Vascular Proliferation and Atherosclerosis: New Perspectives and Therapeutic Strategies

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Abstract and Introduction

Abstract

In atherosclerosis, the vascular smooth muscle cell (VSMC) contributes to vessel wall inflammation and lipoprotein retention, as well as to the formation of the fibrous cap that provides stability to the plaque. The VSMC can undergo a proliferative response that underlies the development of in-stent restenosis, bypass graft occlusion and transplant vasculopathy. Although the benefit/risk of therapeutic inhibition of VSMC proliferation in atherosclerosis is unclear, experimental and human evidence strongly suggests the therapeutic potential of antiproliferative therapy for in-stent restenosis, bypass graft failure and other vascular proliferative disorders.

Introduction

Atherosclerosis involves multiple processes including endothelial dysfunction, inflammation, vascular proliferation and matrix alteration. Vascular proliferation contributes to the pathobiology of atherosclerosis and is linked to other cellular processes such as inflammation, apoptosis and matrix alterations. The contribution of vascular proliferation to the pathophysiology of in-stent restenosis, transplant vasculopathy and vein bypass graft failure is particularly important. Thus, an emerging strategy for the treatment of those conditions is to inhibit cellular proliferation by targeting cell cycle regulation. Here we will review the current understanding of the pathophysiological mechanisms and the status of molecular and gene therapeutic approaches in vascular proliferative diseases. The understanding of the pathophysiology of atherosclerosis and related vascular diseases has changed over the last decade, providing new perspectives for preventive and therapeutic strategies.

Recent studies have emphasized the involvement of inflammation in mediating all stages of atherosclerosis.[1,2] However, in addition to inflammation, a key process of atherosclerosis involves the proliferation of vascular smooth muscle cells (VSMCs)[3-5] (Fig. 1). One precursor of lesion development in humans may be focal accumulation of VSMCs within the intima.[6] The exact function of VSMCs in atherosclerosis is, however, still a subject of debate.[5,6] In early atherosclerosis, VSMCs may contribute to the development of the atheroma through the production of pro-inflammatory mediators such as monocyte chemoattractant protein 1 and vascular cell adhesion molecule, and through the synthesis of matrix molecules required for the retention of lipoproteins.[6] However, VSMCs may also be important in maintaining the stability of the plaque through the formation of a firm fibrous cap. Indeed, in lipid-laden lesions in which the fibrous cap is thin and weak, there is evidence of VSMC apoptosis, especially at the 'shoulder' region, associated with inflammation.[7] In addition, the local inflammatory milieu can induce expression of collagenase and inhibit expression of proteolytic inhibitors, thus rendering the fibrous cap weak and susceptible to rupture.[2,5] In advanced lesions, fibroblasts and VSMCs with extracellular calcification form a fibrocalcific plaque.

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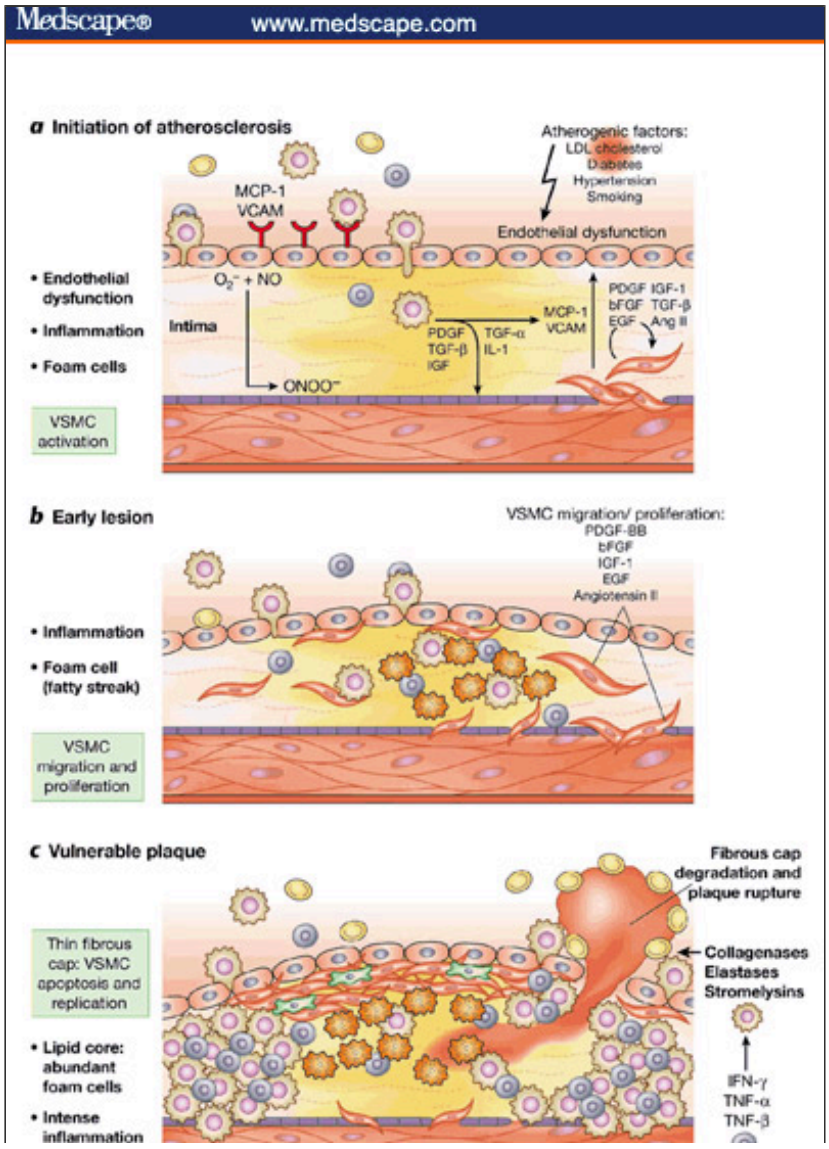


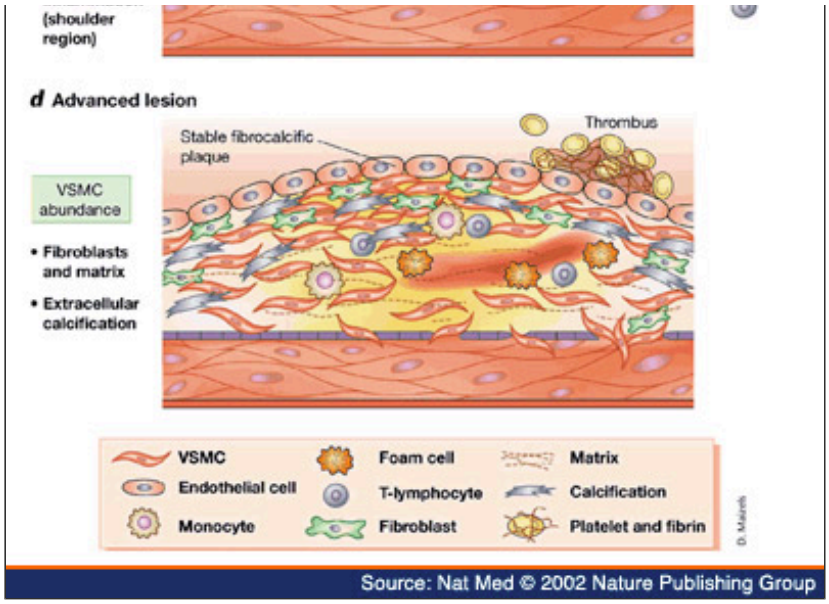


Figure 1. Function of VSMCs during different stages of atherosclerosis. Cardiovascular risk factors alter the vascular endothelium (EC), which triggers a cascade of events, including the recruitment of leukocytes. Cytokines and growth factors are released by inflammatory cells and vascular cells, generating a highly mitogenic milieu. VSMCs migrate, proliferate and synthesize extracellular matrix components on the luminal side of the vessel wall, forming the fibrous cap of the atherosclerotic lesion. Inflammatory mediators ultimately induce thinning of the fibrous cap by expression of proteases, rendering the plaque weak and susceptible to rupture and thrombus formation. In advanced disease, fibroblasts and VSMCs with extracellular calcification give rise to fibrocalcific lesions. LDL, low-density lipoprotein; MCP, monocyte chemoattractant protein; VCAM, vascular cell adhesion molecule; PDGF-BB, platelet-derived growth factor (BB, β-chain homodimer); TNF, tumor necrosis factor; TGF, transforming growth factor; IL, interleukin 1; IGF, insulin-like growth factor; bFGF, basic fibroblast growth factor; Ang II, angiotensin II; EGF, epidermal growth factor; IFN, interferon.

The origin of VSMCs in the atherosclerotic plaque is intriguing. Intimal thickening appears during normal development and aging[8] Intimal VSMCs, including those in atherosclerotic lesions, are reportedly monoclonal in origin.[9] This would indicate that the neointima arises from proliferation of resident pre-existing clonal VSMCs. Although examination of human atherosclerotic lesions has not yielded evidence of extensive replication,[10,11] it may occur very early or at a low rate throughout the development of atherosclerosis or episodically at a high rate. Indeed, VSMCs have been identified in fatty streaks of young individuals.[12] Experimental data also indicate that intimal VSMCs may originate from the media or the adventitia.[13] Furthermore, embryonic endothelial cells are reportedly able to transdifferentiate into mesenchymal cells expressing smooth muscle cell actin.[14] Animal studies have indicated that neointimal cells may also originate from subpopulations of bone marrow- and non-bone marrow-derived circulating cells.[15-18] In models of hyperlipidemia-induced atherosclerosis, as well as in post-angioplasty restenosis and graft vasculopathy, bone marrow cells may give rise to a substantial percentage of the VSMCs that contribute to arterial remodeling. The contribution of these cells to human atherosclerosis has not been proven, although circulating smooth muscle progenitor cells have been identified in human peripheral blood.[19]

In summary, the function of the intimal smooth muscle cell in the natural history of the atherosclerotic lesion seems to be to act as a nidus for development of the lesions, perhaps by accelerating lipid accumulation or macrophage chemotaxis. Proliferation is probably an early event, followed by a chronic process that provides an essential fibrous cap that prevents plaque rupture.

Several vascular diseases involve VSMC proliferation as the primary pathophysiologic mechanism. These clinical conditions include in-stent restenosis, transplant vasculopathy and vein bypass graft failure.[20] Ironically, these conditions develop as consequences of the procedures used to treat occlusive atherosclerotic diseases. Indeed, 30-40% of patients undergoing percutaneous balloon angioplasty will develop restenosis within the first 6 months.[21] With the recent deployment of stents, this incidence is now about 20%, still an unacceptably high rate.[21] Vein graft failure ranges from 10 to 30% per year.[22] These vascular proliferative diseases are initiated by mechanical, biochemical or immunological

injury to the vessel wall. Vascular injury triggers a cascade of events that includes endothelial denudation or dysfunction, inflammation and VSMC activation and proliferation. Myriad growth factors and cytokines can be detected in human vascular lesions. These mediators may be released by dysfunctional endothelial cells, inflammatory cells, platelets and VSMCs, mediating chemoattraction, cell migration, proliferation, apoptosis and matrix modulation.[3]

Understanding of the responses of growth factors and VSMC proliferation to vascular injury is derived mainly from studies involving animal models of arterial injury. Direct data are difficult to obtain from human disease. In the rat model, basic fibroblast growth factor, released from dying vascular cells, can initiate medial proliferation of VSMCs,[23] whereas platelet-derived growth factor may induce subsequent migration of VSMCs toward the intima.[24] Intimal proliferation and matrix accumulation occur under the influence of platelet-derived growth factor, transforming growth factor-β, angiotensin II, epidermal growth factor and insulin-like growth factor 1.[25-28] Furthermore, loss of growth-inhibitory factors, occurring as a result of decreased endothelial cell secretion of nitric oxide (NO), inactivation of NO by reactive oxygen species or altered heparan sulfate proteoglycan synthesis, may also contribute to the migration and proliferation of VSMCs and to the increased inflammatory response.[29,30]

With the recognition of the essential involvement of VSMC proliferation in the conditions described above and the improved understanding of the molecular and cellular mechanisms of cellular proliferation, antiproliferative therapeutic modalities have become a focus of research and development. In the following sections, we review the mechanisms of vascular proliferation and cell cycle regulation and examine the therapeutic potential of targeting these processes.

Cell Cycle and Vascular Disease

Mitogenic growth factors share a final common signalling pathway: the cell cycle (Fig. 2). Quiescent (G0) cells enter a gap period (G1), during which the factors necessary to DNA replication for the subsequent synthetic (S) phase are assembled. After DNA replication is completed, the cells enter another gap phase (G2) in preparation for mitosis (M). Restriction points at the G1-S and G2-M interphases ensure orderly cell cycle progression.[31]

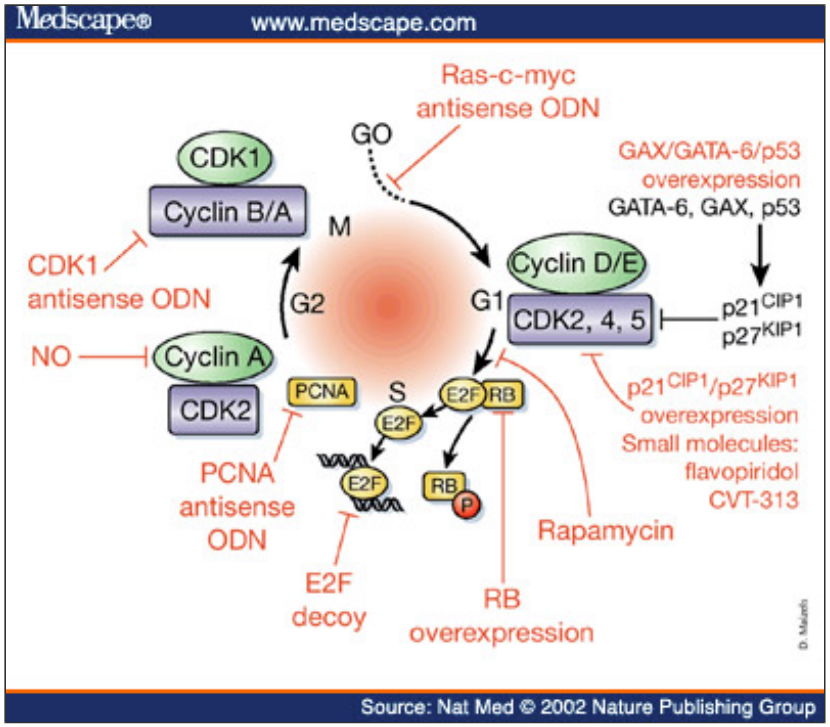


Figure 2. Simplified scheme of the cell cycle. Cell cycle progression is dependent on the orchestrated expression and activation of specific enzymes (CDKs) that form holoenzymes with their regulatory subunits, the cyclins. CKIs ($p27^{KIP1}$ and $p21^{CIP1}$) bind to and inhibit the activation of cyclin–CDK complexes. Therapeutic approaches to vascular proliferative diseases are shown in orange. CDC2, cell division cycle 2.

Cell cycle phases are coordinated by cyclin-dependent kinases (CDKs) that form holoenzymes with their regulatory subunits, the cyclins.[32] The activities of cyclin-CDK complexes depend on the phosphorylation status of CDKs and the

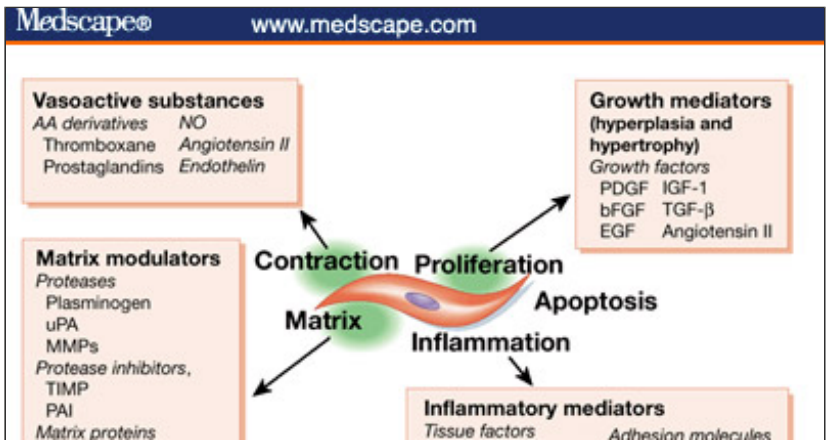
expression level of cyclins. Phase-specific cyclin-CDK complexes confer specificity and orderly progression through the cell cycle. Initially, increasing accumulation of cyclin D-CDK4 and cyclin E-CDK2 complexes, in cooperation with proliferating cell nuclear antigen (PCNA), coordinate DNA replication by regulating the transition through the G1 and S phases.[33] The subsequent G2-M transition is regulated by cyclin A-CDK2 and cyclin B-CDK1 complexes. Furthermore, cell cycle progression is regulated by cyclin-dependent kinase inhibitors (CKIs) such as $p27^{KIP1}$ and $p21^{CIP1}$, which bind to CDKs and prevent their activation.

In addition to being controlled by the regulatory cyclin-CDK complexes, cell cycle progression is modulated by transcription factors that transactivate CDKs and CKIs. Antimitogenic signals activate p53, which induces expression of the CKI $p21^{CIP1}$ and consequently inhibits the activity of the G1 cyclin-CDK complexes, resulting in G1-phase arrest.[34] Conversely, the E2F family of transcription factors controls expression of genes in S-phase.[33] In quiescent conditions, E2F members exist in inactive complexes with retinoblastoma (RB) protein. After mitogenic stimulation, the cyclin D-CDK4 and cyclin E-CDK2 complexes hyperphosphorylate RB, leading to dissociation of E2F, which in turn activates the expression of genes such as those encoding cyclins E and A and CDK1. In addition to p53 and E2F, GAX and GATA-6 are also relevant cell cycle-associated transcription factors in VSMCs. GAX, a homeobox transcription factor that regulates cell differentiation, proliferation and migration, is expressed in quiescent VSMCs.[35] GATA-6 is a transcription factor involved in tissue-specific gene expression including VSMCs.[36] Both stimulate the expression of $p21^{CIP1}$ and induce subsequent cell cycle arrest.[35,37] Both are downregulated by mitogen stimulation *in vitro* and in response to vascular injury *in vivo*.[35,36]

Finally, transcription factors are not the only means by which the cell cycle is regulated in VSMCs. NO represses mitogen-stimulated cyclin A promoter activity, resulting in a cell cycle arrest through blockade of cyclin A mRNA and protein upregulation.[38] In addition NO inhibits proliferation by upregulation of $p21^{CIP1}$.[39] These antiproliferative effects of GAX, GATA-6 and NO make them attractive therapeutic targets for the treatment of vascular proliferative diseases.

Vascular Proliferation and Inflammation Are Linked

VSMCs can synthesize and secrete biologically active mediators that regulate contraction and relaxation, inflammation, proliferation, apoptosis and matrix alterations (Fig. 3). Moreover, there is increasing evidence that connections exist between proliferation and other cellular processes that are important for the pathophysiology of vascular disease. For example, the apparatus governing the cell cycle is able to modulate cell differentiation[40] and programmed cell death (apoptosis).[41] Furthermore, VSMC is inhibited when cell cycle progression is blocked by overexpression of the cell cycle-inhibitory protein $p21^{CIP1}$ or antisense oligonucleotides (ODNs) against c-*myc*.[42,43] Enhanced proliferation has been associated with enhanced extracellular matrix production, which could be prevented by c-*myc* antisense ODNs.[44] The cell cycle machinery may, furthermore, affect cell commitment to hyperplasia or hypertrophy.[45] The processes of proliferation and inflammation are also linked. Cell cycle arrest was able to preserve a non-activated cellular phenotype through prevention of adhesion molecule expression *in vivo*, thereby reducing the susceptibility to atherosclerosis or vasculopathy.[46] CDKs regulate transcriptional gene activation by nuclear factor-κB,[47] providing a mechanism for coordination of adhesion molecule expression with cell cycle progression. Cyclooxygenase 2, essential in inflammation, is also expressed in a cell cycle-dependent way.[48] Conversely, cytokines can influence cell cycle progression. For example, macrophage migration inhibitory factor, which is essential in several inflammatory conditions, stabilizes the cell cycle-inhibitory protein $p27^{KIP1}$ and blocks proliferation.[49]



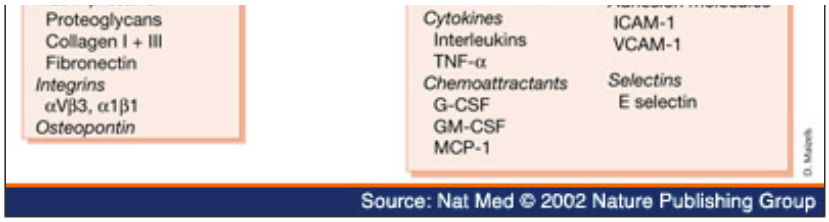


Figure 3. VSMCs mediate proliferation, inflammation, matrix alterations and contraction. Many of the mediators have multiple functions. For example, angiotensin is a vasoconstrictor, but also stimulates proliferation and inflammation. The list here of mediators secreted by VSMCs is not complete. AA, arachidonic acid; TNF, tumor necrosis factor; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte–monocyte colony-stimulating factor; MCP, monocyte chemoattractant protein; ICAM, intracellular adhesion molecule; VCAM, vascular cell adhesion molecule; PDGF, platelet-derived growth factor; bFGF, basic fibroblast growth factor; EGF, epidermal growth factor; IGF, insulin-like growth factor; TGF, transforming growth factor; uPA, urokinase-type plasminogen activator; MMPs, matrix metalloproteinases; TIMP, tissue inhibitor of metalloproteinases; PAI, plasminogen activator inhibitor.

Supporting this link of vascular proliferation and inflammation is the observation that impaired NO bioactivity in vascular disease is associated with VSMC proliferation and inflammation.[1] *In vivo* overexpression of nitric oxide synthase resulted in reduction of atherosclerotic or restenotic lesion formation in rabbits through both inhibition of VSMC proliferation and inhibition of adhesion molecule expression with subsequent reduction of vascular mononuclear cell infiltration.[50,51]

Cell-Cycle Inhibition as a Therapeutic Strategy

With improved understanding of the molecular mechanism of vascular proliferative diseases, opportunities have emerged for the development of treatment strategies that may inhibit or block the pathological processes of vascular proliferation. Antiproliferative drugs have been tested as a means to prevent vascular proliferative diseases. These agents include heparins, angiotensin-converting enzyme inhibitors, antagonists to growth factors such as terbinafine or trapidil (inhibitory to platelet-derived growth factor), angiopeptin (a peptide analog of somatostatin), cytostatic agents such as etoposide or doxorubicin, and calcium-calmodulin antagonists. However, clinical trials have generally failed to recapitulate the efficacy documented in animal studies.[52] These failures could reflect species differences in the response to vascular injury or limitations on matching experimental dosing regimens in humans. Moreover, they could emphasize the improbability that a multifactorial disease can be treated successfully by targeting a single mitogenic factor.

The focus of interest in antiproliferative therapeutics, therefore, has shifted to targeting specific parts of the cell cycle as a 'final common pathway'. Cell-cycle inhibition can be achieved by pharmacological agents, irradiation or gene therapy (Table 1). These can inhibit vascular proliferation by cytotoxic or cytostatic mechanisms. The cytotoxic approach involves the destruction and death of vascular cells, whereas the cytostatic approach blocks cell cycle progression without inducing cell death. Theoretically, a cytostatic strategy would be preferred, as cytotoxic treatment may result in a weakening of the vessel wall, thereby predisposing it to enlargement or aneurysm formation. So far, the therapeutic application of cell cycle inhibition has been limited to in-stent restenosis and bypass graft failure, as these conditions are characterized by substantial proliferative activities that constitute the main basis of these disorders.

Pharmacological Approach

The emphasis of the pharmacological approach is the development of cytostatic molecules. The most promising agent is rapamycin. Rapamycin is a macrocyclic triene antibiotic and natural fermentation product produced by *Streptomyxes hygroscopius* that was isolated from a soil sample collected from Easter Island (Rapa Nui) in the 1970s.[53] After it was studied extensively for its immunosuppressive properties, rapamycin was also reported to block the proliferative response of VSMCs after mechanical or immune-mediated injury, such as balloon angioplasty or allorejection.[54] Recent studies have elucidated the molecular effects of rapamycin in VSMCs and its effects on cell cycle regulation and the prevention of neointimal development[55,56] (Fig. 4*a*).

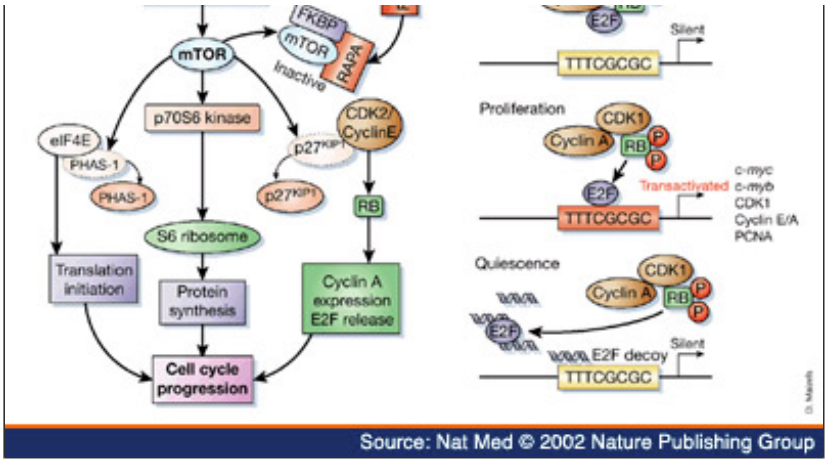


Figure 4. Mechanisms of action of rapamycin and E2F decoy. ***a***, The mTOR–p70S6 kinase signaling pathway. Rapamycin (RAPA) interferes with mTOR and its downstream signaling cascades, involving p70S6 kinase and eIF4E, leading to a reduction in protein synthesis and concomitant cell cycle arrest. FKBP, FK-binding protein; PHAS-1, the EIF4E-binding protein. ***b***, Principle of E2F decoy therapy. Top, the transcription factor E2F is inactivated by its binding to hypophosphorylated (P in red circle; represents monophosphate) RB. Middle, hyperphosphorylation of RB by G1-phase cyclin–CDK complexes liberates E2F, which binds to the *cis* element of cell cycle–regulatory genes and induces their transactivation. Bottom, double-stranded 'decoy' *cis* element binds to free E2F and prevents E2F-dependent gene transactivation.

Rapamycin complexes with FK-binding protein 12, a member of the group of specific intracellular binding proteins called immunophilins. This rapamycin-immunophilin complex inhibits the mammalian target of rapamycin (mTOR). In addition to its regulatory effect on G1-phase CDKs,[57] mTOR activates the 70-kDa S6 protein kinase[58], an enzyme involved in modulating the level of 40D ribosomal protein S6 phosphorylation. Furthermore, mTOR increases the activity of the eukaryotic initiation factor 4E (eIF4E) through phosphorylation and inactivation of its inhibitor, eIF4E-binding protein.[59] The 70-kDa S6 protein kinase and eIF4E are pivotal in translation initiation, which is required to accelerate the rate of protein synthesis in preparation for cell division. Inhibition of translation initiation by mTOR is thought to be the main mechanism of the antiproliferative effect of rapamycin. Indeed, rapamycin exerts favorable effects on other vascular growth processes. For example, rapamycin inhibits migration of VSMCs,[60] hypertrophy in response to angiotensin II[61] and tumor necrosis factor-α-induced expression of the intracellular cell adhesion molecule 1 and vascular cell adhesion molecules (V.J.D., R.C.B.-D. and D.G.S., unpublished data).

In the RAVEL trial, 238 patients were randomly assigned in a double-blinded way to receive either standard or rapamycin-coated stents. No (0%) restenosis occurred in patients receiving rapamycin-coated stents at 1 year compared with 26% in patients receiving placebo (P </= 0.001).[62] Further studies are now underway to verify these early, very encouraging findings in larger controlled, randomized trials (such as the SIRIUS trial; interim analysis reported at the Transcatheter Cardiovascular Therapuetics annual meeting, P.J. Fitzgerald, personal communication, September 2002). Furthermore, ongoing clinical trials should prove rapamycin's effectiveness in preventing accelerated arteriopathy or chronic graft vascular disease due to intimal hyperplasia in transplanted hearts.

Another promising agent is paclitaxel (Taxol), which was first extracted from the bark of the Pacific yew (*Taxus brevifolia*) in 1963.[52] It was found to have cytotoxic activity and is used therapeutically to treat ovarian cancer. Paclitaxel promotes polymerization of tubulin, thereby inhibiting the disassembly of microtubules. This dysfunction inhibits cell division through inhibition of the mitotic spindle and promotes cell death.[63] It prevents neointima formation in animal models.[64] Its clinical efficiency in preventing restenosis is now being evaluated in human trials (the ELUTES, TAXUS I and ASPECT trials) using local delivery through stents coated with this drug (A. Gershlick, E. Gruber and A.E. Raizner, personal communications, September 2002). The first experience with paclitaxel-coated stents in 15 patients with in-stent restenosis showed minimal lesions at the 6-month follow up. However, it is still unclear if the effect is maintained at 12 months.[65]

Small molecules or low-molecular-weight compounds that interfere directly with cell cycle proteins have also been a target of drug development. Two molecules that inhibit CDKs are of particular interest. Flavopiridol blocks the ATP-binding sites of CDKs that are structurally different from the ATP-binding sites of the other kinases.[66] This drug inhibits VSMC

replication *in vitro* and neointima formation *in vivo*.[67] CVT-313, a purine analog, inhibits CDK2 and blocks neointimal hyperplasia *in vivo*.[68] These features may be particularly useful when the drug is delivered locally by drug-coated stents or local application using polymers.

Brachytherapy

Local delivery of either β or γ radiation to the stented artery reduces intimal tissue responses.[69,70] So far, no convincing data have shown any efficacy for brachytherapy in non-stented coronary obstructions. Although the paucity of actively dividing cells in the neointima is of concern, the main effect of vascular brachytherapy may reflect irradiation-induced DNA damage of vascular cells with consequent arrest at the G1 checkpoint or induction of apoptosis through p53-induced $p21^{CIP1}$ upregulation.[20,71] Safety concerns include delayed re-endothelialization with increased risk of stent thrombosis, potential late aneurysm formation and paradoxical excessive tissue proliferation because of injury of some cellular populations.

Gene Therapy

Gene therapy options range from antisense ODNs to transcription factor 'decoys' to overexpression of specific genes through gene transfer to achieve a cytostatic effect (Table 1). Antisense ODNs, designed to inhibit the expression of cell cycle-regulatory genes such as those encoding c-*myc*, c-*myb*, PCNA or different CDKs, have been used successfully in models of vascular lesion formation.[20,72,73] However, a recent trial of intracoronary administration of antisense ODNs against c-*myc* failed to demonstrate prevention of in-stent restenosis in humans.[74] Reduction of stenosis in a porcine model of coronary stent placement has also been achieved through blockade of PCNA expression with ribozymes, RNA molecules designed to cleave mRNA in a sequence-specific way.[75]

Targeting cell cycle molecules by overexpression of the CDK inhibitor $p21^{CIP1}$ using gene therapy achieved a significant inhibition of RB phosphorylation and reduced neointimal hyperplasia in injured rat carotid arteries.[76] Similarly, fusigenic liposome-mediated gene transfer of p53 inhibited neointima formation,[77] as did adenovirus-mediated overexpression of $p27^{KIP1}$, GATA-6 or GAX.[35,78,79] Transduction of porcine femoral or rat carotid arteries with an adenoviral vector expressing a nonphosphorylatable, constitutively active form of RB significantly reduced neointima formation, presumably through the inhibition of E2F activity.[80] Finally, NO potently downregulates cyclin A and upregulates $p21^{CIP1}$, resulting in cell cycle arrest.[38,39] *In vivo* transfer of different isoforms of the gene encoding nitric oxide synthase inhibited injury-induced neointima formation in various animal models,[50,51] and gene therapy trials are underway to show the effectiveness and feasibility of this approach.[81]

Targeting E2F directly using transcription factor decoy ODNs that bind E2F (called 'E2F decoy ODNs' here), responsible for the induction of multiple cell cycle-dependent genes, prevented neointimal hyperplasia in balloon-injured arteries and vein grafts[46,82] (Fig. 4*b*). These encouraging results led to the initiation of the first clinical trial using genetic engineering techniques to inhibit cell cycle activation in vein grafts. A prospective, randomized, controlled trial (PREVENT I) demonstrated the safety and biologic efficacy of intraoperative transfection of human bypass vein grafts with E2F decoy ODNs when they were used in bypass grafts for peripheral arterial occlusion in a high-risk human patient population.[22,83] Although the results are preliminary, this study provided evidence that antiproliferative gene therapy is feasible. Together with the clinical safety, the ease of incorporating the treatment of the vein graft during routine bypass surgery offers a potentially useful approach for the prevention of coronary as well as peripheral bypass failure. Recently, data were reported from the PREVENT II investigators E. Grube, personal communication, November 2001). The PREVENT II trial was a randomized, double-blinded, placebo-controlled phase 2b trial studying the safety and feasibility of the use of E2F decoy ODNs in preventing autologous vein graft failure after coronary artery bypass surgery. The results confirmed the safety and feasibility of using E2F decoys in the treatment of coronary bypass grafts. The analysis of the secondary endpoint using quantitative coronary angiography and three-dimensional intravascular ultrasound demonstrated an increased patency and positive vascular remodeling (inhibition of neointimal size or volume) in the treated group at 12 months after treatment. Further assessment of this encouraging therapeutic approach is needed, which will require adequately powered phase 3 studies.

The antiproliferative strategies used so far have focused on those vascular proliferative diseases that are rapid and localized (such as restenosis or graft failure). Atherosclerosis, however, is a slow and diffuse disease, and thus the present approaches of relatively short-term local delivery of cell cycle-inhibitory agents would probably not be effective for this

condition. However, antiproliferative therapy may result in an increase in acute ischemic events. The view of the smooth muscle cell as being protective raises the concern that long-term antiproliferative treatment would weaken the fibrous cap of atherosclerotic lesions, thereby increasing their susceptibility to rupture.[5] Experimental studies are needed to obtain insights into these questions.

Future Perspectives

In summary, vascular proliferation is central in atherosclerosis and is linked to other cellular processes such as migration, inflammation, apoptosis and extracellular matrix alterations. The contribution of vascular proliferation to the pathophysiology of post-intervention restenosis, transplant vasculopathy and vein bypass graft failure is pivotal. Although the benefits and risks of antiproliferative therapy for atherosclerosis can be debated, inhibition of cellular proliferation by targeting cell cycle regulation for preventing in-stent restenosis and vein bypass graft occlusion is a rational strategy. Indeed, the data at present indicate that cell cycle-inhibitory therapy represents a considerable advancement in the treatment of conditions associated with the consequences of atherosclerosis management.

Tables

Table 1. Targets of cell-cycle inhibition in vascular proliferative disease

Pharmacological approach	Site of action	Ref.
Rapamycin	Protein translation	54,56,62
Paclitaxel	Microtubule assembly	64,65
Flavopiridol	ATP-site inhibitor of CDKs	67
CVT-313	ATP-site inhibitor of CDKs	68
Irradiation		
Brachytherapy	DNA damage, p53	69,70
Gene therapy		
Antisense ODN	CDK1 and CDK2	20,72
	MYC	74
	MYB	20
	PCNA	72,73
Ribozymes	PCNA	75
Transcription factor decoys	E2F	22,46,82,83
Gene transfer	$p21^{CIP1}$	76
	$p27^{KIP1}$	78
	NOS	50,51,81
	p53	77
	RB	80
	GAX	35
	GATA-6	79

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