-bearing (B16/F10-D12) mice (6- to 8-wk-old female nude mice, five mice for each group). Then, the mice were i.v. injected with rhodamine-lectin (100 mg/kg) 2 h later. After 10 min, lung tissues of mice were cryosectioned (30 μm) for fluorescence imaging to visualize the vasculature (red) and circulating tumor cells (green). The tumor cells overlapping with vasculature were considered as circulating cells. Otherwise, the cells without overlap were considered as extravasated ones. Five fields from each mouse were randomly chosen for fluorescence imaging, and three mice for each group. The extravasation index was the ratio of extravasated tumor cells to total tumor cells, and the extravasation that occurred in normal mice was considered as basal level.

**In vitro vascular mimic model.** Transwell chambers were coated with a thin layer of matrix predominantly composed of GFR-Matrigel, with lesser amount of fibronectin, collagen I, collagen IV, and elastin, and HMECs were seeded on top. When endothelial cells grew to confluence in the B16/F10 conditioned medium, 200 ng/mL Angpt2, 50 ng/mL MMP3, and 50 ng/mL MMP10 were supplied alone or in combination for 24 h, and then 100 ng/well luciferase was added into the chamber. One hour later, the media out of chambers were subjected to luciferase activity measurement to represent permeability of the vascular mimic system. Six wells were applied in each group, and the experiments were independently performed thrice.

**In vivo lectin/dextran staining/dermal vascular permeability assay.** Angpt2 (1.5 μg), MMP3 (1.2 μg), and MMP10 (1.2 μg) were i.d. injected into dorsal dermas of mice within prelabeled 100 mm² area. Twenty-four hours later, mice were injected i.v. with rhodamine-conjugated dextran at 100 mg/kg. After 3 h, mice were injected i.v. with FITC-lectin at 10 mg/kg. Ten minutes later, each mouse was anesthetized and perfused with saline and followed by 5 mL of 4% formaldehyde. Tissues of interest were
cubosed and examined by fluorescence microscopy for vascular leakage. There were five mice in each group, and the experiments were independently performed twice.

**Immunohistochemistry.** Mice were anesthetized and perfused with PBS to eliminate circulatory blood components. Then, lung tissues were immunohistochemically stained with indicated primary antibodies and fluorescence-conjugated secondary antibodies as described previously (10, 11).

**Mammary fat pad spontaneous metastasis assay.** Eight- to 10-wk-old female nude mice were anesthetized and perfused with PBS to eliminate circulatory blood components. Then, lung tissues were immunohistochemically stained with indicated primary antibodies and fluorescence-conjugated secondary antibodies as described previously (10, 11).

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**Statistical analysis.** For statistical analysis, the data were expressed as mean ± SD and compared using a Student's t test. A P value of <0.05 was considered significant.

**Results**

**Pulmonary vascular integrity is decreased in the premetastatic phase.** Lung is one of the most preferentially metastatic sites for various types of tumor in murine model, and vascular system is considered as the common route of lung metastasis. To study the responses of local microenvironment to primary tumors in the premetastatic phase, we focused on the lung before occurrence of metastases. B16/F10 melanoma cells were i.d. implanted to C57/BL6 mice as the primary tumor. The pulmonary vasculature was analyzed 12 days after primary tumor implantation, by which time lung metastasis did not happen and could be considered as premetastatic phase based on previous studies (4, 7). The normal mice or tumor-bearing mice were i.v. injected by FITC-lectin and rhodamine-dextran. After perfusion with saline to eliminate circulating dextran, the pulmonary vasculatures were represented by FITC-lectin, and vascular integrity was represented by diffused rhodamine-dextran. We found that more rhodamine-dextran was released from blood vessels in tumor-bearing mice, which indicated that the pulmonary vasculature was destabilized and permeability was increased (Fig. 1A). The relative pulmonary vascular permeability was further quantified by areas of diffused dextran at different days after B16/F10 implantation (Fig. 1B). The remarkable pulmonary vascular destabilization occurred in 1 week and reached the stable phase ~2 weeks (Fig. 1B). As an event in the premetastatic phase, pulmonary vascular destabilization might be correlated with the intrinsic metastatic potential of primary tumor. To verify this point, parallel comparisons of B16/F10 cells with parental B16/F0 and B16/F1 cells were applied to pulmonary vascular analysis. Although both B16/F0 and B16/F1 with low metastatic potential could destabilize the pulmonary vasculatures to some extent, such effects were not as dramatic as B16/F10 cells with high metastatic potential (Supplementary Fig. S1).

Pulmonary microvascular structures were further analyzed by transmission electron microscopy. In normal mice, the pulmonary

**Figure 2.** Premetastatic lungs facilitate extravasation and survival of tumor cells. A, 2 h after i.v. injection, B16/F10-eGFP cells (green) adhered and extravasated from pulmonary blood vessels (red) in control mice or B16/F10-D12 mice. Arrows, extravasated tumor cells. Bar, 20 μm. B, quantification of the relative numbers of extravasated tumor cells was shown. *, P < 0.01. C, 2 d after extravasation, B16/F10-eGFP cells (green dots, arrows) that settled in the lungs of control or “B16/F10-D14” mice were visualized. Insets, enlarged images of B16/F10-eGFP colonies. Bar, 200 μm. D, quantification of the numbers of remaining tumor cells in both control and B16/F10-D14 group. Five mice were analyzed in each group of this study. *, P < 0.01.
endothelium was surrounded by basement membranes. However, in B16/F10-bearing mice, the morphology of endothelium was changed and the integrity of surrounding basement membranes in tunica intima and medium seemed to be disrupted to some extent (Fig. 1C). In addition to pulmonary vasculature, the vasculatures with continuous endothelium in brain and hindlimb were also analyzed in response to primary B16/F10 tumor. Interestingly, B16/F10 tumor had no significant effect on these vasculatures in brain and hindlimb in the premetastatic phase (Supplementary Fig. S2), which indicates that vascular destabilization is specifically associated with lung metastasis. Moreover, the conditioned medium of B16/F10 has a comparable effect on pulmonary vascular destabilization with B16/F10 tumor (Supplementary Fig. S3), which suggests that the B16/F10 cells have greater propensity to destabilize the vasculature compared with its parental cells.

After implantation of primary tumor, we found that fibrinogen was released from blood vessels to deposit in the lung (fibrin deposition) during the premetastatic phase (Supplementary Fig. S4), which further shows that pulmonary vasculatures are destabilized. Interestingly, the process of pulmonary vascular destabilization is accompanied with myeloid cell recruitment to the lung, which is a hallmark of premetastasis niche (4, 5, 7). The recruitment of myeloid cells in response to B16/F10 tumor and its parental B16/F0 and B16/F1 tumors was represented by marker staining are not consistent at day 16, which might be due to the differentiation of recruited myeloid cells after infiltrating to lung. In addition to myeloid cell recruitment, vascular destabilization may be another hallmark of the premetastatic phase, which is also intimately correlated with the intrinsic metastatic potential of primary tumor.

**Pulmonary vascular destabilization facilitates the extravasation of tumor cells.** Although the extravasation of circulating tumor cells and their subsequent survival are critical for metastasis, passage through the vascular barrier is prerequisite for these steps. We wondered whether the vascular destabilization alters the interactions between tumor cells and endothelium and therefore facilitates the extravasation. To verify this point, we performed extravasation assay. Briefly, eGFP-B16/F10 cells were i.v. injected to normal mice or tumor-bearing (B16/F10-D12) mice, and rhodamine-lectin was i.v. injected 2 hours later to visualize the vasculatures. We found that the tumor cell extravasation in the premetastatic lung was more severe than that in the normal lung (Fig. 2A and B). However, the parental B16/F0 and B16/F1 tumors with low metastatic potentials have no significant influence on the extravasation of tumor cells (Supplementary Fig. S6). Two days after the extravasation of eGFP-B16/F10 cells, the lung of mice in each group was cryosectioned for imaging to visualize the remaining extravasated tumor cells (Fig. 2C). More B16/F10-eGFP cells remained in the premetastatic lung than those in the normal lung (Fig. 2C and D). The results suggest that the vascular abnormalities in the premetastatic lung promote the extravasation of circulating tumor cells and subsequent survival of metastatic tumor cells.

**Angpt2, MMP3, and MMP10 are up-regulated in the premetastatic lung.** We hypothesized that vascular remodeling in the premetastatic phase is initiated by the changes of local microenvironment. Therefore, we systematically examined the gene expression of lung mesenchyme by microarrays and found that Angpt2, MMP3, and MMP10 were significantly up-regulated in the premetastatic phase (Supplementary Fig. S7A). Further functional studies, including vascular basement digestion assay and dermal vascular permeability assay, showed that these factors...
might be involved in vascular remodeling (Supplementary Fig. S7B and C). To verify whether Angpt2, MMP3, and MMP10 are the premetastatic factors in regulating pulmonary vasculature, we detected the lung expression patterns for both mRNA and proteins of Angpt2, MMP3, and MMP10 in mice implanted with B16/F10 tumor for 0, 4, 8, 12, and 16 days. We found that the expressions of these factors were significantly up-regulated following progression of primary tumor (Fig. 3A–C). Because other regulatory mechanisms such as microRNA and postsynthesis degradation may exist, the protein expression of these factors did not fully follow their

**Figure 4.** Angpt2 and MMP3/MMP10 synergistically destabilize the blood vessels. A, endothelial monolayers were treated with indicated proteins for 24 h (200 ng/mL of Angpt2; 50 ng/mL of MMP3 and MMP10). Green, the integrity of monolayer was detected by anti-CD31 staining; blue, nuclei were counterstained with 4',6-diamidino-2-phenylindole. Arrows, disconnections in endothelial monolayers. Bar, 20 μm. B, the permeability of in vitro vascular mimic system, induced by indicated proteins (200 ng/mL of Angpt2; 50 ng/mL of MMP3 and MMP10) for 24 h, was quantified. ***, P < 0.001. Inset, in vitro vascular mimic system. C, mice were i.d. treated with indicated proteins (1.5 μg/mouse of Angpt2; 1.2 μg/mouse of MMP3/MMP10) for 24 h, and the dermal vasculatures were analyzed. Green, dermal blood vessels; blue, vascular permeability stained with extravasated dextran. Right, lectin and dextran staining were merged. Bar, 100 μm. Insets, higher magnifications. D, the dermal vascular permeability in each group was further quantified and shown. Five mice were analyzed in each group of this study. **, P < 0.005; ***, P < 0.001.
These observations indicate that local microenvironment in the lung is indeed influenced by primary tumor during premetastatic phase, and the alterations may lead to pulmonary vascular destabilization.

**Angpt2, MMP3, and MMP10 synergistically destabilize the blood vessels.** To further investigate the role of Angpt2, MMP3, and MMP10 in vascular destabilization, in vitro vascular mimic model and in vivo mice dermal microvascular model were applied. MMP3 and MMP10 (stromelysin 1 and stromelysin 2) belong to the same kind of MMP subfamilies and share high sequence and functional similarities (12). Because they show similar degrading patterns in vascular remodeling (data not shown) and both were up-regulated in the premetastatic phase, we applied them together in this study. The dosages for Angpt2, MMP3, and MMP10 used in the following experiments were predetermined by the titration assays in each model (Supplementary Fig. S8). In the vascular mimic system, MMP3/MMP10 dramatically facilitated the Angpt2-induced leakages via degrading the perimatrix of endothelial layer.

*Figure 5.* Knocking down Angpt2, MMP3, and MMP10 inhibits the vascular permeability and myeloid cell infiltration in the lung. A, the pulmonary vasculatures were stained with FITC-lectin (Lectin) in B16/F10-D12 mice treated with lentivirus-based mock RNAi (Mock) and lentivirus-based RNAi for Angpt2 (Angpt2-RNAi), MMP3/MMP10 (MMP3/10-RNAi), and Angpt2/MMP3/MMP10 (Angpt2/MMP3/10-RNAi), respectively. Lentivirus was applied weekly after tumor inoculation (twice). Vascular integrities were represented by the diffused modamine-dextran (Dextran). Bottom, lectin and dextran staining were merged. Bar, 20 μm. B, the permeability of pulmonary vasculatures in each group was quantified. *, P < 0.01; ***, P < 0.001. C, myeloid cells (red) infiltrated out of pulmonary vasculatures (green) in B16/F10-D12 mice treated with mock, VEGFR1/AMD3100, and lentivirus-based RNAi of Angpt2/MMP3/MMP10. Arrowheads, infiltrated cells; arrows, adhered cells inside the vasculature. Bars, 50 μm (three left panels). The rightmost panels are the magnified images. Bar, 20 μm. D, the statistic chart showed the relative ratios of extravasated myeloid cells to the total myeloid cells in the lung of indicated groups. Five mice were analyzed in each group of both experiments. *, P < 0.01; ***, P < 0.001.
(Fig. 4A and B). The synergistic effect indicates that during vascular remodeling, Angpt2 disrupts the organization of endothelium (Fig. 4A), whereas MMP3/MMP10 simultaneously degrades the basement membrane surrounding the endothelium.

The effects of Angpt2 and MMP3/MMP10 on vascular remodeling were further analyzed in the mice dorsal dermal vasculatures. Compared with control groups, both Angpt2 and MMP3/MMP10 induced the permeability of dermal vessels to some extent. However, the enlarged images showed that the leaky patterns induced by different treatments varied. The diffused dextran, induced by Angpt2, closely surrounded the blood vessel, whereas the diffused dextran in MMP3/MMP10-treated group, although in less amount, spread far away from blood vessel. Consistent with the in vitro assay, Angpt2 and MMP3/MMP10 showed a synergistic effect on inducing the permeability of dermal vasculatures in vivo (Fig. 4C and D).

Collectively, both in vitro and in vivo studies show that Angpt2, MMP3, and MMP10 can disrupt the vascular integrity in a synergistic manner, which indicates that up-regulation of these factors in the lung may contribute to the pulmonary vascular destabilization.

Interfering Angpt2 and MMP3/MMP10 attenuates the pulmonary vascular permeability and inhibits lung metastasis. To further investigate the functions of Angpt2, MMP3, and MMP10 in the pulmonary vascular destabilization, lentivirus-based in vitro RNA interference (RNAi) was applied. Lentiviruses encoding interfering sequences against Angpt2, MMP3, and MMP10 were intrapleurally injected weekly to knock down them in the lung, and the interfering efficiency was evaluated (Supplementary Fig. S9). Compared with the vasculatures in the premetastatic phase, the pulmonary vascular permeability was significantly decreased on silencing Angpt2, MMP3, and MMP10 in the premetastatic lung (Fig. 5A and B).

Because myeloid cell recruitment is a hallmark of premetastatic niche and certain critical chemokines are involved in this process (4, 7, 13), we wondered whether the vascular destabilization induced by Angpt2 and MMP3/MMP10 affects the recruitment of myeloid cells. To address this point, we analyzed the infiltration of myeloid cells in the lung. Myeloid cells, isolated from peripheral blood and labeled by CellTracker Red, were i.v. injected to tumor-bearing mice treated with mock RNAi, vascular endothelial growth factor receptor 1 (VEGFR1)/AMD3100, and Angpt2/MMP3/MMP10 RNAi, respectively. Of note, interfering Angpt2, MMP3, and MMP10 also attenuated the infiltration of myeloid cells (Fig. 5C and D), which indicates that the permeable vasculatures induced by these premetastatic factors promote the recruitment of myeloid cells via facilitating the infiltration. Soluble VEGFR1 and AMD3100 served as a positive control here to block the chemotraction of myeloid cells.

The formation of premetastatic niche is an early event of the subsequent metastasis. As aforementioned, the pulmonary microenvironment was modulated to facilitate extravasation and survival of metastatic tumor cells. To examine the contribution of pulmonary vascular destabilization to the lung metastasis, the spontaneous metastasis model was applied with implantation of luciferase-labeled MDA-MB-231 cells (MDA-MB-231-Luc-D3H1) in mammary fat pad. Interfering Angpt2/MMP3/MMP10 also significantly inhibited the spontaneous lung metastasis of MDA-MB-231 (Fig. 6A and B). These results indicate that melioration of pulmonary vasculature can retard the occurrence of subsequent lung metastasis.

Based on our previous descriptions (Figs. 4 and 5), Angpt2, MMP3, and MMP10 destabilize the pulmonary vasculatures in the premetastatic phase. How are these factors regulated by primary tumors? Do the intrinsic characters of primary tumors affect the expressions of these factors? To address these points, we constructed the promoter-driven luciferase reporter vectors of each gene. The conditioned media of invasive breast cancer cells (MDA-MB-231), noninvasive breast cancer cells (MCF7), and HMepiC were subjected to stimulate the lung fibroblast cell (MDA-MB-231), which was pretransfected with reporter vectors of those
premetastatic factors. It was shown that invasive tumor cells (MDA-MB-231) were more potent in up-regulating the premetastatic factors than either noninvasive ones (MCF7) or normal cells (HMEpiC; Supplementary Fig. S10A). Consistently, the invasive B16 cells (B16/F10) stimulated the expression of premetastatic factors more significantly than the noninvasive B16/F0 and B16/F1 cells (Supplementary Fig. S10B). The study shows that the malignancies of primary tumors are positively correlated with their capabilities to induce the expression of these premetastatic factors.

In the following study, we found that transforming growth factor-β1 (TGF-β1) and tumor necrosis factor-α (TNF-α), both of which were secreted by B16/F10 and MDA-MB-231 cells (data not shown), showed significant effects on up-regulating the genes of Angpt2, MMP3, and MMP10 (Supplementary Fig. S11A). Interactions between the promoters of these premetastatic factors and downstream transcription factors of TGF-β1 and TNF-α were detected by chromatin immunoprecipitation. Consistent with the luciferase reporter assay, Smad4, downstream transcription factor of TGF-β1, binds to the promoter regions of Angpt2 and MMP10, and P65, downstream transcription factor of TNF-α, binds to the promoter regions of Angpt2, MMP3, and MMP10 (Supplementary Fig. S11B and C). These observations indicate that TGF-β1 and TNF-α secreted by primary tumors may be involved in the alterations of pulmonary microenvironment, which is consistent with previous studies about the roles of TGF-β and TNF-α in the premetastatic niche and lung metastasis (7, 9).

Taken together, in the premetastatic phase, the primary tumor initiates the alterations of lung microenvironment and destabilizes the pulmonary vasculatures. These alterations lead to enhanced extravasation and retention of circulating tumor cells and facilitate the recruitment of myeloid cells, which finally promote the subsequent lung metastasis.

Discussion

The process of cancer metastasis is complex and consists of a series of interrelated steps. Extravasation of metastatic tumor cells in specific organs is a checkpoint to produce clinically relevant lesions. The metastasis depends on both the intrinsic properties of the tumor cells and their interactions with the host microenvironment (14, 15). In this study, we show that the premetastatic vascular remodeling contributes greatly to extravasation-survival steps of metastatic cells. Angpt2, MMP3, and MMP10 are induced in local microenvironment to destabilize the vasculatures of lung in response to primary tumor. Recently, Padua and colleagues (9) found that Angpt4, secreted by circulating tumor cells, increased the permeability of lung capillaries, and our study also showed that B16/F10 induced pulmonary vascular permeability to a stronger degree compared with B16/F0 and F1, which indicates that the intrinsic properties of metastatic cells also contribute to destabilize pulmonary vasculature. Therefore, microenvironment alterations in the premetastatic organs depend on both intrinsic characteristics of tumor cells and host factors.

Vascular integrity is maintained by tight junctions between adjacent endothelial cells. Surrounded basement membranes and pericytes also contribute to the maintenance of integrity (16–18). Although VEGF is a crucial factor in inducing angiogenesis and disrupting vascular barriers in diseased tissues (19–21), we did not observe the significant up-regulation of VEGF in lung mesenchyme of tumor-bearing mice. Angpt2 has been reported to work by blocking the Tie2 signal and antagonizing the actions of Angpt1 (22, 23). Compared with Angpt1 that is constitutively expressed in normal adult tissues, Angpt2 is up-regulated only at sites of vascular remodeling to allow the vessels to be transformed into a more plastic state (24, 25). Angpt2 causes the loosening of endothelial junctions and makes the endothelium of the blood vessel separate from the periendothelial cells (23, 26–28).

MMPs, which degrade the extracellular matrix including the junctional complex, play important roles in tumor microenvironment, malignancy, and vascular remodeling (29–31). Basement membrane in the tunica of mature blood vessels consists of fibronectin and collagens, which facilitate maintenance of vascular integrity, and is the potential substrate for MMP3 and MMP10 (30, 32, 33). In concert with Angpt2, MMP3 and MMP10 would be expected to further exacerbate the abnormalities in vascular integrity. Additionally, MMP9, shown by Kaplan and colleagues (4) as an important mediator in the premetastatic niche, may also have a similar effect. However, because MMP9 is mainly produced by the recruited hematopoietic cells but not by the local microenvironment, the potential vascular remodeling effect of MMP9 may occur posterior to the recruitment of myeloid cells.

In the premetastatic phase, primary tumors alter the host microenvironment, including destabilizing vasculature and recruiting myeloid cells. Our results show that mollerization of the vasculatures can retard the occurrence of metastasis in the lung, which strongly suggests that pulmonary vascular destabilization has clinical relevance as potential targets for prevention of metastasis at very early stage.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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References


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