Angiogenesis is the formation of new blood vessels from existing endothelial cell–lined vessels. It occurs during normal development, and in many pathological conditions including tumor growth, wound healing, inflammation, ischemia, and atherosclerosis. The development of new vessels can occur by abluminal sprouting of endothelial cells to form new branches or by the longitudinal splitting of existing vessels in a process termed “intussusception.” The activation phase of angiogenesis begins with increased vascular permeability leading to extravasation of fibrin, degradation of the endothelial cell basement membrane, and migration and proliferation of endothelial cells to form new vascular channels. The resolution phase of angiogenesis involves the cessation of endothelial cell proliferation and migration, synthesis of new basement membrane, junctional complex maturation, and recruitment and differentiation of pericytes or smooth muscle cells. It is increasingly recognized that the structure and composition of the extracellular matrix in the microenvironment surrounding the cells plays an important role regulating the process of angiogenesis.

Matrix metalloproteinases (MMPs) are a family of enzymes that have in common the ability to degrade many molecules of the extracellular matrix (>25 MMPs have been identified). MMP activity can be inhibited by endogenous tissue inhibitors of metalloproteinases (TIMPs), and the net proteolytic activity within a tissue is a function of the balance of MMPs/TIMPs. Numerous studies have shown that various MMPs and TIMPs are expressed by endothelial cells during angiogenesis. Furthermore, experiments using broad-spectrum synthetic or natural inhibitors of the MMPs have shown that blocking MMPs can inhibit angiogenesis; however, the role of any given MMP remains uncertain, since these inhibitors target several different MMPs.1

Studies using knockout mice provide evidence for the importance of individual MMPs and clues about the mechanisms by which the MMPs influence angiogenesis. MMP-2, MMP-9, and the MT1-MMP have all been implicated in angiogenesis.2–5 In particular, there is very interesting data emerging on the role of MMP-9 in angiogenesis. MMP-9 is known to degrade gelatins and basement membrane collagens; therefore, at least in part, its function is to clear the matrix surrounding endothelial cells allowing for proliferation and migration. However, MMP-9 may also act via indirect mechanisms. It cleaves type IV collagen in the basement membrane, leading to exposure of a cryptic regulatory sequence that can stimulate endothelial cell growth and migration.5,7 Another recent study suggests that MMP-9 triggers an angiogenic switch for tumor progression by releasing matrix-bound vascular endothelial growth factor (VEGF), making it available to interact with vascular endothelial growth factor receptor (VEGFR) and activate angiogenesis in pancreatic tumors.8 MMP-9 may also influence angiogenesis through the release of circulating endothelial precursor stem cells (CEPs) from the bone marrow, since it is required for cleavage and release of soluble Kit ligand, a factor that regulates stem cell release and differentiation.9 Thus, the list of mechanisms for MMP-9 contributing to angiogenesis is expanding.

In an article published in this issue of Circulation Research, Johnson et al10 have used the MMP-9–null mouse to show that MMP-9 mediates angiogenesis in ischemic muscle. They ligated the femoral arteries and veins of wild-type and MMP-9–null mice as a model for peripheral occlusive vascular disease. This procedure creates ischemia in the upper hindlimb muscle beds and results in the formation of new capillaries to revascularize the tissue. In wild-type mice, both MMP-2 and MMP-9 expression and activity were rapidly and dramatically increased after femoral artery ligation. Capillary density in the muscle was measured by infusing a fluorescent-labeled lectin that binds to endothelial cells, and they found that capillary density was doubled in wild-type but not MMP-9–null mice. Fluorescent microspheres were infused into the vasculature postmortem to visualize open capillary structures and to quantitate branchpoints in the microvascular network. The authors noted decreased capillary branching in the MMP-9–null mice. Transgenic mice expressing LacZ under the control of the MMP-9 promoter were used to demonstrate MMP-9 promoter activity in cells localized perivascularly at capillary branchpoints. Surprisingly, immunostaining revealed that the MMP-9–expressing cells at the branchpoints were macrophages. Finally, a bone marrow transplant from wild-type to MMP-9–null mice was performed before femoral ligation, and MMP-9–producing donor macrophages were identified in the ischemic tissue. Furthermore, the angiogenic response was rescued in the transplanted animals, since capillary density and branching were restored to wild-type values.

These data support a novel paradigm for the role of MMP-9 in angiogenesis: that protease production by perivascular...
cular cells can influence the development of vascular channels. The present results parallel the findings in studies of tumor angiogenesis, where MMP-9 produced by perivascular neutrophils and mast cells was shown to contribute to angiogenesis in squamous cell and pancreatic carcinomas.\textsuperscript{3,5,11,12} In the present study, transplantation of bone marrow from wild-type mice to MMP-9\textsuperscript{−/−} null mice partially restored the invasion of macrophages into the ischemic tissues, and enhanced neovascularization, which suggests that bone marrow--derived cells contributed to angiogenesis. However, the identity of the MMP-9--expressing perivascular cells may be questioned, since bone marrow--derived cells may differentiate along different lineages, including hematopoietic, endothelial, and smooth muscle. Although some cells stained positive for the leukocyte antigen CD45.1, immunohistochemistry for the CEP marker Flk-1 was inconclusive, since the antibody did not efficiently stain any cells. It is quite possible that at least some of the MMP-9--expressing cells in the new vessels originated from CEPs, especially since MMP-9 mobilizes CEPs from the bone marrow\textsuperscript{9} and it is well established that CEPs contribute to neovascularization in ischemic muscle beds.\textsuperscript{13}

Another caveat of these studies is that the mechanism by which MMP-9 acts locally in the ischemic tissue to promote angiogenesis was not determined. The authors indicate that MMP-9--producing perivascular cells tended to be localized at branchpoints of the newly formed vessels and therefore proposed a role for macrophages mediating branching angiogenesis. A previous study suggested that macrophages may tunnel through the matrix clearing a path for endothelial or CEPs to follow.\textsuperscript{14} This might be a mechanism by which macrophages conduct endothelial morphogenesis. However, the observation of macrophages at capillary branchpoints is merely a correlation, and further studies will be necessary to confirm a role for macrophages in the process of branching of the capillary tubes.

Moreover, the molecular mechanisms of the MMP-9 effect are not clear. There were no differences in tissue VEGF levels between wild-type and MMP-9--knockout mice, so MMP-9 was not acting through release of VEGF from the extracellular matrix. Other possible mechanisms include mediating the release of other growth factors (transforming growth factor-β, fibroblast growth factor-2), clearing fibrin deposits or interstitial matrix to facilitate endothelial cell migration, clearing cadherins or integrins to release endothelial cells from attachments, or uncovering cryptic proangiogenic matrix fragments.

Johnson et al.\textsuperscript{10} observed an early and dramatic increase in MMP-2 activity after ischemia; in fact, the amount of active MMP-2 seemed to far exceed the amount of active MMP-9. The potential importance of this MMP-2 upregulation was not addressed in these studies but is of interest given previous research demonstrating an important role for MMP-2 mediating angiogenesis.\textsuperscript{3,5} Moreover, it is puzzling that very little active MMP-9 was obtained from the tissues. Although the attenuation of angiogenesis in the MMP-9\textsuperscript{−/−} mouse provides evidence for the importance of this proteinase, knockout of this gene may have resulted in the misregulation of other functionally similar enzymes. Thus, confirmation of the role of MMP-9 using specific inhibitors or targeted RNA inhibitors to knock down expression in a specific and controlled fashion is necessary.

In conclusion, the study by Johnson et al.\textsuperscript{10} demonstrates a role for MMP-9 in mediating the progression of angiogenesis in the ischemic hindlimb and suggests that macrophages regulate neovessel branching. Future research will determine whether this paradigm of macrophage conduction of endothelial cells is important in other forms of pathological angiogenesis.

References


Key Words: matrix metalloproteinases, angiogenesis, macrophages, extracellular matrix, vascular biology
Macrophage Matrix Metalloproteinase-9 Regulates Angiogenesis in Ischemic Muscle
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Circ Res. 2004;94:138-139
doi: 10.1161/01.RES.0000117525.23089.1A
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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