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Nitric oxide-induced collagen IV expression and angiogenesis: FAK or fiction? Focus on "Collagen IV contributes to nitric oxide-induced angiogenesis of lung endothelial cells"

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TYPE IV COLLAGEN IS A MAJOR basement membrane component of blood vessels and plays a key role in the regulation of endothelial cell adhesion, migration, and angiogenesis. Assembly of the type IV collagen network dictates the organization of the vascular basement membrane, which is required for endothelial cell adhesion to the extracellular matrix via interactions with cell surface $\alpha_1\beta_1$- and $\alpha_v\beta_3$-integrins (10). Collagen IV peptides generated by proteolytic cleavage can be either proangiogenic (HepI, HepIII) (10) or antiangiogenic (tumstatin). Thus collagen cleavage is tightly regulated during angiogenesis to promote endothelial sprouting and lumen formation during the early stages and vessel maturation in the later stages (see Ref. 6 for a recent review).

Given the critical role of collagen IV in angiogenesis, surprisingly little is known about the molecular mechanisms regulating its synthesis. Low-density lipoproteins increase pathologic collagen IV expression by human aortic endothelial cells by a transforming growth factor-β (TGF-β) and MAP kinase-dependent mechanism (15). High shear stress also upregulates type IV collagen synthesis in cultured human umbilical vein endothelial cells (HUVECs; 18), and cAMP-protein kinase A (PKA) promotes collagen IV deposition by cultured rat adrenal medullary endothelial cells (11).

In this issue, Wang and Su (17) demonstrate a novel regulation of collagen IV synthesis and secretion by nitric oxide (NO) in porcine pulmonary artery endothelial cells and aortic endothelial cells. Interestingly, NO concomitantly increased the expression of $\alpha_v\beta_3$, a major collagen IV receptor. This coordinated response results in increased focal adhesion kinase (FAK) phosphorylation and alterations in cell adhesion, migration, and proliferation. Further evidence to link the coordinated regulation of integrin and collagen IV expression comes from the finding that NO stimulated endothelial monolayer wound repair, proliferation, and tube formation that was inhibited by collagen IV small interfering RNA (siRNA) or a neutralizing antibody against $\alpha_v\beta_3$-integrin (17). NO regulation of collagen IV expression was mediated by the cGMP-protein kinase G (PKG) pathway since both the PKG inhibitor KT5823 and PKG siRNA decreased NO-induced changes in collagen IV protein and mRNA expression as well as endothelial angiogenesis.

NO has pleiotrophic effects on endothelial cell function, including cell growth regulation, proliferation, apoptosis, and migration. The effects of NO on endothelial gene expression are largely driven by PKG, although an NO-dependent, PKG-independent regulation can also occur under shear stress conditions (2). Koomans' group, using cDNA microarrays in HUVECs, also reported that NO donors predominantly reduced the expression of genes that were associated with cell growth, adhesion, and structure through PKG-dependent mechanisms (3). These changes in gene expression were associated with decreased levels of the egr-1, MSX-1, and v-Rel transcription factors. PKG-dependent regulation of the antioxidant enzymes catalase and glutathione peroxidase is also evident in lung microvascular endothelial cells (16). Finally, NO-cGMP-PKG disrupts TGF-β-Smad2 signaling by promoting the proteasomal degradation of activated Smad (14).

Collagen IV interacts with both $\alpha_1\beta_1$- and $\alpha_v\beta_3$-integrins; unlike traditional extracellular matrix binding, which is mediated by specific Arg-Gly-Asp (RGD) sequences, binding of collagen IV to integrins is RGD independent and relies predominantly on the triple helical structure (6). Interestingly, during angiogenesis, cleavage of collagen IV by matrix metalloproteinase (MMP)-9 exposes a cryptic binding site that favors a loss of binding to $\alpha_1\beta_1$ and increased binding to $\alpha_v\beta_3$, which may limit angiogenesis (5). Low levels of NO are capable of increasing MMP-9 activity indirectly by reducing the expression of its tissue inhibitor TIMP1 (13). Upon integrin engagement, FAK, a cytoplasmic tyrosine kinase that localizes to focal adhesion sites, becomes phosphorylated and activated to transduce the extracellular to intracellular ("outside-in") signaling cascade (4). There is also evidence that a synergistic relationship exists between integrins and growth factor receptors. In a study by Masson-Gadais et al. (9), HUVEC migration was dependent on $\alpha_v\beta_3$-integrin-vascular endothelial growth factor (VEGF) receptor cross talk to potentiate FAK phosphorylation. A subsequent study by Mahabeleshwar et al. (8) demonstrated that activation of the β_3-cytoplasmic domain is critical for cross-activation of the VEGFR2 receptor.

From the above discussion, it is clear that the present study by Wang and Su (17) has begun to dissect the role of the NO-cGMP-PKG and collagen IV-integrin-FAK pathways in endothelial cell regulation. This study and others (7) suggest that NO-cGMP-PKG initiates a proangiogenic phenotype that positively regulates collagen IV synthesis, $\alpha_1\beta_1$- and $\alpha_v\beta_3$-integrin-FAK activation, and angiogenesis (Fig. 1). Overall, these findings along with previous reports that NO potentiates VEGF (12) and angiopoetin (1) suggest two possible scenarios for NO-dependent angiogenesis: *1*) protective: during disease progression and wound healing, tight regulation must be maintained over the expansion or suppression of collagen IV to promote controlled angiogenesis; and *2*) detrimental: if coordination between collagen IV synthesis/proteolytic processing becomes dissociated from the regulation of integrin-binding, VEGF signaling, aberrant angiogenesis could occur. Further elucidation of the molecular mechanisms involved in NO-dependent regulation of collagen IV expression and cross talk

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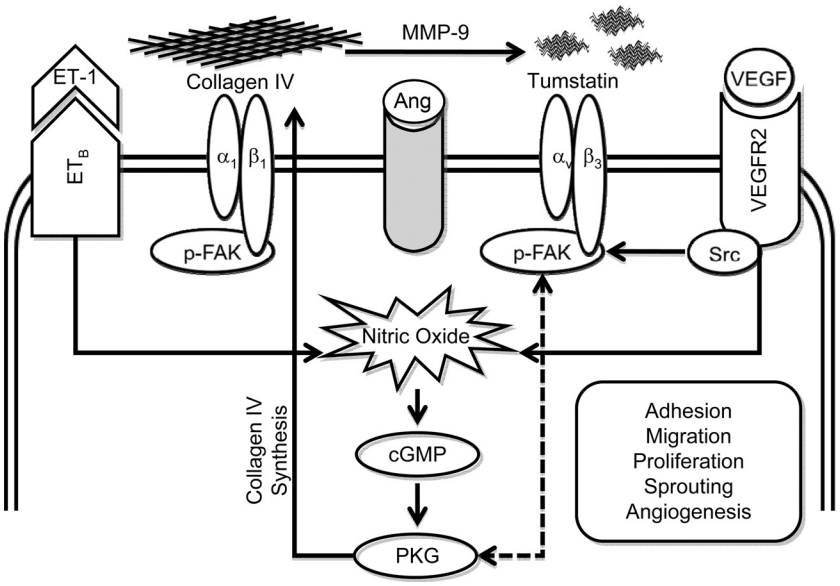

Fig. 1. A central role for nitric oxide (NO) in angiogenesis. NO production via endothelial NO synthase (eNOS) is regulated by endothelin (ET-1) interactions with the ET_B receptor, VEGF/VEGF receptor (VEGFR2), as well as shear stress (not shown). NO-cGMP regulates the expression of collagen IV, matrix metalloproteinase (MMP)-9, and $\alpha_1\beta_1$ and $\alpha_v\beta_3$ and modulates VEGF, integrin, and angiopoietin (Ang) signaling. Collagen IV interacts with $\alpha_1\beta_1$; MMP-9 cleavage exposes an $\alpha_v\beta_3$ cryptic binding site (tumstatin) that may limit angiogenesis. p-FAK, phosphorylated focal adhesion kinase.

between the NO-cGMP-PKG, collagen IV-integrin-FAK, and VEGF signaling cascades is necessary to understand how NO regulates both physiological and pathophysiological angiogenesis.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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