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Angiogenic and lymphangiogenic cascades in the tumor microenvironment

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1. ABSTRACT

Blood and lymphatic vessels in tumor tissue are major components of the tumor microenvironment. These vessels are newly formed from pre-existing host vessels stimulated by pro-blood-angiogenic and pro-lymph-angiogenic (pro-blood/lymph-angiogenic) factors expressed in tumor cells. Tumor cells establish a specific stromal microenvironment fostering tumor growth, in which blood/lymph-angiogenesis are involved. The tumor-associated blood/lymph-angiogenesis is continually induced by complicated cytokine networks, namely pro-blood/lymph-angiogenic factor-mediated paracrine and autocrine interactions among tumor cells and stromal cells including endothelial cells (ECs) and non-endothelial mesenchymal cells (neMCs). In this review, we provide an overview of the features of tumor-associated blood/lymph-angiogenesis based on recent and updated information obtained mainly from our studies. With regard to the constituent cell-dependent molecular mechanisms that regulate tumor blood/lymph-angiogenesis, we focus on: 1) the role of blood/lymph-angiogenesis-related factors/receptors expressed in tumor cells; and 2) the role of blood/lymph-angiogenesis-related factors/receptors expressed in stromal cells (ECs and neMCs). Finally, we discuss the features of tumor-associated blood/lymph-anigogenesis, especially a vessel abnormality through the viewpoint of blood/lymph-angiogenic cascades in tumor microenvironment for better understanding of the tumor vascular biology.

2. INTRODUCTION

Blood/lymph-angiogenesis are pathophysiological events, and are necessary for pathological conditions such as inflammation, wound healing, and tumor progression to occur in adults (1-3). Newly formed blood and lymphatic vessels in pathological tissue play a critical role not only in the blood supply and drainage of tissue fluid but also in the recruitment and subsequent regression of inflammatory/immune cells through these vessels. The blood/lymph-angiogenic response in human pathological conditions leads to beneficial or occasionally harmful results. For example, the response in impaired lesions such as myocardial infarction plays an essential role in tissue repair and regeneration. In contrast, the response in tumors promotes the growth and metastasis of tumor cells, and that in diabetic retinopathy causes vision loss. For this reason, the therapeutic strategies for mediating blood/lymph-angiogenesis among diseases should be considered in a wide range of view (1-7).

Tumor cells establish a specific stromal microenvironment fostering tumor growth (Figure 1A). Tumor-associated angiogenesis is thought to be an essential pathophysiological phenomenon for sustaining the viability of tumor cells during tumor progression, and it leads to an increased incidence of hematogenous metastasis (4, 5). On the other hand, the implication of tumor-associated lymphangiogenesis for the viability of tumor cells is unclear. It is known that such lymphangiogenesis leads to

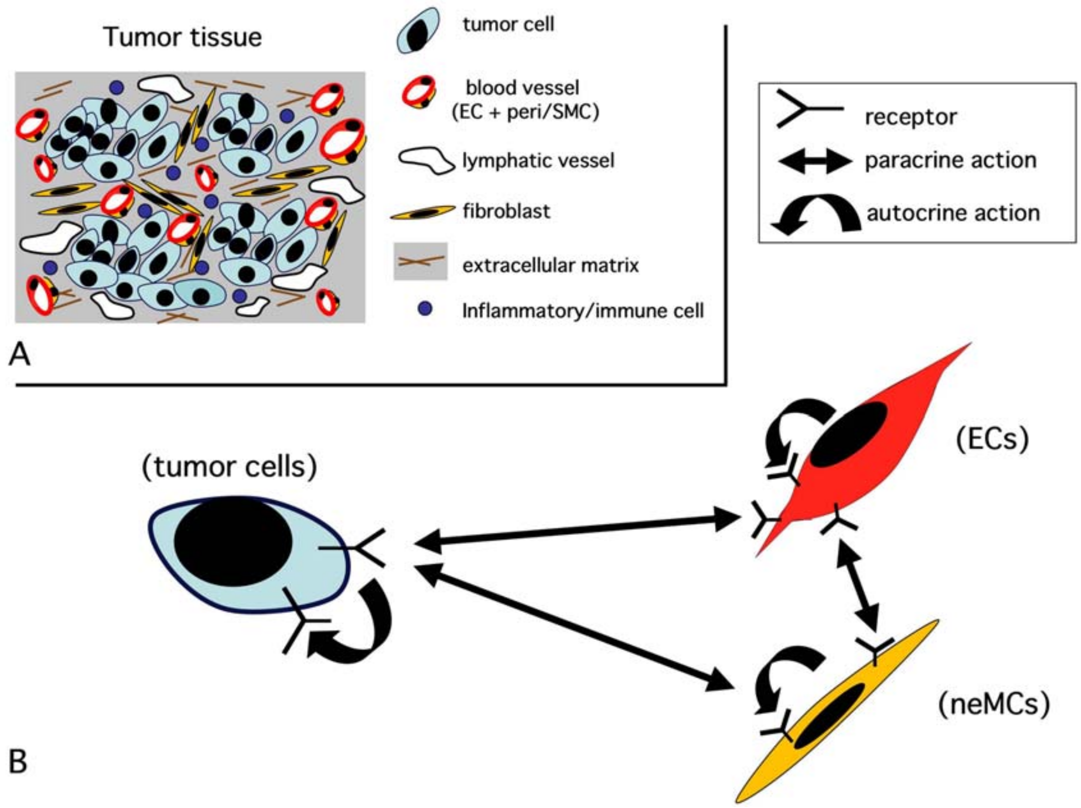

Figure 1. A, Scheme of constituent tissue components in tumor microenvironment. The tumor tissue consists of tumor cells, fibroblasts, blood vessels, lymphatic vessels and infiltrated inflammatory/immune cells and an extracellular matrix. B, Autocrine and paracrine interactions via the ligand/receptor systems among tumor cells and host stromal cells in the tumor microenvironment. The host stromal cells consist of blood or lymphatic endothelial cells (ECs) and non-endothelial mesenchymal cells (neMCs) such as fibroblasts, vascular smooth muscle cells and pericytes.

an increased incidence of lymphogenous metastasis (3, 8). Tumor blood/lymph-angiogenesis is induced by pro-blood/lymph-angiogenic factors expressed in tumor cells that have different characteristics compared to those of normal cells due to genetic transformation (9). In many cases, several key blood/lymph-angiogenesis-related factors, including receptors, are expressed in tumor cells, and these factors form cytokine networks in the tumor microenvironment (Figure 1B). In particular, vascular endothelial growth factor (VEGF)-A and VEGF-C are well known to be crucial pro-blood/lymph-angiogenic factors, respectively (3, 7, 8, 10). VEGF-A is upregulated by various stimuli in growing tumors and directly activates host endothelial cells (ECs) expressing the cognate receptors VEGF receptor (VEGFR)-1 and -2 (10). Especially, the upregulation of the VEGF-A in response to hypoxia in tumor cells is a well known phenomenon, and it is advantageous to tumor progression. A chronic ischemic condition in the tumor tissue with an unlimited/autonomic proliferation of tumor cells can induce constitutive and increased expression of VEGF-A in tumor cells, which causes sustained blood-angiogenesis. In contrast, VEGF-C gene expression does not respond to hypoxic stimulation (3, 11). We herein describe advantageous and hypoxia-independent molecular systems relevant to VEGF-A and VEGF-C regulation in tumor cells for promoting tumor-associated blood/lymph-angiogenesis.

Apart from tumor angiogenesis, “therapeutic angiogenesis” is an approach to the clinical treatment of atherosclerosis and blood vessel-related ischemic diseases such as myocardial and cerebral infarctions, arteriosclerosis obliterans (ASO) and Berger’s disease (12). The end goal of this therapy is to supply sufficient blood flow to the ischemic organ through focal and effective induction of angiogenesis and arteriogenesis. At present, there are two main ways to induce angiogenesis: 1) focal administration of an appropriate pro-blood/lymph-angiogenic factor (cytokine therapy) (12, 13); and 2) focal administration of peripheral blood- or bone-marrow-derived mononuclear or endothelial progenitor cells (cell therapy) (13-15). The former method aims to actively induce blood/lymph-angiogenesis through the effect of an exogenous cytokine, and the latter aims to actively induce vasculogenesis through the endothelial differentiation of inoculated stem/progenitor cells. Initially, VEGF-A attracted considerable attention as a useful therapeutic agent in cytokine therapy (12). However, a number of subsequent studies demonstrated that high-dose administration of VEGF-A induced angioma-like, robust and aberrant angiogenesis (16, 17), and that VEGF-A-induced neovessels were leaky and poorly organized (18-20). At

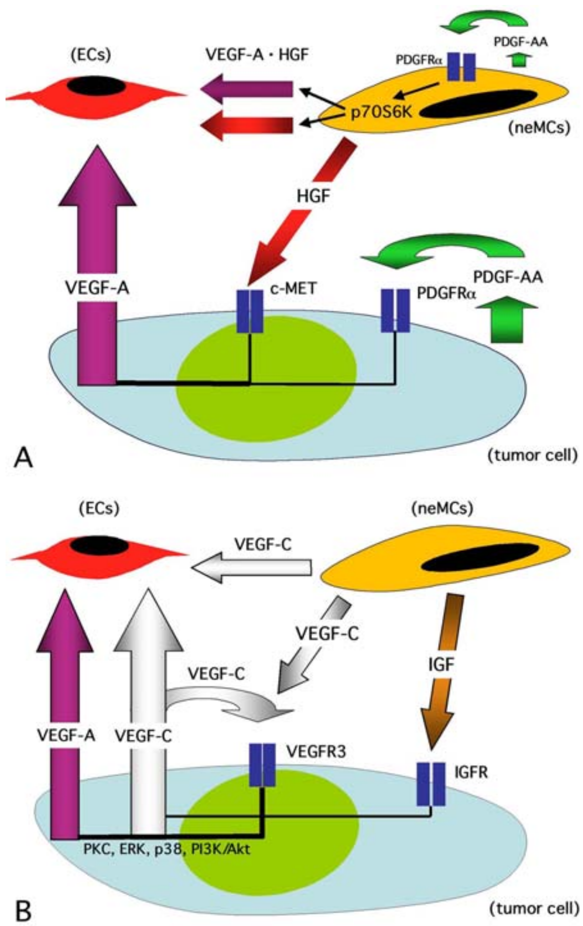

Figure 2. Autocrine and paracrine systems to activate tumor-associated growth factor receptors expressed in tumor cells. A) Scheme of antocrine and paracrine systems to augment VEGF-A secretion in tumor cells, and VEGF-A and HGF secretions in neMCs. The PDGF-AA/PDGFR-alfa autocrine system in tumor cells and neMCs, and the HGF/c-MET paracrine system between tumor and host stromal cells are indicated. B) Scheme of antocrine and paracrine systems to augment VEGF-A and VEGF-C secretion in tumor cells. The VEGF-C/VEGFR-3 autocrine system (positive feedback loop) in tumor cells and the IGF/IGFR paracrine system between tumor and host stromal cells are indicated. PKC (protein kinase C), ERK, p38 (p38 MAPK) and PI3K-Akt are VEGFR-3-asscoaited downstream signals for upregulation of VEGF-A and -C.

present, functional and well-organized new blood vessels, which can provide sufficient blood flow to ischemic tissues, are expected to be induced by the harmonized effects of several blood/lymph-angiogenesis-related factors (11, 21-23). We gave the name "functional" angiogenesis to the harmonization-induced blood/lymph-angiogenic process.

In this review, the features of blood/lymph-angiogenesis in solid tumors and therapeutic angiogenesis in ischemic diseases are discussed. We focus on the similarity between these two pathological events and provide a new insight into the mechanisms underlying vessel abnormality in the tumor microenvironment. Our overview is based on up-to-date information regarding: 1) the molecular systems in tumor cells, by which blood/lymph-angiogenesis is actively induced, and 2) spatiotemporally coordinated molecular systems among stromal cells, by which functional and well-organized blood vessels are newly formed ("functional" angiogenesis).

3. MOLECULAR SYSTEMS IN TUMOR CELLS FOR PROMOTING BLOOD/LYMPH ANGIOGENESIS

3.1. An expression profile of blood/lymph-angiogenesis-related factors in tumor cells

Tumor cells show uncontrolled and heterotopic expression of various factors related to blood/lymph-angiogenesis, the degradation of extracellular matrix and intracellular signaling pathway(s). In our study, we measured the spontaneous gene expression of blood/lymph-angiogenesis-related factors in a variety of cancer cell types, including squamous cell carcinoma (SCC) of the oral cavity, SCC and adenocarcinoma of the lung and adenoid cystic carcinoma of the salivary glands. We revealed that these cancer cell lines frequently expressed soluble factors such as VEGF-A, VEGF-C, platelet-derived growth factor (PDGF)-A, and also the receptors such as c-MET, PDGF receptor (PDGFR)-alfa , epidermal growth factor (EGF) receptor (EGFR), insulin-like growth factor (IGF) receptor (IGFR), and VEGFR-3 *in vitro* (24). Notably, a large number of cell types possess autocrine systems such as PDGF-AA/PDGFR-alfa and VEGF-C/VEGFR-3. The roles of these growth factors and their-mediated signaling crosstalks in tumor progression, including tumor-associated blood/lymph-angiogenesis, are described below.

3.2. Activation systems of tumor cell-associated receptors in a paracrine manner

As mentioned in section 3-1, a number of cancer cell lines express IGFR and c-MET (24). Since the respective corresponding ligands IGF and hepatocyte growth factor (HGF) are not expressed at significant levels in the tumor cells (24), these receptors in tumor cells may not be activated in an autocrine manner. However, these receptors can be activated by the host stromal cell-derived corresponding ligands in a paracrine manner (Figure 2), thereby promoting proliferative, migratory and pro-blood/lymph-angiogenic activities of tumor cells. Several recent reports have demonstrated that the c-MET signaling pathways in tumor cells can stimulate the VEGF-A gene (25, 26) (Figure 2A), and that IGFR signaling pathways in tumor cells can stimulate the VEGF-C gene, promoting lymphogenous metastasis in animal models (27, 28) (Figure 2B). Therefore, the tumor cell-associated growth factor receptor, if the corresponding ligands are simultaneously not expressed in tumor cells, can be activated in a host context-dependent manner, and contribute to tumor-associated blood/lymph-angiogenesis.

3.3. Activation systems of tumor cell-associated receptors in an autocrine manner

3.3.1. The VEGF-A/VEGFR-2 autocrine system

Dias *et al.* first reported on the detailed pathological role of the autocrine system of angiogenesis-related factors in tumor cells (29). They found that the VEGF-A/VEGFR-2 autocrine system was active in leukemia cells. Subsequently, Masood *et al.* demonstrated that several tumor cell lines such as Kaposi's sarcoma, melanoma, and ovarian and prostatic carcinomas had the VEGF-A/VEGFR-2 autocrine system (30). These studies suggest that VEGF-A in tumor cells contributes not only to angiogenesis but also to increased proliferation/migration activities of tumor cells via the autocrine system, thus enhancing tumor progression (29, 30). In contrast, our study revealed that the expression of VEGF-A was detected, but not with the expression of VEGFR-2, in various types of cancer cell lines including SCC of the oral cavity, SCC and adenocarcinoma of the lung and adenoid cystic carcinoma of the salivary glands (24). Therefore, it is suggested that the VEGF-A/VEGFR-2 autocrine system is relatively limited, if it is present at all, in many tumor cell lines (24).

3.3.2. The PDGF-AA/PDGFR-alfa autocrine system

PDGF-A peptide is a monomeric subunit derived from the PDGF-A gene. The PDGF-A subunit dimerizes in cytoplasm, and dimeric PDGF-AA is secreted from cells and binds the cognate receptor PDGFR-alfa, thus inducing receptor autophosphorylation (31). Until recently, the role of PDGF-AA during angiogenesis has not been well characterized. It is believed that PDGF-AA neither accelerates the proliferation nor induces the migration of ECs *in vitro* (32). On the other hand, it was suggested that PDGF-AA stimulated angiogenesis *in vivo* via indirect effect (33). We clarified the mechanism of PDGF-AA–mediated angiogenesis in part. In our studies, non-endothelial mesenchymal cells (neMCs) such as vascular smooth muscle cells and fibroblasts produced PDGF-AA endogenously, and these neMCs expressed its cognate receptor PDGFR-alfa (22, 34) simultaneously *in vitro*. VEGF-A and HGF protein secretion were largely dependent on endogenous PDGF-AA function in neMCs *in vitro* (22, 34) (Figure 2A), suggesting that the system played an important role in VEGF-A and HGF expressions. A variety of cancer cell lines as well as neMCs possess the PDGF-AA/PDGFR-alfa autocrine system (24, 35), and their spontaneous VEGF-A expression is also partly dependent on this system (34) (Figure 2A). Blockade of the system in cancer cells was shown to suppress tumor-associated angiogenesis in an *in vivo* mouse tumor implantation model (35). Furthermore, a clinicopathological study revealed a positive correlation between VEGF-A and PDGF-AA expressions in cancer cells in non-small cell lung carcinomas (NSCLCs). The study showed that tumor sizes were larger in PDGF-AA-positive NSCLC patients than in PDGF-AA-negative patients, and that the 5-year survival rates were significantly lower in positive cases than in negative cases (35). Interestingly, epithelial cells in atypical adenomatous hyperplasia (AAH), a precancerous lesion of the lung, did not express PDGF-AA, whereas they often expressed VEGF-A (35). These findings suggest that a phenotypic change from the absence to the presence of PDGF-AA expression in pre-cancerous cells may be critical for obtaining malignant potential, and that the enhanced VEGF-A expression via the PDGF-AA/PDGFR-alfa autocrine system in tumor cells plays a crucial role in stimulating tumor-associated angiogenesis.

3.3.3. The VEGF-C/VEGFR-3 autocrine system

Along with the VEGF-A/VEGFR-2 autocrine system, the VEGF-C/VEGFR-3 autocrine system plays a critical role in some types of tumor cells (36, 37). The functions of the VEGF-C/VEGFR-3 autocrine system were somewhat similar to those of the VEGF-A/VEGFR-2 autocrine system—namely, the promotion of proliferative and anti-apoptotic activities. Moreover, Su *et al.* demonstrated that the VEGF-C/VEGFR-3 autocrine system played a role in promoting the invasive activity of pulmonary adenocarcinoma cells via VEGFR-3-associated src/p38 mitogen-activated protein kinase (MAPK)-dependent upregulation of contactin-1 (37). In support of these findings, some clinicopathological studies have revealed that VEGF-C and VEGFR-3 were simultaneously expressed in human cancer cells, and that the expression levels of VEGF-C and VEGFR-3 positively correlated with a higher incidence of lymph node metastasis and/or poor prognosis (38, 39). Recently, we found that constitutive activation of VEGFR-3 was caused by autocrine action of VEGF-C in a number of tumor cells, leading to sustained activation of its downstream signaling pathways PKC, ERK, PI3K-Akt and p38 MAPK (Figure 2B) (24). These intracellular signals are linked with upregulation of VEGF-A and VEGF-C. The autocrine loop between VEGF-C and VEGFR-3 can induce high-level expression of VEGF-A and VEGF-C in tumor cells (24) (Figure 2B). The blockade of the VEGF-C/VEGFR-3 autocrine system in tumor cells successfully suppressed tumor-associated blood/lymph-angiogenesis in a mouse tumor implantation model (24).

Throughout section 3, it is noted that growth factor receptors expressed in tumor cells are activated in both context-dependent (paracrine action of cognate ligands secreted from stromal cells) and context-independent (autocrine action of cognate ligands secreted from tumor cells) manners in the tumor microenvironment, thereby enhancing the malignant potential of tumor cells. In particular, these systems-dependent productions of high-level pro-blood/lymph-angiogenic factors actively stimulate blood/lymph-angiogenesis in the tumor microenvironment.

4. MOLECULAR SYSTEMS AMONG STROMAL CELLS FOR INDUCING "FUNCTIONAL" ANGIOGENESIS

4.1. Differential therapeutic effects between VEGF-A and FGF-2 in cytokine therapy

Focal and high-dose administration of an angiogenic factor into an ischemic organ is one of the strategies used to alleviate hypoxic damage and improve tissue function (4). To establish an effective therapeutic strategy, it is important to scientifically explore which angiogenic factors are the most available for use as therapeutic agents. VEGF-A, a specific mitogenic stimulator of ECs, had once been expected as a promising candidate. However, some studies demonstrated that focal administration of exogenous VEGF-A induced angioma-

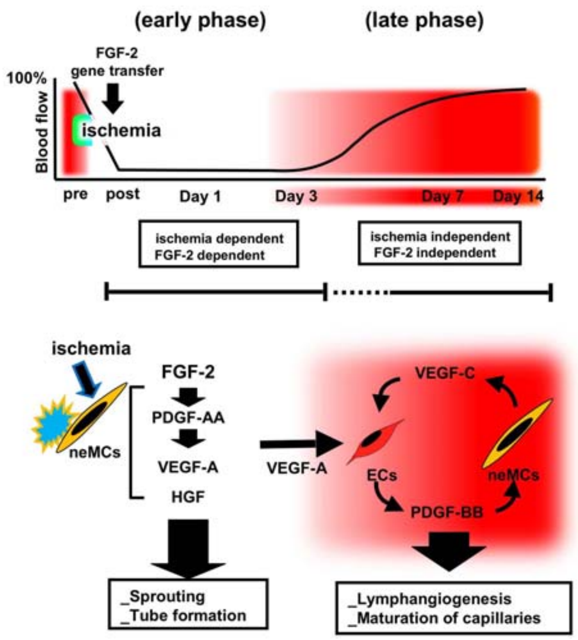

Figure 3. Spatiotemporal blood/lymph-angiogenic mechanisms during FGF-2-mediated therapeutic angiogenesis. Scheme indicates the time-dependent recovery of blood flow in a mouse hindlimb ischemia model after SeV-FGF-2-mediated gene transfer (upper graph), and the temporally corresponding interactions of critical blood/lymph-angiogenesis-related factors to induce "functional" angiogenesis (lower schemes). In the early phase, in addition to the effect of hypoxia on VEGF-A upregulation, FGF-2-induced endogenous systems—namely, the upregulation of VEGF and HGF induced by the PDGF-AA/PDGFR-alfa system in neMCs—are critical in the endothelial sprouting and tube formation. In the late phase (red), the spatial ECs/neMCs interaction-based positive feedback loop between the VEGF-C/VEGFR3 and the PDGF-BB/PDGFR-beta systems causes them to stimulate each other to simultaneously enhance lymphangiogenesis and the maturation of blood vessels. The VEGF-A/VEGFR2 system play the role of synergistically enhancing the PDGF-BB expression in ECs in cooperation with the VEGFC/VEGFR3 system (Reproduced with permission from ref. #11).

like robust and structurally aberrant blood vessels in murine ischemic myocardium (16-18). Our study also revealed that focal and high-dose administration of VEGF-A mediated by a Sendai virus vector (SeV) had poor therapeutic effects in a murine hindlimb ischemia model (22). Immunohistochemical analysis in the study showed that the number of microvessels in VEGF-A-administrated hindlimb increased five-fold in number compared to that in the control hindlimb (22). The results suggest that an increase of blood vessels by VEGF-A does not lead to a therapeutic effect, and that some other molecules may be required to induce functional blood vessels. We found that SeV-mediated FGF-2 gene therapy showed better therapeutic effects in the ischemia model. The number of microvessels in the FGF-2-administrated group was almost equivalent to that in the VEGF-A-administrated group (22). Furthermore, the newly formed blood vessels in the FGF-2-treated hindlimb looked well-organized with sufficient pericyte coverage compared to that treated with VEGF-A (22). Taken together, these findings suggest that the induction of newly formed blood vessels with structural maturation is critical for therapeutic angiogenesis, and that FGF-2 is a candidate to be an effective therapeutic agent.

4.2. The essential role of endogenous blood/lymph-angiogenesis-related factors in cytokine therapy mediated by FGF-2

We have revealed that FGF-2 gene transfer was accompanied by the upregulation of mRNAs of several endogenous blood/lymph-angiogenesis-related growth factors, such as VEGF-A, HGF, PDGF-A, PDGF-B and VEGF-C (11, 22, 23, 34). Notably, the blockade of either one of these molecules or VEGFR-3, the receptor for VEGF-C, by systemic administration of the respective neutralizing antibody diminished the FGF-2-mediated therapeutic effects either partially or completely (11, 22, 23, 34). The phenomena in the experiments provided important information. As described in section 4-1, high-dose administration of exogenous VEGF-A showed no therapeutic effect, and FGF-2-mediated upsurge of endogenous VEGF-A successfully led to therapeutic effect. These findings suggest that each of endogenous blood/lymph-angiogenesis-related factors including VEGF-A plays an essential role in the FGF-2-mediated cytokine therapy in a harmonized manner, and that the essential factor is not always available as a therapeutic agent.

4.3. Spatiotemporal coordination of blood/lymph-angiogenesis-related systems in cytokine therapy mediated by FGF-2

4.3.1. Temporal coordination

Ischemia-induced angiogenesis is vital to avoiding hypoxia-induced tissue damage, and this pathophysiological phenomenon is probably regulated by a harmonized interaction among angiogenic growth factors. Therefore, the investigation of ischemia-dependent temporal expression pattern of angiogenic growth factors would provide important information for understanding the finely harmonized systems among these factors. Our studies in a mouse hindlimb ischemia model demonstrated that the reduced blood flow just after ischemic surgery was gradually restored and reached a plateau by ten days after the surgery (Figure 3 upper graph) and that the expression levels of the mRNAs of representative endogenous pro-blood/lymph-angiogenic factors in the thigh muscles were upregulated compared to those in untreated controls (11, 22, 23, 34). The expression peaks of FGF-2, VEGF, HGF and PDGF-A occurred in the early phase (one day after ischemic surgery) of the blood flow recovery process, whereas the peaks of VEGF-C and PDGF-B occurred in the late phase (seven days after ischemic surgery) (11, 34). FGF-2, VEGF-A and HGF, which showed expression peaks in the early phase, are generally known to be angiogenic initiation factors, and can directly stimulate ECs and induce tube formation *in vitro*. In contrast, PDGF-BB, which showed an expression peak in the late phase, is well known to be an essential factor for vessel maturation (11, 40, 41).

Therefore, ischemia-induced angiogenesis is regulated by temporally different angiogenic signals, namely the initiation signals in the early phase and the maturation signals in the late phase. In our studies, SeV-mediated FGF-2 gene transfer to the ischemic hindlimb further upregulated these endogenous factors without disturbing the ischemia-dependent temporal expression pattern (11, 34) (Figure 3). These findings suggest that FGF-2 can augment the ischemia-induced vital angiogenic response, thereby inducing "functional" angiogenesis. As we will discuss later, spatial interactions between ECs and neMCs are critical in the mechanism underlying the temporally well-balanced upregulation of endogenous angiogenic factors by FGF-2.

4.3.2. Spatial coordination

Several blood/lymph-angiogenesis-related factors, including VEGF-A, HGF, PDGF-AA and VEGF-C, are more abundantly secreted from neMCs than from ECs, and the respective cognate receptors are expressed mainly in ECs. On the other hand, PDGF-BB is specifically secreted from ECs, and its cognate receptor PDGFR-beta is specifically expressed in neMCs. Considering the cell-type dominant/specific expression profiles of these blood/lymph-angiogenesis-related factors, ECs and neMCs spatially interact via paracrine systems (11, 21, 42). Our *in vitro* studies and other past studies revealed that FGF-2 was a direct stimulator of several angiogenesis-related factor genes such as VEGF-A, HGF, PDGF-A and VEGF-C in neMCs , and the effect of FGF-2 was mediated by p42/44 MAPK in our ischemic model (11, 23, 34, 43). Our studies also revealed that the FGF-2-depedent significant upregulation of VEGF-A and HGF in neMCs involved PDGF-AA and p70 S6 kinase (p70S6K) signaling pathways (23, 34). The mechanism was explained as follows; in neMCs, PDGF-AA induced by FGF-2 acted on PDGFR-alfa in an autocrine manner, and the activated PDGFR-alfa accelerated p70S6K signaling pathways to produce VEGF and HGF (23, 34) (Figure 3). These increased VEGF and HGF in neMCs then began to stimulate ECs, which mediated an essential spatial interaction between the two cell types.

In our investigation, FGF-2 did not stimulate the PDGF-B gene in ECs *in vitro*. However, PDGF-B mRNA in ECs was upregulated in response to FGF-2 gene transfer in a mouse hindlimb ischemia model *in vivo*, particularly in the late phase of ischemia (11). We found that the VEGF-C/VEGFR-3 system mediated the upregulation of PDGF-B mRNA in ECs (11). Upregulated VEGF-A by FGF-2 via the PDGF-AA/PDGFR-alfa autocrine system in neMCs in the early phase subsequently triggers a group of reactions in the late phase (Figure 3). VEGF-A-mediated VEGFR-2 activation enhances the PDGF-BB production in ECs. The secreted PDGF-BB from ECs then interacts with PDGFR-beta in neMCs and instigates production of VEGF-C via the PDGFR-beta-mediated signaling pathways. Increased VEGF-C in neMCs by the paracrine system further stimulates VEGFR-2 and VEGFR-3 in ECs, and synergistically activates the signaling pathways for the production of PDGF-BB. These spatial crosstalks between ECs and neMCs amplify the production of VEGF-C and PDGF-BB, which gradually replaced the temporal coordination of the PDGF-AA/PDGFR-alfa autocrine system in neMCs of the early phase and sustained FGF-2-mediated mature blood vessel formation in the late phase of ischemia (11) (Figure 3).

5. THE FEATURES OF ANGIOGENIC RESPONSE IN THE TUMOR MICROENVIRONMENT

5.1. Pro-blood/lymph-angiogenic factor-rich microenvironment in tumors

Tumor-associated blood/lymph-angiogenesis is stimulated by pro-blood/lymph-angiogenic factors secreted from tumor cells in a paracrine manner (Figure 1B). As described in section 3, the expression levels of these factors in tumor cells are regulated not only by hypoxic stimuli but also by autocrine- and paracrine- cytokine networks (Figure 2). In many cases, the high-level secretion of these factors, especially VEGF-A and VEGF-C, from tumor cells, stimulates host blood and lymphatic vessels in the tumor microenvironment. For example, a mouse tumor implantation model using a human oral SCC cell line SAS revealed that the levels of human VEGF-A and VEGF-C derived from SAS cells are considerably higher than those derived from host stromal cells in implanted tumors (25, 34). The paracrine/autocrine activation systems of tumor-cell-associated growth factor receptors are one of the mechanisms underlying the pro-blood/lymph-angiogenic growth factor-rich microenvironment in tumors.

A certain amount of pro-blood/lymph-angiogenic factors is spontaneously present in normal tissues without a blood/lymph-angiogenic reaction. For example, mRNAs and/or proteins of VEGF-A, VEGF-C, VEGF-D, HGF, PDGF-A (PDGF-AA), PDGF-B (PDGF-BB) and FGF-2 are detectable in non-ischemic thigh muscles (11, 22, 23, 34, 44). While it is not entirely clear why the existing pro-blood/lymph-angiogenic factors do not stimulate blood/lymph-angiogenesis in normal quiescent condition, the concentrations of these factors may be at levels below the stimulus threshold and contribute only to the maintenance of vascular cells. For the initiation of blood/lymph-angiogenesis, suprathreshold levels of these factors would be required. If this is the case, the tumor-cell-associated systems to upregulate pro-blood/lymph-angiogenic factors may be advantageous to tumors in the induction of blood/lymph-angiogenesis. It should be noted that tumor cells are able to enhance the expression levels of the key pro-blood/lymph-angiogenic factors VEGF-A and VEGF-C by the autocrine system in a context-independent manner (25, 35). Through these systems, the tumor establishes a pro-blood/lymph-angiogenic factor-rich microenvironment, and the enriched factors stimulate the pre-existing or newly formed vessels in a paracrine manner, resulting in aberrant and non-functional blood/lymph-angiogenesis.

5.2. The essential role of spatiotemporal coordination among host stromal cells during tumor-associated angiogenesis

As described in section 4, the spatiotemporal coordination of endogenous blood/lymph-angiogenesis-related factors among ECs and neMCs is critical for inducing "functional" angiogenesis in cytokine therapy for ischemic disorders (Figure 3), and each of the factors plays an essential role in FGF-2-mediated therapeutic effects. In the case of tumor-associated angiogenesis, the coordination among these host stromal cells also influences the function of newly formed blood vessels (34). It is known that rapamycin, which specifically inhibits p70S6K by reducing the activity of the mammalian target of rapamycin (mTOR), is an effective agent for cancer therapy (45, 46). In our study using a mouse tumor implantation model, we clarified one of the possible mechanisms underlying the suppressive effect of rapamycin on tumor progression (34). We demonstrated in the tumor implantation study that systemic and sustained administration of rapamycin reduced intratumoral blood flow (34). The mechanism was attributed to the blockade of the PDGF-AA/PDGFR-alfa autocrine system in neMCs. Rapamycin suppressed p70S6K and its downstream pathways mediating VEGF-A and HGF production in this cell type (23, 34). Although the PDGF-AA/PDGFR-alfa autocrine system also existed in tumor cells and upregulated VEGF-A as described in section 3, rapamycin did not show inhibitory effect on VEGF-A expression in tumor cells *in vitro*. The result suggests that the VEGF-A upregulation mediated by PDGF-AA/PDGFR-alfa autocrine-system may be independent of p70S6K signals in tumor cells (34). Taken together, rapamycin probably exerted the inhibitory effect on neMCs via antiangiogenic mechanism and not on tumor cells directly *in vivo*. The effect of rapamycin was seen in implanted tumors of several cancer cell lines with various expression profiles of angiogenic growth factors including a mouse hepatocellular carcinoma cell line MH134 with low-level of VEGF-A, and a human oral SCC cell line SAS with high-level of VEGF-A. These findings strongly suggest that the increases of VEGF-A and HGF in neMCs mediated by p70S6K are essential for promoting blood flow in tumor tissue irrespective of the expression level of tumor cell-derived VEGF-A, and that tumor-cell-derived VEGF-A cannot compensate for neMC-derived VEGF-A.

6. PERSPECTIVE

Many recent reports have demonstrated that tumor-associated blood vessels showed poor hierarchical vasculature with structural immaturity in many cases of human malignancies (47, 48). However, the reason why the newly formed blood vessels show such abnormalities in tumors has remained unclear. The features of the angiogenic response in the tumor microenvironment described in this review provide one of the pathological mechanisms underlying tumor-associated abnormal angiogenesis. Here, we re-emphasize that the blood/lymph-angiogenic response in tumors has several features in common with that in therapeutic angiogenesis. The tumor angiogenic response is caused by high-level pro-blood/lymph-angiogenic factors secreted from tumor cells. In a similar fashion, the angiogenic response in therapeutic angiogenesis is caused by high-dose administration of an exogenous pro-blood/lymph-angiogenic growth factor as a therapeutic agent. Based on our findings regarding effective therapeutic angiogenesis, the tumor microenvironment has two specific features: 1) VEGF-A, an ineffective growth factor in therapeutic angiogenesis, is often continually rich; and 2) FGF-2 and HGF (49), effective agents for therapeutic angiogenesis, are often poor (24). Although we did not discuss the detailed mechanism of the latter feature in this review, we investigated that the expression of these factors was absent or extremely low in a variety of tumor cell lines (24). Newly formed blood vessels in tumors, therefore, may be potentially aberrant and immature, as are those in VEGF-A gene therapy for ischemic organs. The tumor with the VEGF-A-rich and FGF-2/HGF-poor microenvironment would impair spatiotemporal coordination of blood/lymph-angiogenesis-related factors among the host stromal cells. Based on our understanding of the mechanism underlying "functional" angiogenesis (Figure 3), these aberrant vessels might make a minor contribution to "sufficient" blood flow to the tumor tissue.

Finally, we discuss the prospects for anticancer therapy based on pro-blood/lymph-angiogenic cascades in the tumor microenvironment. In order to establish an effective strategy for cancer therapy, tumor-associated blood/lymph-angiogenesis has been targeted in order to induce tumor dormancy or to suppress hematogenous/lymphogenous metastasis (4-6). However, considering the features of the tumor microenvironment, the effects of such anti-angiogenic therapy, especially of anti-blood angiogenic therapy, are thought to be slight. This is explained in part by the fact that human tumors actually continue growing under hypoxic and poorly nutritious conditions caused by insufficient blood flow. In addition, two critical issues related to anti-blood/lymph-angiogenic therapy must be considered: 1) blood/lymph-angiogenesis is not a tumor-specific biological phenomenon, and anti-blood/lymph-angiogenic therapy would therefore cause severe side effects, including unfavorable effects on the viability of pre-existing vessels; 2) it would be difficult to eradicate all of the tumor cells or alter them from active to dormant status even if complete suppression of the tumor-associated angiogenesis could be achieved, because certain populations of tumor cells are located at the periphery of the tumor tissue or invade the surrounding host tissue, thereby receiving oxygen and nutrients from pre-existing blood vessels. Recently, a new concept has been proposed in the research on cancer therapy targeting tumor angiogenesis, namely that tumor-associated blood vessels should be rather normalized (well-organized/functional) to increase the effects of chemotherapy, radiotherapy and immune response (47, 48, 50, 51). This counterintuitive idea may be worth mentioning for establishing more effective cancer therapy targeting tumor angiogenesis. Although the strategies for the normalization of abnormal vessels have not been established yet, blockade of VEGF-A may improve vessel normalization in tumor tissue (48). However, as we discussed in this review, we should keep in mind that VEGF-A derived from host stromal cells is an essential factor in tumor-associated angiogenesis. Delicate control of the VEGF-A level in the tumor microenvironment would be necessary to achieve vessel normalization, but it would seem to be clinically difficult to apply the strategy to malignant neoplasms with various characteristics, including the VEGF-A level. Based on our findings relevant to effective angiogenesis by FGF-2 administration in an ischemic model, therapeutic administration of FGF-2 into tumor tissues may be expected as a strategy for normalizing dysfunctional angiogenic vessels. We hope that our review on the blood/lymph-angiogenesis associated with solid tumors and ischemic conditions will be helpful in improving cancer therapy and further investigation of vascular biology in various diseases.

7. ACKNOWLEDGMENT

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