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Perspective



# Perspectives Series: Cell Adhesion in Vascular Biology

## The Extracellular Matrix as a Cell Cycle Control Element in Atherosclerosis and Restenosis

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*Introduction*

The process of atherosclerosis is thought to be initiated by injury to the arterial wall, followed by a reparative response by the vascular smooth muscle cells (vSMCs).[1] This response consists of migration and proliferation of vSMCs within the arterial intima, leading to the great intimal expansion seen in atherosclerotic plaques. One of the mainstays of current therapy for patients who have coronary artery obstruction with myocardial ischemia is balloon angioplasty, which often leads to a temporary relief, followed by a restenosis of the vessel. To address this problem, a number of animal models have been developed in which an intimal smooth muscle lesion is developed after injury from a balloon catheter. These models show that a large number of vSMCs proliferate in the first 72 h after injury (1). This proliferative state is concomitant with the phenotypic modulation of the vSMCs from a contractile to a synthetic phenotype (2). In addition to cell growth, this phenotypic modulation leads to an increased production of a number of extracellular matrix (ECM) molecules (2), which may explain the increased collagen deposition seen in advanced intimal lesions, particularly in coronary artery plaques.

While a clear pathogenic role for smooth muscle proliferation in atherosclerotic plaque development has yet to be shown, some studies have used injury models to show that antiproliferative therapies can be effective in preventing the development of smooth muscle intimal lesions (1, 3). In particular, infection of the aorta with adenoviruses encoding a constitutively active mutant of the retinoblastoma protein (pRb) leads to a dramatic prevention of the smooth muscle intimal lesion after balloon injury (3). These results demonstrate that smooth muscle proliferation, as in most cell types, is controlled by phosphorylation of pRb. Since recent work has also demonstrated that pRb phosphorylation is jointly regulated by growth factors and the extracellular matrix (see references 5 and 6), we have considered the idea of the ECM as a cell cycle control element, regulating pRb function during normal and abnormal smooth muscle cell proliferation.

*Regulation of pRb function*

pRb is a critical regulator of cell cycle progression, and pRb function is controlled by reversible phosphorylation (4). In quiescent cells (G0) and in early G1, pRb is found in a hypophosphorylated state, and this form of the protein binds to several growth regulatory factors among which the E2F family of transcription factors is the best studied. E2F–pRb complexes repress the transcription of E2F-regulated genes, several of which are involved in cell cycle progression. The hyperphosphorylation of pRb allows for the release of E2F and loss of E2F/pRb repression. Hypophosphorylated pRb also binds to other proteins, e.g., the c-abl protein, and the bound c-abl is inactive as a kinase.

pRb is phosphorylated by the G1 phase cyclin-dependent kinases (cdks) (4). These enzymes are typically expressed constitutively, but they are inactive in the absence of their cyclin partners. There are several cdks that mediate distinct transitions in the cell cycle; the cdks thought to phosphorylate pRb are cdk4 (or its homologue cdk6) in combination with the D-type cyclins (D1, D2, or D3) and cdk2 in combination with cyclin E. It now seems that mitogenic growth factors and the ECM are required for cell proliferation because they allow for G1 phase induction of cyclin D–cdk4/6 and cyclin E–cdk2 kinase activities. Growth factors and the ECM are both required to induce the expression of cyclin D1 mRNA in normal human fibroblasts, and the matrix is further required for translation of the mRNA (5, 6). The ECM-dependent expression of cyclin D1 is causal for cell cycle progression because forced expression of cyclin D1 in suspended cells rescues pRb phosphorylation and entry into S phase (6, 7).

In contrast to the behavior of cyclin D1, cyclin E levels and the number of cyclin E–cdk2 complexes are not strongly induced during G1 phase. However, cyclin E–cdk2 activity is strongly induced in late G1 phase, and this induction seems to result from the fact that growth factors and the ECM are controlling the p21 family of cdk inhibitors (8). The p21-like cdk inhibitors (p21, p27, and p57) can bind to and inhibit cyclin E–cdk2. Three recent studies show that growth factors and the

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1. *Abbreviations used in this paper:* cdk, cyclin-dependent kinase; ECM, extracellular matrix; pRb, retinoblastoma protein; vSMC, vascular smooth muscle cell.

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ECM cooperate to regulate the steady state levels of both p21 and p27 and thereby allow for proper activation of cyclin E–cdk2 in G1 phase (6, 7, 9). Although most of the studies to date have been performed in fibroblasts and fibroblastic cell lines, we find that pRb phosphorylation is also adhesion dependent in aortic smooth muscle cells (Zhu, X., and R.K. Assoian, unpublished data).

The deregulated proliferation of vSMCs in atherogenesis and restenosis, and the inhibition by dominant negative RB genes, raises the tempting idea that the ECM surrounding vSMCs is working as a partner with local mitogenic growth factors to allow for pRb phosphorylation and cell cycle progression. Selective inhibition of the ECM effect, presumably by blocking the integrin signaling cascade that the ECM controls, would therefore be one means of inhibiting both Rb phosphorylation and vSMC proliferation. Cyclin D1 is a particularly attractive target in this regard because its expression is ECM dependent and it (together with cdk4/6) plays an essential role in pRb phosphorylation.

Albanese et al. (10) showed that a dominant negative ERK2 (MAP kinase) antagonized EGF-dependent activation of the cyclin D1 promoter, and a recent paper by Lavoie et al. (11) shows that cyclin D1 reporter activity requires the sustained activation of the ERK subfamily of MAP kinases. Since integrins are involved in maintaining active ERKs in G1 phase, it seems quite reasonable that integrin-dependent activation of MAP kinase may play an important role in the adhesion-dependent expression of cyclin D1 and phosphorylation of pRb in vSMCs. As outlined below, changes in the nature of the vSMC extracellular matrix and/or the integrins that are expressed by vSMCs may therefore affect MAP kinase activity, cyclin D1 expression, pRb phosphorylation and, ultimately, cell proliferation.

*The ECM and integrins of vSMC*

In vivo, vSMCs are surrounded by a simple basement membrane that contains collagen IV and laminin, with small amounts of fibronectin. As mentioned above, vSMCs undergo a phenotypic transformation from a contractile to a synthetic state as they begin to proliferate. In vitro, this phenotypic modulation can be shown to be dependent on the nature of the substratum. Aortic vSMCs rapidly become synthetic when cultured on a fibronectin substratum, and they will proliferate if they are also exposed to appropriate growth factors (12). However, if these cells are cultured on laminin, they will remain in a contractile (and nonproliferative) state for some time (12). These results suggest that specific ECM substrates will have different signaling responses within the cell. Similar results are seen in skeletal myoblasts (13), suggesting that the integrin receptors for these two matrix molecules may evoke distinct signals that account for the two vSMC phenotypes.

Integrins are transmembrane heterodimers that bind to a number of ligands, primarily ECM molecules, and stimulate a number of signal transduction pathways (14). There is evidence for a role of integrins in cell survival promoted by ECM, and blockade of integrins can trigger apoptosis of endothelial cells in vivo (15). A number of studies have shown that fibronectin can provide the necessary signals for adhesion-dependent growth. Since most cells adhere to fibronectin via integrins, it is presumed that these receptors can induce signal transduction pathways that are involved in stimulating cell growth.

*Integrin signaling pathways*

A central signaling pathway for integrin receptors appears to be their ability to stimulate transphosphorylation of focal adhesion kinase (16). However, studies to date have not yet dem-

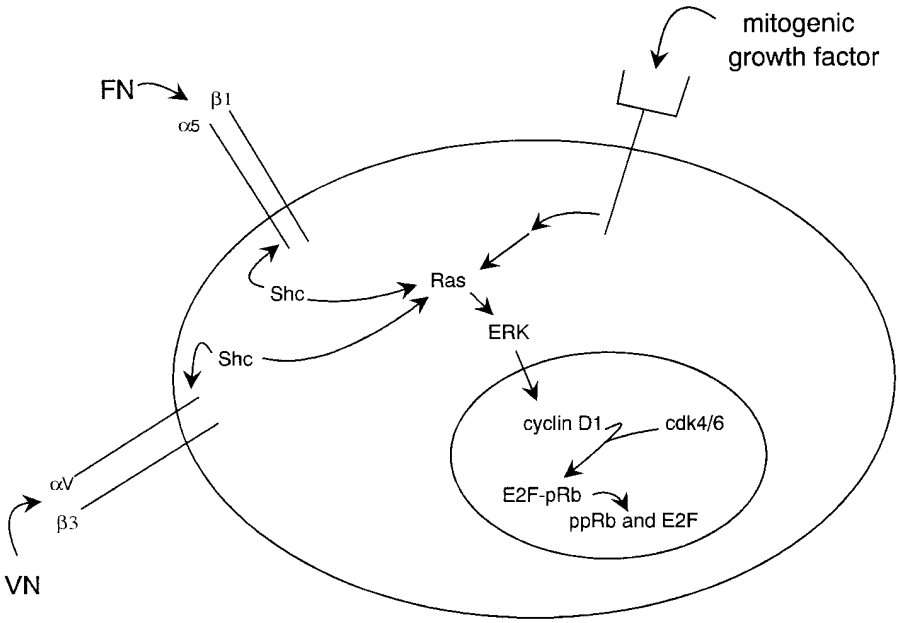


*Figure 1.* Coordinate regulation of cyclin D1 expression and pRb phosphorylation by the extracellular matrix and growth factors. The diagram summarizes the studies reviewed here and proposes a working model for explaining how specific matrix proteins and growth factors might cooperate to control cyclin D1 expression and pRb phosphorylation in vSMCs. Fibronectin (*FN*) or vitronectin (*VN*) binding to α5β1 and αVβ3 integrins, respectively, would lead to Shc association and an ras-dependent activation of ERKs. This effect would cooperate with the ERK activation mediated by mitogenic growth factors, allowing for sustained ERK activity and translocation of the enzyme to the nucleus. Nuclear ERK activity is required for transcription of the cyclin D1 gene, and cyclin D1 (upon binding to cdk4/6) is required for phosphorylation of the retinoblastoma protein. This model also provides a potential explanation for the fact that the expression of cyclin D1 and phosphorylation of pRb require cell anchorage to the extracellular matrix as well as stimulation by soluble mitogenic growth factors.

onstrated a major role for focal adhesion kinase in adhesion-dependent growth. Integrin-dependent adhesion can also lead to an activation of the ERK-type MAP kinases. While there now seems to be several mechanisms by which integrins lead to MAP kinase activation (17–21), the recent study by Wary et al. (21) has elucidated an Ras-dependent pathway that has the potential to explain the ECM-specific changes in vSMC proliferation as described previously. Specifically, MAP kinase activation was found to be α subunit dependent, occurring with integrins containing α1, α5, or αV subunits but not with integrins containing α2 or α3 subunits. Interestingly, the α1, α5, and αV-containing integrins associated with phosphorylated Shc upon clustering. Although the mechanism of Shc association with these integrins remains somewhat unclear, it is clear that MAP kinase activation on fibronectin in fibroblasts was blocked by dominant negative Shc as well as dominant negative Ras. Thus, the model proposed is that the few integrin heterodimers which lead to association and phosphorylation of Shc can then activate Ras through the Shc/Grb2/SOS complex, leading to MAP kinase activation.

Using this model, we can speculate that the difference in the rates at which vSMCs proliferate when cultured on fibronectin and laminin is due to differences in Ras activation. vSMCs in culture will use α5β1 as their primary receptor for fibronectin, which should lead to Shc association and subsequent Ras activation. This activation could lead to the proliferative/synthetic phenotype seen on fibronectin. Cultured vSMCs probably use α3β1 as their laminin receptor, and this receptor would not lead to Ras activation. Thus, adhesion to laminin would permit persistence of the contractile/nonproliferative phenotype. While this model has not been tested in vSMCs, similar experiments have been done using human umbilical vein endothelial cells (21). In these experiments, cell adhesion to fibronectin (through α5β1) led to Shc association and proliferation whereas adhesion to laminin (through α2β1) led to neither Shc recruitment nor proliferation. It will be important to examine the role of Shc-dependent MAP kinase activation in the phenotypic modulation of contractile versus synthetic SMCs.

We also need to consider the nature of the ECM after vSMC injury in vivo. Injured (synthetic) vSMCs increase their production of fibrillar collagens, particularly type I, and they undergo a switch in their collagen receptors from α1β1 to α2β1 (2, 22). The pathway outlined by Wary et al. (21) would suggest that this switch may not be directly related to the changes in vSMC proliferation. Rather, it may play a role in the ability of synthetic vSMCs to migrate on a collagen I matrix (22). Injured vSMCs also have α5β1 and αV-containing integrins, so fibronectin or vitronectin, which are likely to be present in the injured vessel, could be providing the signal for Shc-dependent MAP kinase activation. This idea is supported by the intriguing finding that treatment of patients with anti-β3 blocking antibodies at angioplasty (to prevent platelet thrombi formation) leads to protection against restenosis, presumably by blockade of αVβ3 (23).

Fig. 1 shows a working model that summarizes the papers reviewed here and tries to link the recent studies on integrin-specific ERK activation to those showing that cell adhesion is required for expression of cyclin D1 and phosphorylation of pRb. Clearly, much work needs to be done to test the ideas proposed in this model. Nevertheless, the newly found relationships between: (*a*) integrin-specific signaling and MAP kinase activation; (*b*) MAP kinase activation and cyclin D1 expression; and (*c*) cyclin D1 expression, pRb phosphorylation, and anchorage-dependent growth all suggest a consistent molecular mechanism which emphasizes that subtle changes in the local matrix can have profound consequences on pRb phosphorylation and vSMC proliferation during injury. If these links can be documented in vivo, then interventions that inhibit this pathway would have potential adjuvant therapeutic value in coronary angioplasty.

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