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CONTROL OF VASCULAR SMOOTH MUSCLE CELL GROWTH BY CYCLIN-DEPENDENT KINASE INHIBITORY PROTEINS AND ITS IMPLICATION IN CARDIOVASCULAR DISEASE

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1. ABSTRACT

While quiescence is a defining characteristic of differentiated vascular smooth muscle cells (VSMCs) residing within the medial layer of elastic arteries in the adult organism, mature VSMCs can undergo phenotypic modulation and reenter the cell cycle in response to several physiological and pathological stimuli. Abnormal VSMC proliferation is thought to contribute to the pathogenesis of vascular occlusive lesions, including atherosclerosis, vessel renarrowing after successful angioplasty (restenosis), and graft atherosclerosis after coronary transplantation. Therefore, elucidating the molecular mechanisms limiting VSMC growth is currently the subject of active research. This review will focus on the role of cyclin-dependent kinase inhibitory proteins in the regulation of VSMC proliferation and its implication in intimal lesion formation during the pathogenesis of vascular proliferative diseases.

2. INTRODUCTION

In the adult organism, the vessel wall in a healthy artery is composed of an outer layer of connective tissue (adventitia), a medial layer of VSMCs (media) and an inner monolayer of endothelial cells (ECs) (intima). Accumulation of cellular and extracellular substances in the space between the EC lining and the underlying VSMCs leads to neointimal lesion formation and the ensuing progressive reduction of arterial patency. According to the response-to-injury hypothesis, atherosclerosis is triggered by different risk factors (hypercholesterolemia, aging, hypertension, smoking and diabetes) that can somehow lead to endothelial dysfunction (1, 2). Studies in hypercholesterolemic animals and in human atherosclerotic specimens have identified three processes involved in the formation of the neointimal lesion once the normal properties of the endothelium have been altered (1, 2): 1) the proliferation of VSMCs, macrophages and possibly lymphocytes; 2) the formation by VSMCs of a connective tissue matrix comprising elastic fibre proteins, collagen and proteoglycans; and 3) the accumulation of lipid and mostly free and esterified cholesterol in the surrounding matrix and the associated cells. Figure 1 shows an example of diet-induced pathological proliferation of medial VSMCs and macrophages within the intimal lesion of hypercholesterolemic rabbits. Numerous observations suggest that VSMCs in atherosclerotic lesions have changed from a *contractile* to a *synthetic* state (3), in which they can respond to different growth factors and synthetize extracellular matrix (4). “Activated” VSMCs can also migrate toward the arterial lumen and express abundant levels of novel matrix components and proteases that modify the surrounding matrix. This “growth and synthetic” response of VSMCs contributes to atheroma formation.

Excessive VSMC proliferation also contributes to restenosis, the recurrence of arterial narrowing at the site of balloon angioplasty that occurs in 20-55% of coronary artery disease patients after successful angioplasty (1, 5, 6). Acute disruption of the protective endothelial lining at the site of angioplasty appears to trigger this aggressive form of atherosclerosis, which is typically characterized by exuberant VSMC hyperplastic response (7-9), extracellular matrix accumulation (10, 11) and local "remodeling" (elastic recoil) of the dilated vessel (12, 13).

Cell cycle progression and cellular proliferation in mammals requires the activation of cyclin-dependent kinases (CDKs) through their association with regulatory subunits called cyclins (14, 15). Different CDK/cyclin holoenzymes are orderly activated at specific phases of the cell cycle. Active CDK/cyclin complexes are presumed to hyperphosphorylate pRb and the related pocket proteins p107

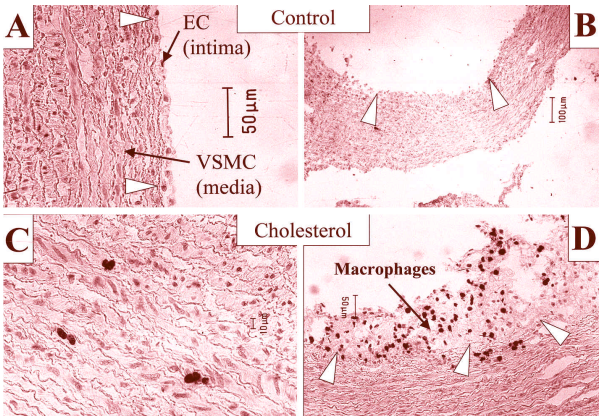

Figure 1: Hypercholesterolemia induces abnormal cell proliferation in the aortic arch. Male New Zealand rabbits were fed a control diet (A, B) or a cholesterol-rich diet for 2 months (C, D). Animals received 4 intraperitoneal injections of 5-bromodeoxyuridine (BrdU) during the last 2 days prior to sacrifice to identify proliferating cells. The aortic arch was embedded in paraffin and cut in 5-micron sections for immunohistochemistry using a mouse monoclonal anti-BrdU antibody and an streptavidin-peroxidase detection system. Dark nuclei indicate BrdU-immunoreactive cells. Specimens were countestained with hematoxylin. Arrowheads point to the internal elastic lamina, which marks the boundary between the tunica media (composed of VSMCs and elastic fibers) and the intima (composed of a monolayer of endothelial cells in control arteries). Immunohistochemistry using mouse monoclonal anti-RAM11 antibody (not shown) demonstrated that the intimal lesion at these early time points is mainly composed of macrophages. Note the presence of BrdU-positive VSMCs in the media (C) and macrophages in the intimal lesion (D) of hypercholesterolemic rabbits.

and p130 from mid G1 to mitosis. The interaction among members of the E2F family of transcription factors and individual pocket proteins is a complex regulatory event that determines whether E2F proteins function as transcriptional activators or repressors (16-20). VSMC proliferation in the balloon-injured rat carotid artery is associated with a temporally and spatially coordinated expression of CDK2 and its regulatory subunits, cyclin E and cyclin A (21). Induction of these factors correlated with increased CDK2-, cyclin E- and cyclin A-dependent kinase activity, indicating the assembly of functional CDK2/cyclin E and CDK2/cyclin A holoenzymes in the injured arterial wall. Expression of CDK2 and cyclin E was also detected in human VSMCs within atherosclerotic and restenotic tissue (21-23), suggesting that induction of positive cell-cycle control genes is a hallmark of vascular proliferative diseases.

CDK activity is negatively regulated by specific cell cycle regulators, termed CDK inhibitors (CKIs), which associate with and inhibit the activity of CDKs (15, 24-26). CKIs of the CIP/KIP family (p21, p27 and p57) bind to and inactivate CDK2-containing holoenzymes, while members of the INK4 family (p15, p16, p18, p19) are specific for CDK4- and CDK6-containing holoenzymes. In addition to its inhibitory effect on CDKs, p21 can also inhibit DNA replication through direct interaction with proliferating cell nuclear antigen (PCNA) (27, 28). Separate domains of p21 are involved in the inhibition of CDK and PCNA (29, 30), and reversible phosphorylation at the C-terminal regulatory domain of p21 modulates PCNA binding (31). In the next sections, we will discuss the role of CKIs in the pathogenesis of atherosclerotic cardiovascular diseases.

3. DISCUSSION

3.1. Inhibition of VSMC proliferation by CKIs

While proliferating cells are present at all stages of development of atherosclerotic lesions (1, 2), studies with Watanabe heritable hyperlipidemic and hypercholesterolemic fat-fed rabbits have demonstrated an inverse relationship between lesion size (and severity) and the proliferative index in the arterial wall (32-35). These findings suggest that cell proliferation may be a relatively early event in the atherogenic process. Likewise, balloon angioplasty leads to a rapid proliferative response of VSMCs within the media, followed by a second peak of proliferation in neointimal VSMCs which then resume a quiescent phenotype within 2-6 weeks after angioplasty (36-40). Thus, both atherosclerosis and restenosis are characterized by the reestablishment of the quiescent phenotype after the initial burst of proliferation.

Recent studies suggest that p27 and p21 are physiological regulators of VSMC proliferation that contribute to limiting neointimal hyperplasia during arterial repair. Balloon angioplasty in rat and porcine arteries resulted in the induction of p21 and p27 in VSMCs at time points that correlated with reduced CDK2 activity and the decline in VSMC proliferation (41-43). Moreover, overexpression of p27 efficiently blocked mitogen- and c-fos-dependent induction of cyclin A promoter activity in cultured VSMCs (41, 44). Thus, upregulation of p21 and p27 may limit VSMC growth at late time points after angioplasty (Figure 2). In agreement with this hypothesis, Chang et al. (45) and Yang et al. (43) first demonstrated that adenovirus-mediated overexpression of p21 attenuated neointimal thickening in balloon-injured rat and porcine arteries. Likewise, Chen et al. reported that local delivery of adenovirus encoding for p27 at the time of angioplasty reduced neointimal hyperplasia in the rat carotid artery (41). Additional studies by other investigators have corroborated the ability of p21 and p27 to inhibit the development of injury-induced vascular occlusive lesions (46, 47). These studies also showed that overexpression of p16 failed to inhibit neointimal VSMC proliferation (47). Of note is that induction of p27, but not p21, is associated with inhibition of VSMC proliferation in cells stably transfected with PKC delta (48). Whether PKC delta is involved in the upregulation of p27 after angioplasty *in vivo* remains to be explored.

Tanner *et al* (42) analyzed CKI expression in human coronary arteries ranging from normal to advanced atherosclerosis. Expression of p27 was abundant within normal and atherosclerotic arteries. While p21 was undetectable in normal arteries, its expression was elevated in atherosclerotic tissue. In this same study, p16 could not be detected in normal or atherosclerotic specimens, demonstrating that the CIP/KIP and INK4 families of CKIs have different temporal patterns of expression in VSMCs in

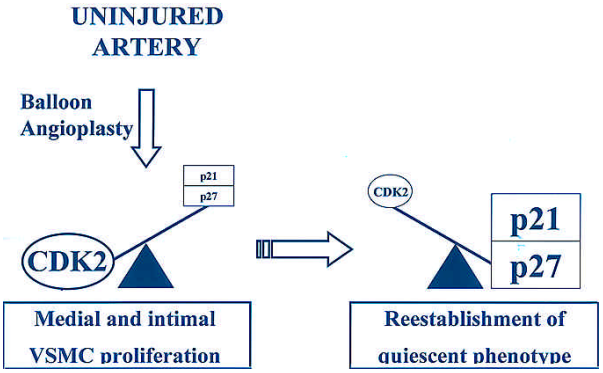

Figure 2: Role of p21 and p27 in the regulation of VSMC growth after angioplasty. Using several animal models of balloon angioplasty, it has been shown that injury-induced vascular remodeling is characterized by a rapid proliferative response of VSMCs within the media, which migrate towards the arterial lumen and initiate a second wave of proliferation within the intimal lesion. Two to four weeks after angioplasty, VSMC proliferation returns to basal levels. Medial and intimal VSMC proliferation correlates with low level of expression of p21 and p27 and high CDK2 activity. Reduced CDK2 activity and low proliferation at later time points coincides with upregulation of p21 and p27, suggesting that induction of these CKIs may contribute to the reestablishment of the quiescent phenotype. Consistent with this notion, adenovirus-mediated overexpression of p21 and p27 following angioplasty limited intimal thickening (See text for details).

balloon-injured arteries and atherosclerotic lesions. More recently, Ihling *et al* (23) have demonstrated coexpression of type I and II transforming growth factor-beta (TGF-beta) receptors in virtually all cells positive for p27 within human atherosclerotic tissue. In the atherosclerotic specimens, approximately 13% of the nuclei were positive for cyclin E, whereas in the control tissue cyclin E staining was restricted to 0.19% of the cells. Importantly, about 5% of p27-positive cells disclosed cyclin E immunoreactivity. These results suggest that TGF-beta present in human atherosclerotic tissue may mediate its growth suppressive activity through p27-dependent blockade of cyclin E-CDK2 activity.

Internal mammary artery (IMA) bypass grafts have a higher patency than saphenous vein (SV) grafts. Yang et al. (49) examined the growth properties of human VSMCs isolated from IMAs and SVs. Cell outgrowth from explants over a 20-day period and serum-induced increase in cell number over an 8-day period were more pronounced in SV than in IMA of the same patient. These differences in the response to growth stimuli were observed despite functional growth factor receptor expression and MAPK activation in VSMCs isolated from both SVs and IMAs. Platelet-derived growth factor-BB (PDGF-BB) markedly downregulated p27 protein level in SV, but this was much less pronounced in IMA. Thus, sustained p27 expression in spite of growth stimuli may contribute to the resistance to growth of VSMCs from IMA and to the longer patency of arterial versus venous grafts.

Fibroblast growth factor 2 (FGF2 or basic FGF) plays a critical role in the induction of medial VSMC proliferation in balloon-injured arteries (50-52). In marked contrast, neutralizing antibodies to FGF2 failed to inhibit intimal VSMC proliferation after balloon angioplasty (53), and only a small increase in proliferation was seen when FGF2 was added to arteries with existing intimal lesions (50, 52). Attenuated FGF2-dependent proliferation of intimal VSMCs occurred despite a robust activation of the MAPK pathway and induction of positive cell cycle regulators (i. e., cyclin D, cyclin E, CDK2 and CDK4) (52). Interestingly, intimal VSMCs expressed high levels of p15 and p27 compared with medial VSMCs, and FGF2 infusion did not reduce the level of these inhibitors in arteries with established intimal lesion. Collectively, the studies by Yang et al. (49) and Olson *et al* (52) using different sources of VSMCs suggest that high level of expression of p15 and p27 can attenuate VSMC proliferation in spite of the activation of the MAPK pathway and expression of cell cycle activators.

3.2. Role of p21 and p27 in the control of VSMC growth by extracellular matrix components

Accumulating evidence indicates that specific components of the extracellular matrix (ECM) and integrins are physiological cell-cycle control elements in atherosclerosis and restenosis (54). Neointimal VSMCs within atherosclerotic lesions synthesize novel ECM components and induce the expression of matrix-degrading proteases that remodel the surrounding ECM. For example, matrix-degrading metalloproteinase (MMP) expression is induced within atherosclerotic plaques and after balloon angioplasty (55-58). Moreover, MMP inhibitors repressed VSMC proliferation *in vitro* and after angioplasty *in vivo* (59-61). Accordingly, these ECM enzymes have been implicated in the induction of neointimal VSMC hyperplasia during atherosclerosis and restenosis.

Integrins are transmembrane heterodimers that bind to a number of ligands, primarily ECM molecules, and stimulate a variety of transduction pathways (62). One integrin in particular, $alpha_v beta_3$, is thought to interact with osteopontin and play a critical role in regulating cellular functions deemed essential for restenosis including migration, ECM invasion and proliferation of VSMCs (63). $Alpha_v beta_3$ has been found to be expressed by VSMCs in the intima of diseased human coronary arteries (64) and is upregulated following balloon injury of baboon brachial arteries (65). Further evidence of the importance of this integrin in the pathogenesis of restenosis has been provided by showing that selective $alpha_v beta_3$ blockade could potently limit neointimal hyperplasia in animal models of arterial injury (66, 67). Interestingly, it has been suggested that inhibition of $alpha_v beta_3$ could constitute a potential mechanism for the beneficial effects on clinical restenosis of abciximab (an inhibitor of platelet glycoprotein IIb/IIIa) in patients undergoing high-risk percutaneous coronary interventions (68).

Changes in collagen content have been well documented in different animal models of atherosclerosis and angioplasty (11, 69, 70). To investigate whether changes in

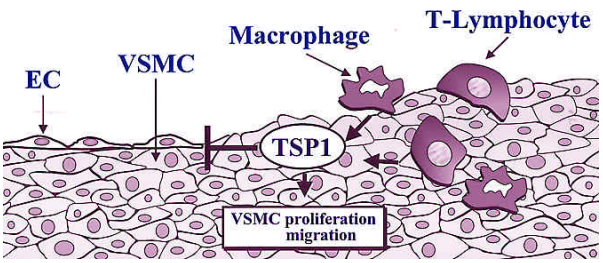

Figure 3: Thrombospondin 1 expression is induced in atherosclerotic plaques and restenotic lesions. Schematic showing the deleterious effects of TSP1 accumulation within injured arteries. TSP1 is secreted at the sites of arterial injury by macrophages and T-lymphocytes. In the left part is show the inhibitory effect of TSP1 on endothelial cell (EC) migration and proliferation, which delays reendothelialization after balloon angioplasty. On the other hand, TSP1 stimulates VSMC proliferation and migration, therefore contributing to intimal thickening. Consistent with these harmful effects of TSP1, antibody blockade of TSP1 promotes reendothelialization and reduces intimal lesion development after angioplasty in the rat carotid artery. *In vitro* experiments have shown that p21 is essential for TSP1-dependent regulation of cellular proliferation (See text for details).

collagen may regulate VSMC proliferation, Koyama et al. studied the growth properties of VSMCs cultured on monomer collagen fibers and on polymerized collagen (71). The rationale for these studies is that polymerized collagen may resemble the scenario of a normal artery composed of quiescent VSMCs, and monomer collagen might mimic the ECM surrounding proliferating VSMCs within atherosclerotic and restenotic plaques. Consistent with this interpretation, mitogen-stimulated VSMCs proliferated in culture dishes coated with monomer collagen, but were arrested in G1 when grown on polymerized collagen. The inhibitory effect of polymerized collagen on VSMC growth appeared to be mediated by $alpha_2$ integrins, and was associated with suppression of p70 S6 kinase and upregulation of p21 and p27. These findings indicate that the ability of VSMCs to respond to growth signals *in vitro* is highly dependent on changes in specific ECM components through regulation of CKIs. Of note is that lack of proliferation in nonadherent NRK fibroblasts can be linked to an increased association of p21 and p27 to cyclin E-containing holoenzymes (72). Further studies are required to determine whether cell cycle control in the arterial wall is linked *in vivo* to integrins and ECM components through changes in CKI expression.

The glycoprotein thrombospondin 1 (TSP1) is a component of the ECM synthesized and secreted by activated platelets (73) and a variety of cell types including ECs (74, 75), macrophages (76), fibroblasts (77) and VSMCs (78). TSP1 is a 450 kD homotrimer that interacts with multiple extracellular macromolecules and cell surface receptors, thus exerting a wide range of functions (79, 80). TSP1 can induce EC growth arrest *in vitro* (81, 82), and inhibits the spontaneous development of angiogenic tube-like structures both *in vitro* and *in vivo* (83-85). In marked contrast, TSP1 promotes VSMC proliferation and migration (86, 87), and plays a stimulatory role in platelet activation and aggregation (88, 89). TSP1 expression has been associated with atherosclerotic lesions, acute vascular injury, hypercholesterolemia and hypertension (75, 90-95). Thus, TSP1 may have detrimental effects of the vessel wall (Figure 3). Consistent with this notion, antibody blockade of TSP1 accelerated reendothelialization and reduced neointima formation in balloon-injured rat carotid artery (96). Neutralizing A4.1 anti-TSP1 antibody inhibited CDK2 activity and blocked the induction of S-phase entry which normally occurs in serum-stimulated VSMCs (97). This growth inhibitory effect was associated with a marked induction of total cellular p21 expression and increased level of CDK2-bound p21 in A4.1-treated VSMCs. A4.1 antibody inhibited [^{3}H]-thymidine incorporation in wild-type, but not in p21-deficient mouse embryonic fibroblasts, suggesting that p21 plays an essential role in TSP1-mediated control of cellular proliferation.

3.3. Role of CKIs in nitric oxide-dependent suppression of VSMC proliferation

Nitric oxide (NO) has critical roles in the maintenance of vascular homeostasis. In addition to its role as a vasodilator, NO inhibits platelet function, leukocyte adhesion to ECs, and VSMC growth (98, 99). Teleologically, the lack of endothelium-derived NO production due to disruption of the protective endothelial lining after balloon angioplasty might be expected to contribute to VSMC hyperplasia. Consistent with this notion, arterial delivery of EC mitogens that accelerated reendothelization also attenuated neointimal hyperplasia after vascular injury (100-102). Studies with endothelial NO sinthase (eNOS)-deficient mice have provided direct evidence for the importance of endothelium-derived NO in vascular response to injury (103, 104). Moreover, high production of NO by intimal VSMCs via inducible NOS (iNOS) may contribute to the restoration of the quiescent phenotype after balloon angioplasty (105, 106). Of note is that NO from VSMCs can reduce eNOS protein expression by ECs via a tumor necrosis factor (TNF) alpha-dependent mechanism (107).

Administration of the NO precursor L-arginine (108-111), or *in vivo* transfer of NO synthase gene (112-116) inhibited neointimal lesion development in several animal models, including balloon angioplasty, cholesterol-induced atherosclerosis and allograft atherosclerosis. Conversely, inhibition of NO production by treatment with NG-nitro-L-arginine methyl ester (L-NAME) accelerated neointima formation in hypercholesterolemic rabbits (117) and apolipoprotein E-deficient mice (118). These findings are consistent with the observation that resistance of VSMCs to NO contributes to abnormal endothelium-dependent vasodilation during hypercholesterolemia (119), and suggest that NO plays a critical role during the pathogenesis of vascular proliferative diseases.

Recent studies have shed significant insight into the mechanisms involved in the antiproliferative effect of NO. When starvation-synchronized human VSMCs were serum-restimulated, the mRNA and protein levels of p21 were high in early G1 and then rapidly decreased prior to the induction of CDK2 activity (120). Addition of the NO donor S-nitroso-N-acetylpenicillamine (SNAP) to serum-restimulated VSMCs

inhibited DNA synthesis assessed by [^{3}H]thymidine incorporation. The antiproliferative effect of SNAP was associated with enhanced and sustained p21 expression, increased amount of CDK2-associated p21 and inhibition of CDK2 activity (120). Moreover, evidence has been presented suggesting that NO-dependent VSMC growth arrest results, at least in part, from the repression of cyclin A gene transcription (121).

The involvement of cGMP in NO-dependent regulation of CKI expression is controversial. Loss of NO responsiveness in aged rats due to the lack of the beta subunit of soluble guanylyl cyclase may contribute to the enhanced intimal thickening in response to injury in old animals (122). Gu *et al* suggested that NO increases p21 expression by a cGMP-dependent mechanism that includes activation of extracellular signal-regulated kinase (Erk) and p70 S6 kinase (123). In contrast, Sarkar et al. reported that NO inhibition of VSMC proliferation is associated with two distinct and reversible cell cyle arrests, an immediate cGMP-independent S-phase block followed by a shift back in the cell cycle from the G1-S boundary to a quiescent G0-like state (124). Likewise, Tanner et al. suggested that upregulation of p21 in VSMCs treated with the NO donor diethylenetriamineNONOate may occur independent of cGMP (125). It is important to note that diethylenetriamineNONOate did not change p27 expression, whereas a transient increase in p27 in CDK2 immunoprecipitates, without changes in total cellular p27, correlated with the delay in CDK2 activation caused by cGMP in human VSMCs (126). cGMP-elevating agents inhibited EGF-induced VSMC proliferation by a mechanism that appears to involve the repression of Ras-dependent activation of Raf-1 (127). While the above studies clearly suggest a role of p21 and p27 in NO-dependent VSMC growth arrest, additional studies are required to clarify the role of cGMP in this pathway.

Several studies have suggested the contribution of adventitial myofibroblasts to vascular remodeling and intimal lesion formation after experimental angioplasty (128). In this regard, it is noteworthy to point out that NO has been implicated as a potential regulator of the cell cycle in aortic adventitial myofibroblasts through a cGMP-mediated transcriptional mechanism involving the induction of p21 (129).

3.4. p27 as a regulator of the phenotypic response of VSMCs to mitogenic and hypertrophic stimuli

VSMC hypertrophy is associated with cardiovascular disease in elderly and hypertensive individuals. Therefore, a better understanding of the molecular mechanisms underlying the onset of VSMC hypertrophy may have implications for the design of novel therapeutic interventions in cardiovascular disease. Angiotensin II (Ang II) has been shown to stimulate hypertrophy but not hyperplasia of quiescent VSMCs in serum-free media, in spite of increased expression of several protooncogenes and autocrine growth factors (130-133). While both serum and Ang II treatment of quiescent VSMCs led to upregulation of positive cell-cycle regulators, including proliferating cell nuclear antigen, cyclin D1, CDK2 and CDK1, only serum-treated VSMCs induced CDK2 and CDK1 activity (134). Braun-Dullaeus et al. provided compelling evidence implicating p27 as a molecular switch that regulates the phenotypic response of VSMCs to mitogenic and hypertrophic stimuli (134). Their experiments show that Ang II-induced hypertrophy of quiescent VSMCs correlated with sustained expression of p27, unlike serum-dependent cell-cycle reentry of starvation-synchronized cells, which correlated with a marked downregulation of p27 protein level. Importantly, forced overexpression of p27 inhibited serum-stimulated proliferation and induced VSMC hypertrophy. Moreover, inhibition of p27 expression in VSMCs treated with antisense oligodeoxynucleotides increased [^{3}H]-thymidine incorporation and the percentage of S-phase cells in Ang II-treated cultures. These results demonstrate that Ang II treatment of quiescent VSMCs is associated with cell-cycle entry, but hypertrophic rather than hyperplastic growth may prevail by the failure of cells to downregulate p27. In another study, Servant et al. (135) compared the effects of Ang II and the mitogenic factor PDGF-BB on cultured VSMCs. While both factors stimulated the accumulation of G1 cyclins and CDKs, only PDGF-BB activated CDK2 in late G1. Lack of CDK2 activity in Ang II-treated cells correlated with sustained p27 protein level. In contrast, PDGF-BB downregulated p27 expression, and this effect correlated with a reduced rate of synthesis and an increased rate of degradation of p27. Moreover, the reduction in p27 synthesis by PDGF-BB was associated with diminished p27 gene transcription and decreased mRNA accumulation. Collectively, these studies identify p27 as an important regulator of the phenotypic response of VSMCs to mitogenic and hypertrophic stimuli.

4. PERSPECTIVES

Abnormal VSMC hyperplastic and hypertrophic growth play an important role in the pathogenesis of cardiovascular diseases, including atherosclerosis and restenosis. Because of the public health importance and economic impact of these pathological processes, elucidating the regulatory factors and molecular mechanisms that control VSMC growth is currently the subject of active research. In this review, we have discussed the role of CKIs in the regulation of VSMC proliferation. *In vitro* studies have implicated p27 as a molecular switch that regulates the phenotypic response of VSMCs to mitogenic and hypertrophic stimuli. Moreover, induction of endogenous p21 and p27 at late time points after balloon-angioplasty may contribute to the restoration of the quiescent phenotype during vascular remodeling. Consistent with this notion, adenovirus-mediated overexpression of p21 and p27 inhibited VSMC hyperplasia and prevented arterial narrowing in balloon-injured rat and porcine arteries. In a recent study (136), cDNA array hybridization techniques showed that p21 induces the expression of genes implicated in atherosclerosis, including serum amyloid A (137), connective tissue growth factor (138), and galectin-3 (139). It is therefore essential to continue our efforts to elucidate the molecular mechanisms governing the control of CKI expression in the vessel wall. Ultimately, a thorough understanding of these regulatory networks may lead to the development of novel therapies for the treatment of vascular proliferative diseases in human patients.

5. ACKNOWLEDGMENTS

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