9 Reciprocal Relationship Between VE-Cadherin and Matrix Metalloproteinases Expression in Endothelial Cells and Its Implications to Angiogenesis

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Abstract

Angiogenesis, the process of new blood vessel formation from preexisting ones, is critical in the development and progression of tumor. Since metastasis is favored by increased neovascularization, understanding the molecular mechanism governing angiogenesis gains utmost importance. Endothelial cells respond to numerous angiogenic factors like VEGF and switch over to angiogenic phenotype. Apart from VEGF, another key molecule involved is matrix metalloproteinases which are the enzymes involved in pericellular proteolysis, a process critically important in initiating angiogenesis. But during the later stages, when cell–cell contact formation occurs, MMP expression is downregulated. Regulation of MMPs by cell–cell contact formation was found. This article focuses on a reciprocal relationship between the expression of cell adhesion molecules that modulates cell–cell contact formation and MMP expression.

Keywords

Matrix metalloproteinases • VE-cadherin • β-Catenin • Angiogenesis • Pericellular proteolysis • Cell adhesion molecules

9.1 Introduction

Angiogenesis, the formation of new blood vessels from preexisting ones, is one of the most pervasive and fundamentally essential biological processes required for the maintenance of

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functional and structural integrity of organism. Endothelial cells are the key players in angiogenesis. All tissues develop a vascular network that provides cells with nutrients and oxygen. The vascular network, once formed, is a stable system that regenerates slowly. Normal angiogenesis is an essential process during wound healing, fetal development, ovulation, as well as growth and development. When angiogenesis is deranged, pathological problems often follow. Angiogenesis-dependent diseases result when new blood vessels either grow excessively or insufficiently. Insufficient angiogenesis occurs in diseases such as coronary artery disease, stroke, and chronic wounds. In these conditions, blood vessel growth is less due to inadequate production of angiogenic growth factors; hence circulation is not properly restored, leading to the risk of tissue death. Excessive angiogenesis occurs when abnormal amounts of angiogenic growth factors are produced by the diseased cells which overwhelm the effects of natural angiogenesis inhibitors. Excessive angiogenesis occurs in diseases such as cancer (Charlesworth and Harres 2006), inflammation (Chade et al. 2004), atherosclerosis (Hermann et al. 2006; Lip and Blann 2004), diabetic blindness, age-related macular degeneration, rheumatoid arthritis, and psoriasis (Folkman 1997).

Angiogenesis plays a critical role in the development of cancer. Vascularization is not observed in solid tumors which are smaller than 1–2 mm^3. To metastasize, they need to be supplied by blood vessels that bring oxygen and nutrients and remove metabolic wastes. Beyond the critical volume of 2 mm^3, oxygen and nutrients have difficulty in diffusing to the cells in the center of the tumor, causing a state of cellular hypoxia, which is an important factor stimulating the production of proangiogenic molecules (Shweiki et al. 1992). It is the shifting of balance from the anti- to the proangiogenic factors, which causes the transition from the dormant to the angiogenic phase (Hanahan and Folkman 1996). New blood vessel development is an important process in tumor progression, which favors its transition from hyperplasia to neoplasia, i.e., the passage from a state of cellular multiplication to a state of uncontrolled proliferation. Neovascularization also influences the spreading of cancer cells throughout the entire body eventually leading to metastasis formation. The vascularization level of a solid tumor is an indicator of its metastatic potential.

For angiogenesis to proceed, first the diseased or injured tissues must produce and release angiogenic growth factors that diffuse into the nearby tissues. The angiogenic growth factors bind to specific receptors located on the endothelial cells of nearby preexisting blood vessels. Once they bind to their receptors, the endothelial cells become activated. Signals sent from the cell's surface to the nucleus trigger the production of new molecules including enzymes like MMPs, which degrade the basement membrane surrounding existing blood vessels that helps the endothelial cells to proliferate and migrate toward the diseased tissue (Folkman 1997; Risau 1997).

9.2 Role of Proteases in Angiogenesis

Three major groups of endoproteases participate in the processes that regulate angiogenesis, including the remodeling of the extracellular matrix, cell migration and invasion, and the liberation and modification of growth factors. They comprise the metalloproteinases, in particular the matrix metalloproteinases, the cathepsin cysteine proteases, and the serine proteases. The activities of these proteases are controlled by specific activation mechanisms and specific inhibitors, particularly tissue inhibitor of matrix metalloproteinases, cystatins, and inhibitors of serine proteases called serpins (Victor et al. 2006).

Of these endopeptidases, matrix metalloproteinases play a major role in angiogenesis. MMPs are a diverse family of enzymes capable of degrading various components of the extra cellular matrix. They require zinc for catalytic activity and are synthesized as inactive zymogens which have to be proteolytically cleaved to be active. Normally, MMPs are expressed only when and where needed for tissue remodeling. However, aberrant expression of various MMPs has been

correlated with pathological conditions, such as tumor cell invasion and metastasis, periodontitis, and rheumatoid arthritis (Woessner 1991). Matrix metalloproteinase can be classified into two groups: as soluble MMPs and membrane-type MMPs. The soluble MMPs consist of collagenases (MMP 1, MMP 8, and MMP 13), gelatinases (MMP 2 and MMP 9), stromelysins (MMP 3, MMP 10 and MMP 11), matrilysins (MMP 7 and MMP 25), and MMP 12 and MMP 26 (Visse and Nagase 2003). The membrane-type MMPs encompass 6 members, MT1-MMP to MT6-MMP, that are activated intracellularly by furin-like enzymes. The MT-MMP family members were first identified as activators of soluble MMPs and subsequently also shown to be able to degrade extracellular matrix components such as fibrillar collagen, laminin-1 and laminin-5, aggrecan and fibronectin, and the plasma-derived matrix proteins vitronectin and fibrin (Visse and Nagase 2003; Seiki et al. 2003). The MMPs are inhibited by their endogenous inhibitors, the TIMPs (TIMP 1, 2, 3, 4), each capable of inhibiting virtually all members of the MMP family (Henriet et al. 1999). The equilibrium between TIMPs and MMPs is important in localized proteolysis.

MMPs not only degrade basement membrane and other ECM components allowing endothelial cells to detach and migrate into the tissues but also release ECM-bound proangiogenic factors like bFGF, VEGF, and TGF β. They also trigger integrin intracellular signaling by degrading ECM components, thereby generating fragments with integrin binding site. However, MMP 2 also generates endogenous angiogenic inhibitors from larger precursors. Cleavage of plasminogen by MMPs releases angiostatin; endostatin is the COOH terminal fragment of the basement membrane collagen XVIII, which can be generated by cleavage by cathepsins and MMPs; and generation of the hemopexin domain of MMP-2 from MMP-2 may be through autocatalysis. Thus, the MMPs have both pro- and antiangiogenic functions. On the whole, however, MMPs are required for angiogenesis, and inhibitors of MMPs have been shown to inhibit angiogenesis in animal models (Naglich et al. 2001).

Reports suggest that cultures of endothelial cells, which are used as model cell systems to study angiogenesis, expressed MMP 1, 2, 3, 9, and 14 and TIMP 1, 2 (Moses 1997). The requirement of MMPs produced by endothelial cells for angiogenesis is evidenced by the studies where naturally occurring (Moses et al. 1990; Fisher et al. 1994; Schnaper et al. 1993; Anand-Apte et al. 1997) or synthetic inhibitors (Montesano and Orci 1985; Hass et al. 1998; Maekawa et al. 1999) of MMPs caused inhibition of angiogenesis or various events thereof. Additional evidence for the requirement of MMPs during angiogenesis comes from genetic studies in mice. With regard to the MMP knockout mice deficient in MMP-2, tumor-induced angiogenesis was markedly reduced in dorsal sac assay with B16-BL6 melanoma cells, and in MMP-9-deficient mice, there was reduced bone growth plate angiogenesis (Itoh et al. 1998; Vu et al. 1998).

Recent reports suggest a temporal relationship between MMP production and angiogenic process in HUVEC in culture. When endothelial cells establish cell–cell contact formation resembling angiogenic process, downregulation in the production of MMP-2 and MMP-9 occurs, which suggests that MMPs are produced during initial stages of angiogenesis before cell–cell contacts are established (Kiran et al. 2006). Agents that promote angiogenesis such as curcumin (in serum free conditions) induces capillary network-like structure formation and cell–cell contact formation at a faster rate (within 48 h for curcumin) compared to control cells. We observed an inverse relation between cell–cell contact formation and MMP expression. There are increasing number of reports demonstrating that, apart from requirement of MMPs in proteolysis of extracellular matrix, endothelial cell requires MMPs for cell movement, proliferation, migration, and attachment to one another as well as to the ECM (Gingras et al. 2001; Qian et al. 1997; Yu and Stamenkovic 1999). Further, MMPs have been reported to alter with change in endothelial cell shape where maximum activity was reported when the cells were spherical in shape (Yan et al. 2000).

9.3 Cell Adhesion Molecules and Angiogenesis

Apart from MMPs, cell adhesion molecules are also important regulators of angiogenesis. They give endothelium the ability to control the passage of solute and circulating cells by forming intercellular junction between endothelial cells (Simionescu and Simionescu 1991). They also provide endothelial surface polarity and regulate initiation and maturation of newly formed vessels during angiogenesis (Muller and Gimbrone 1986). During the initiation phase of angiogenesis, the continuity of endothelial layers is interrupted due to loosening of cell–cell contacts enabling the endothelial cells to proliferate and migrate to free area (Schwartz et al. 1978; Sholley et al. 1977). But during the maturation phase, the endothelial cells establish the intercellular contacts in order to maintain the morphological integrity and quiescence of newly formed vessel (Yang et al. 1999; Lampugnani et al. 1992). Hence, the molecular mechanism that governs the formation and stabilization of cell–cell contacts and pericellular proteolysis must be suitably coordinated and regulated.

Cell adhesion molecules can be classified into four families depending on their biochemical and structural characteristics. These include the cadherins, selectins, immunoglobulin supergene family, and the integrins. Members of each family are implicated in neovascularization. Endothelial cells express several distinct types of integrins, allowing attachment to a wide variety of ECM proteins (Eliceiri and Cheresh 1999). It is reported that αvβ3 binds to MMP 2, thereby localizing MMP-2-mediated matrix degradation to the endothelial cell surface (Brooks et al. 1996). It is nearly undetectable on quiescent endothelium but is highly upregulated during cytokine or tumor-induced angiogenesis. Besides integrins, members of immunoglobulin superfamily also mediate heterophilic cell–cell adhesion. ICAM-1 and VCAM-1 are expressed on quiescent endothelium but are upregulated after stimulation with TNF-α, IL-1, or interferon-α (Brooks et al. 1996). VCAM-1 can induce chemotaxis in endothelial cells in vitro and angiogenesis in vivo. Members of the selectin family, in particular P-selectin and E-selectin, promote adhesion of leukocytes to cytokine-activated vascular endothelium, which plays a major role in angiogenesis (Koch et al. 1995). E-selectin induces endothelial migration and tube formation in vitro and angiogenesis in vivo (Nguyen et al. 1993).

Recent reports indicate that the components of the intercellular adherence junctions also function in intracellular signaling during angiogenesis (Bazzoni and Dejana 2004). Cadherin is the major endothelial specific cell adhesion molecule that plays important role in vascular morphogenesis and growth control (Lampugnani and Dejana 1997). They establish direct molecular connections with cytoplasmic partners that bind to different and specific domains of their cytoplasmic region. Classical cytoplasmic partners of cadherins are the catenins, namely, α, β, and p120 catenin (Anastasiadis and Reynolds 2000), which, besides promoting anchorage to actin cytoskeleton, when released into the cytoplasm, may translocate to the nucleus and influence gene transcription (Ben-Ze'ev and Geiger 1998). Moreover, cadherins associate to growth factor receptors (Carmeliet et al. 1999; Pece and Gutkind 2000) and some components of their signaling cascade such as Shc (Xu et al. 1997) phosphatidylinositol 3-kinase (Carmeliet et al. 1999; Pece et al. 1999) and various protein phosphatases (Zondag et al. 2000). These types of interactions may play a role in controlling growth factor signaling. VE-cadherin is exclusively expressed in the endothelium and regulates fundamental activities of this tissue (Dejana et al. 1999). Inactivation of VE-cadherin expression by gene targeting results in early embryonic lethality due to impairment of vascular organization and remodeling (Carmeliet et al. 1999). Antibodies blocking VE-cadherin adhesion and clustering strongly affect formation of new vascular structures in adult animals (Liao et al. 2000) and increase permeability of constitutive vessels (Corada et al. 1999).

We examined the molecular mechanisms involved in cell–cell contact-dependent regulation of MMPs in endothelial cells undergoing angiogenic process using HUVECs in culture and the results showed a reciprocal change in the expression of MMPs (MMP 2 and MMP 9) and VE-cadherin as the cells undergo angiogenic transition. A significant decrease in the production and secretion of MMP 2 and MMP 9 was seen with the progression of culture, when grouping of cells and tubular network-like structure developed. Development of extensive tubular network-like structure involves cell–cell contact formation, which correlated with the expression of endothelial cell markers like CD 31, ICAM 1, and E-selectin, which mediates cell adhesion, and VEGF and FGF, which are the biochemical markers of angiogenesis. Thus, a reciprocal relationship between the expression of markers of angiogenesis, cell adhesion molecules, and MMPs was evident. Further proof for the relationship between MMP 2, MMP 9, and angiogenesis came from the results of the experiments where endothelial cells were treated with curcumin and ursolic acid. Angiogenic effect of ursolic acid was shown by upregulation of the expression of angiogenic markers ICAM-1 and angiogenic factors like VEGF and fibroblast growth factor-2 by endothelial cells (Kiran et al. 2008a). Opposing effects of curcumin on angiogenesis was shown using different model systems and the proangiogenic effect was mediated through VEGF and PI3K-Akt pathway (Kiran et al. 2008b). Treatment of endothelial cells with substances which promote angiogenesis caused downregulation of MMP 2 and MMP 9. But such downregulation was not produced by aspirin which inhibited angiogenesis and reduced the production of cell adhesion molecules that promote cell–cell contact formation (Kiran et al. 2006).

During the initial stages when cells remained mostly as individual ones, there was more MMP-2 and MMP-9 and less VE-cadherin, and at later stages, when grouping of cells and network-like structures developed, increase in VE-cadherin and decrease in MMP-2 and MMP-9 production were observed. In serum-free conditions, curcumin accelerated angiogenic phenotype, which caused a significant upregulation of VE-cadherin and downregulation of MMPs. Further investigations on the signaling pathway downstream to VE-cadherin suggested the involvement of β catenin. During the initial stages of the culture, β-catenin was less in the cytosol, but there was a rapid translocation of β-catenin to the cytosol during the later stages, when cell–cell contacts were established. The activity of β-catenin is regulated by its phosphorylation status. We observed an increased tyrosine phosphorylation, when catenin remained in the nucleus during the initial stages, and relative increase in serine phosphorylation during the later stages of the culture, when cell–cell contact formation occurred and β-catenin was translocated to the cytosol. A correlation between nuclear localization of β-catenin and MMPs was observed, i.e., when β-catenin remained in the nucleus, there was an upregulation in the production of MMPs, and when β-catenin was translocated to cytosol, MMP expression was downregulated. Agents which cause inhibition of angiogenesis (curcumin in presence of FCS) affect the downregulation of MMPs as well as the phosphorylation of beta catenin. Such downregulation of MMP was not seen in cells treated with lithium chloride and SB 216763, which caused increased nuclear localization of β-catenin. The downregulation of MMP 2 and MMP 9 caused by curcumin was also reversed by lithium (Kiran et al. 2011). Lithium and SB 216763 inhibits GSK 3β, which is involved in the serine phosphorylation of β-catenin. Since serine phosphorylation was inhibited, catenin remained in the nucleus which correlated with the upregulation of MMPs and decreased cell–cell contact formation. MMP genes are under the influence of the transcriptional activity of β-catenin as β-catenin responsive elements are reported to be present in the MMP genes (Munshi and Stack 2006; Overall and Lopez-Otin 2002). We observed the downregulation of MMPs when the level of VE-cadherin increased and β-catenin remained in the nucleus. This suggests the possibility that VE-cadherin modulates MMP expression through β-catenin-dependent mechanism.

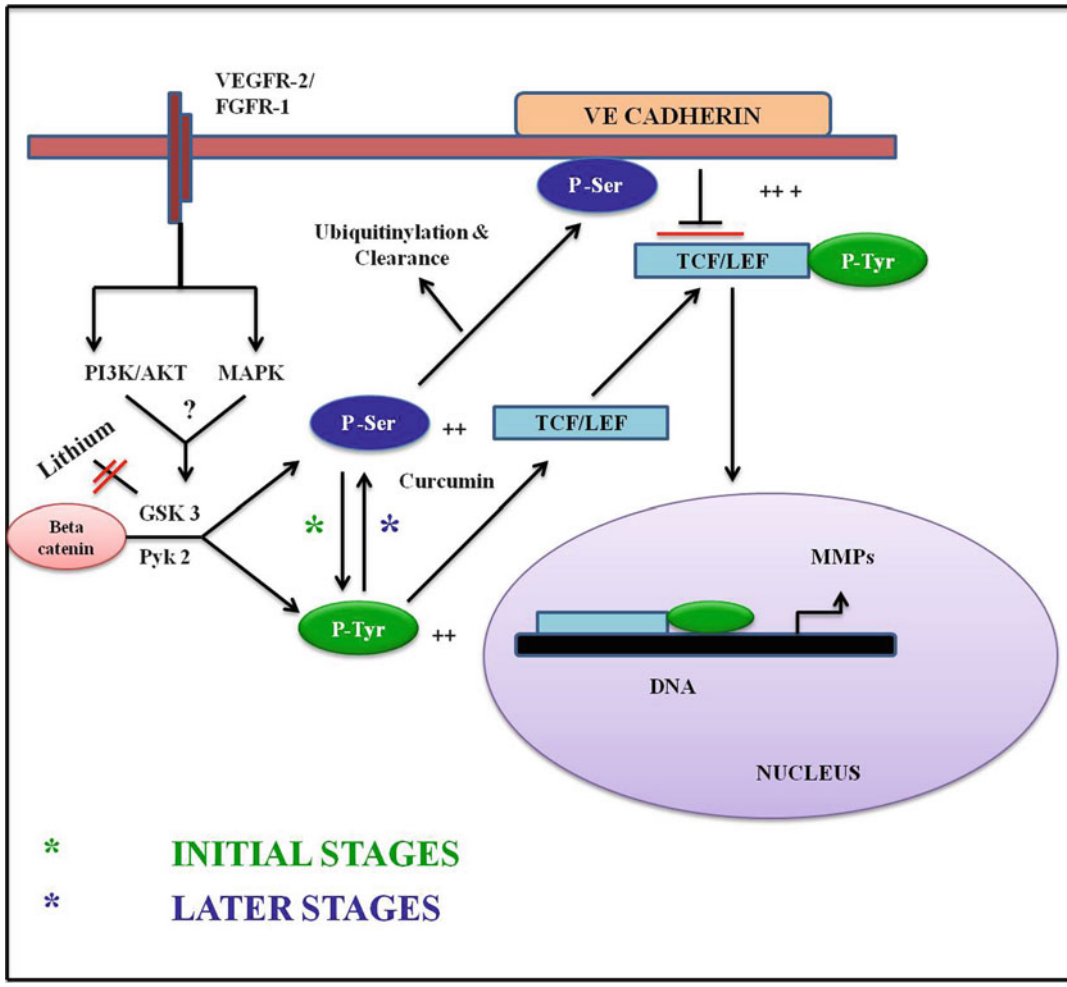

Fig. 9.1 *Scheme showing VE-cadherin–β-catenin signaling in the regulation of MMPs*. The transcriptional activity of β-catenin is regulated by its phosphorylation status. Serine phosphorylation increases its affinity to VE-cadherin and prevents its nuclear translocation. In the absence of VE-cadherin Ser-phosphorylated β-catenin is cleared from the cytosol by ubiquitinylation. Tyrosine phosphorylation reduces its affinity to VE-cadherin, and it binds with Tcf/Lef protein and translocates into the nucleus and regulates gene expression. Higher levels of VE-cadherin inhibit the translocation of Tyr-phosphorylated β-catenin. The role of PI3K-AKT and MAPK pathways (as was observed to be involved in mediating the angiogenesis) in regulating the activities of GSK-3 and Pyk2 is unknown. We observed higher levels Tyr-phosphorylated β-catenin during the initial 48 h when the level of VE-cadherin was significantly low and concomitantly MMP levels were high. During the later stages, high levels of Ser-phosphorylated β-catenin was observed when the levels of VE-cadherin increased and a significantly low level of MMPs

9.4 Conclusion

Our results suggested a reciprocal relationship between VE-cadherin and MMP production. The production and secretion of MMP-2 and MMP-9 was high in the initial stages of HUVECs in culture, when they remained as individual cells, which was associated with the decreased expression of VE-cadherin and increased nuclear localization of β-catenin. But during the later stages, when cell–cell contact formation was established, there was a downregulation in the production of MMPs, which was associated with increased

expression of VE-cadherin and decreased nuclear localization of β-catenin. These results suggest that VE-cadherin may mediate the expression of MMP production through a β-catenin-dependent mechanism (Fig. 9.1).

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