Over the last decade, percutaneous coronary interventions (PCIs) have become the standard therapeutic approach to symptomatic coronary artery disease. Currently, more than 1 million PCI procedures, including coronary balloon angioplasty, stenting, and rotational atherectomy, are performed every year worldwide, with one half conducted in the United States. Compared with bypass surgery for multivessel disease, PCIs offer similar protection against major ischemic events and are less expensive. Despite great advances in device technology and adjunctive pharmacotherapy, restenosis (i.e., late renarrowing of the target lesion) remains as the limitation of PCI.

The incidence of angiographic restenosis (greater than 50% reduction in luminal diameter) is 40% to 50%. Approximately one half of patients with angiographic restenosis experience clinical restenosis with recurrent symptoms that lead to major adverse cardiac events (MACEs) or target vessel revascularization (TVR). Thus, stent implantation, which decreases the rate of angiographic restenosis to 20% to 30%, has become the mainstream intervention, currently used in more than 70% of PCIs. Nevertheless, the rate of repeat revascularization is still unacceptably high (9% to 15%) with intracoronary stents. There are four primary pathologic processes responsible for restenosis after overdistension of the diseased vessel by angioplasty: elastic recoil, thrombogenesis, neointimal formation, and remodeling. The results of intravascular ultrasound studies suggest that coronary stenting reduces restenosis rate, compared with coronary balloon angioplasty, by preventing both elastic recoil and negative remodeling as luminal scaffolds. In addition, aggressive antiplatelet therapy and various stent coating materials have reduced subacute thrombosis that had once been the major issue with early stent devices. Neointimal formation, however, has remained a major obstacle. Neointimal formation is responsible for both in-stent restenosis (ISR) and postballoon angioplasty restenosis (Fig. 25-1). This chapter reviews molecular and cellular events involved in neointimal formation.

NEOINTIMIAL FORMATION

The neointima is rich in smooth muscle cells (SMCs) that are surrounded by extensive extracellular matrix. Neointimal formation is believed to be an exaggerated wound healing process in response to various forms of vascular injuries associated with PCI, including vascular wall distension, endothelial denudation, atherosclerotic plaque rupture, medial dissection, and fracture of the internal elastic lamina. Balloon injury induces apoptosis of medial SMCs in an early phase, which may exacerbate subsequent neointimal formation by provoking a greater wound healing response to overcome the cellular deficit. Neointimal formation is seen in coronary artery bypass grafts, transplanted hearts, arteriovenous fistulas, and angioplastied vessels. Although this complex process is not completely understood, longstanding research efforts have provided critical clues about the therapeutics of restenosis that have led to a number of ongoing clinical trials.

Inflammation

Leukocytes and other mediators of inflammation appear to play a major role in the development of the neointima. In balloon-injured coronary arteries, leukopenia decreases neointimal formation and adventitial fibrosis. Balloon angioplasty upregulates selectins, integrins, VCAM-1, and ICAM-1 on the remaining endothelium of the injured artery, resulting in leukocyte adhesion to the injured vessel wall within 24 to 48 hours. Leukocytes, recruited by selectin-mediated attachment and rolling, activate and express surface integrins that facilitate the adherence to ICAMs on the endothelial cells, thereby promoting leukocyte transendothelial migration to the sites of inflammation. Perturbation of the selectin-mediated process reduces the adhesion of platelets and leukocytes to injured arteries and prevents subsequent neointimal formation, adventitial inflammation, and negative vascular remodeling. ICAM-1 and VCAM-1 are receptors that mediate adhesion of leukocytes to endothelial cells. The serum levels of ICAM-1 and also a neutrophil integrin Mac-1 (CD11b/CD18) after PCI correlate with the late restenotic lumen loss. Coronary stenting is associated with a persistent increase in plasma ICAM-1 levels, which may explain the mechanism of increased neointimal formation with stent devices compared with balloon angioplasty. Inhibition of ICAM-1 or Mac-1 diminishes medial leukocyte accumulation, SMC proliferation, and neointimal formation after PCI. In addition, a single nucleotide polymorphism of the Mac-1 gene is associated with a lower
The development of the neointima is also mediated by various inflammatory cytokines. Balloon angioplasty upregulates IL-1 and TNF-α, which increase adhesiveness of cultured endothelial monolayers for circulating immature bone marrow cells and mature leukocytes and promote transendothelial migration of leukocytes. Both IL-1α and TNF-α stimulate SMC proliferation by inducing PDGF. In experimental models, external application of IL-1 and TNF-α increases the endothelial expression of both ICAM-1 and VCAM-1, thereby promoting leukocyte adhesion to the vessel wall and neointimal thickening and luminal narrowing. There is also a positive correlation between the restenosis rate and the preprocedural plasma level of IL-6 or C-reactive protein. In addition, inhibition of IL-6 significantly reduces TNF-α-induced SMC proliferation in vitro.

**Thrombogenesis**

The role of thrombogenesis in neointimal formation has been recognized since an early study reported that thrombocytopenic rats develop little neointimal formation in balloon-injured carotid arteries. Platelet adherence and aggregation occur at the vascular surface components that are exposed by angioplasty, such as collagen, von Willebrand factor, fibronectin, and laminin. Thrombus formation is observed on stent struts within 10 days after stent deployment. Thrombus formation is stimulated by tissue factor (TF), an endogenous procoagulant upregulated in balloon-injured vessels. TF initiates the extrinsic coagulation pathway by activating factor VII, which subsequently activates factors IX and X, resulting in thrombin formation. Thrombin stimulates release of PDGF, proliferation of SMCs, and alteration of extracellular matrix composition.
monocytes, and SMCs upregulate the gene expression of TF further perpetuating the thrombogenic cycle.\textsuperscript{57} Termination of the thrombogenic cycle with the recombinant TF pathway inhibitor (rTFPI) or with hirudin, a direct thrombin inhibitor, has been demonstrated in experimental models. rTFPI markedly inhibits fibrin formation and subsequent neointimal development on the balloon-injured arteries, whereas hirudin curtails persistent expression of TF following balloon injury and diminishes restenosis.\textsuperscript{57-60} However, hirudin did not affect the late luminal loss or event-free survival in patients treated with coronary angioplasty.\textsuperscript{70} In addition to hirudin, a number of agents that interrupt thrombogenesis, including glycoprotein IIb/IIa inhibitors, were examined in clinical trials and failed to show benefit in preventing postangioplasty restenosis.\textsuperscript{71,72} Failure of the antithrombotic approach to affect the outcome of restenosis may reflect the complexity of the pathogenesis of the human neointima.

**Growth Factors**

Growth factors released after arterial injury play critical roles in neointimal formation. Thrombus formation and endothelial denudation promote platelet degranulation within the thrombi, releasing growth factors such as PDGF, FGF, and TGF-β. These factors act in a complementary and interdependent fashion. PDGF is a potent mitogen that is immediately released in response to injury or inflammation, and thus regarded as one of the most important initiators of neointimal formation. In carotid artery injured rats, PDGF increases the migration of SMCs from the media to the intima and the proliferation of medial SMCs.\textsuperscript{60,63} PDGF also induces expression of MCP-1, which may contribute to monocyte activation. Overexpression of PDGF-B by direct gene transfer into porcine arteries induces neointimal formation \textit{in vivo} with prominent SMC proliferation.\textsuperscript{74} In animal balloon injury models, disruption of the interaction between PDGF and its receptors decreases neointimal formation.\textsuperscript{75-77} A clinical trial has shown that PDGF antagonists prevented angiographic and clinical restenosis after balloon angioplasty, but the benefit was modest.\textsuperscript{78} FGF is another potent mitogen that initiates SMC proliferation. Augmentation of acidic FGF (aFGF) or basic FGF (bFGF) results in neointimal formation with accentuated intimal angio genesis \textit{in vivo}.\textsuperscript{79,80} Antibodies against bFGF before balloon injury significantly decrease SMC proliferation but do not affect subsequent neointimal formation.\textsuperscript{81,82} TGF-β, another putative culprit of neointimal formation, is believed to be a cell-type-specific regulator of proteoglycan synthesis in human blood vessels. TGF-β stimulates synthesis of proteoglycans specifically in human adult arterial SMCs \textit{in vitro} but does not significantly stimulate proliferation of quiescent SMCs or inhibit proliferating cells.\textsuperscript{83} Overexpression of TGF-β1 \textit{in vivo} promotes procollagen synthesis and neointimal formation, both of which are inhibited by ribozyme oligonucleotides against TGF-β.\textsuperscript{84,85} Human neointimal SMCs from atherectomy tissues contain a high level of another growth factor IGF-1 and type 1 IGF receptor and IGF binding proteins.\textsuperscript{86} IGF-1 promotes cell-cycle progression and mitogenesis, resulting in SMC proliferation and migration.\textsuperscript{87,91} IGF-1 stimulates SMC proliferation synergistically with PDGF, and overexpression of IGF-1 in SMCs leads to SMC hyperplasia \textit{in vivo}.\textsuperscript{92,93} Contribution of IGF-1 in neointimal formation is also suggested by elevated levels of insulin and IGF-1 in diabetic patients, who have a significantly high incidence of restenosis after PCI.\textsuperscript{94,95} Although IGF-1 inhibitors, such as somatostatin analogs octreotide and angiopeptin, inhibit SMC proliferation and neointimal formation in animal studies, clinical trials with IGF-1 inhibitors failed to show consistent benefits.\textsuperscript{96-102}

**Smooth Muscle Cells**

\textit{Origins of Neointimal Smooth Muscle Cells}

Neointimal SMCs appear to represent a distinct cell population of heterogeneous origins. Neointimal SMCs are different from medial SMCs in phenotype and gene-expression patterns.\textsuperscript{103-105} Neointimal SMCs (\textit{synthetic phenotype}) appear less mature than medial SMCs (\textit{contractile phenotype}). The synthetic phenotype is epithelioid shaped as opposed to the spindle-shaped contractile phenotype and contains prominent endoplasmic reticulum and Golgi complex, which suggests active protein synthesis and cell proliferation.\textsuperscript{106,107} The synthetic phenotype also exhibits a reduced expression of α-smooth muscle actin (α-SMA) and laminin, and an increase in fibronectin, tropoelastin, and α1 procollagen (type I). In addition, the synthetic phenotype expresses a large number of growth factor receptors and produces PDGF-B, TGF-β1, IGF-1, and osteopontin.\textsuperscript{108,109}

These observations have led to the concept of \textit{phenotypic modulation} or dedifferentiation, that is, neointimal SMCs originate from normal SMCs in the tunica media at the injury site through regression to a less mature phenotype, followed by proliferation, migration, and synthesis of extracellular matrix in the tunica intima.\textsuperscript{103,104,110,111} Neointimal SMCs may also derive from remnant precursor cells in the tunica media that become activated in response to injury.\textsuperscript{112}

Fibroblasts in the tunica adventitia have also been suggested as another possible origin of neointimal SMCs. Fibroblasts are relatively undifferentiated and can assume a particular phenotype in response to physiologic needs and/or microenvironmental stimuli.\textsuperscript{113} Adventitial fibroblasts acquire α-SMA after angioplasty and are transformed into myofibroblasts, which are phenotypically similar to synthetic SMCs and proliferate earlier than medial SMCs.\textsuperscript{114-117} Fibroblasts, myofibroblasts, and synthetic SMCs may be part of the same spectrum of cells, deriving from a common progenitor cell as well.\textsuperscript{115,116,121} In fact, both SMCs and fibroblasts originate from epicardially derived mesenchymal cells in the developing coronary arteries.\textsuperscript{122} A number of cytokines and growth factors influence proliferation and phenotypic transitions of fibroblasts and myofibroblasts, including TGF-β1, IFN-γ, and heparin.\textsuperscript{120,121,123-125} In rats, TGF-β1 receptor antagonists almost completely inhibit the induction of α-SMA expression in adventitial cells and decrease neointimal formation after balloon injury,
primarily through prevention of negative remodeling, in parallel with reduced adventitial fibrosis and collagen deposition. The migratory capacity of myofibroblasts in injured vessels, however, has not been definitively established. Although early studies have suggested that at least some of the neointimal SMCs may originate from the adventitia, two recent studies have reached opposite conclusions. When lacZ-transfected myofibroblasts are introduced into the adventitia after balloon injury in rat carotid arteries, β-galactosidase expression is observed in the adventitia, media, and neointima, suggesting that myofibroblasts have a viable migratory capacity. In contrast, adventitial cells stained with a fluorescent dye are not detected in the media or the neointima but are found exclusively in the adventitia after balloon angioplasty in the same animal model. Therefore, whether myofibroblasts migrate from the adventitia to the subendothelial layer to participate in neointimal formation remains unclear.

Finally, accumulated evidence strongly suggests that neointimal SMCs originate, at least in part, from circulating progenitor cells. Hematopoietic stem cells rapidly and constitutively migrate through the blood. Bone marrow cells migrate to the infarcted lesion of the heart, replicate, differentiate, and ultimately promote myocardial repair. Blood cells contain progenitors that have the potential to differentiate into SMCs or endothelial cells in vitro. In addition, neointimal SMCs express a number of hematopoietic lineage markers. In transplant vasculopathy observed in the murine heterotopic cardiac allograft, most neointimal cells derive from the recipient. Neointimal cells developed in the murine aortic transplant allograft derive almost exclusively from the recipient, and at least a subset originates from recipient bone marrow cells. Recently, it has been discovered that purified hematopoietic stem cells differentiate into SMCs in vitro and in vivo and that bone marrow cells engender most SMCs and endothelial cells that contribute to neointimal formation in mouse models of atherosclerosis, transplant vasculopathy, and postangioplasty restenosis. These different origins of neointimal SMCs are most likely not mutually exclusive but participate together in creating the cell population in the neointima after arterial injury.

Smooth Muscle Cell Proliferation

Cell proliferation is ultimately dependent on key cell-cycle events (Fig. 25-2). A number of positive and negative regulatory molecules play crucial roles in proper progression of the cell cycle, including cyclins, cyclin-dependent kinases (cdks), and cyclin-dependent kinase inhibitors (cdkis). Cyclins form a complex with cdks to initiate the cell cycle, whereas cdkis maintain the quiescent status by inhibiting cyclin/cdk complexes. The cdkis are structurally divided into two families: the Ink4 family (p14, p15ink4a, p16ink4a, p18ink4c, p19ink4d) and the Kip/Cip family (p21cip1, p27kip1, p57kip2). The Ink4 family controls the G1 phase through inhibition of cyclin D/cdk4 and cyclin D/cdk6 complexes, whereas the Kip/Cip family is induced by a tumor suppressor protein p53 and controls all phases of the cell cycle. Among the cdkis, p27kip1 is the pivotal molecule that regulates the transition from the G1 to S phase. Growth factors activate early cyclins (D1, 2, 3), which form a complex with early cdks (cdk4,5,6) and proliferating cell nuclear antigen (PCNA), a cofactor of DNA polymerase δ. The activated cyclin D/cdk/PCNA complexes reduce p27kip1, which

![Cell cycle](image-url)
maintained the cell in G\textsubscript{0} by inhibiting these complexes, and the cell enters the S phase.\textsuperscript{139} Ablation of p\textsuperscript{2\textsubscript{7}Kip\textsubscript{1}} gene leads to overproliferation of cells in most organs because of uncontrolled cell-cycle regulation.\textsuperscript{140–142} Another negative regulator that maintains the quiescent status is the retinoblastoma gene product RB.\textsuperscript{143,144} The active form of RB binds to a family of transcription factors E2F and maintains the cell cycle in G\textsubscript{1}. Uprogessed G\textsubscript{1} cyclin/cdk complexes phosphorylate and deactivate RB, which then releases E2Fs that interact with chromosomal DNA and RB-related proteins, upregulating genes required for the transition from the G\textsubscript{1} to S phase.\textsuperscript{145–150}

SMCs in the adult artery normally exist in the G\textsubscript{0}-1 phase of the cell cycle and do not proliferate. The quiescent state of SMCs may also be regulated by surrounding polymerized type I collagen fibrils via integrin signaling pathways.\textsuperscript{151} A growth arrest homeobox gene \textit{gax}; mainly expressed in cardiovascular tissues, is downregulated within hours after angioplasty.\textsuperscript{152} Within 24 hours after balloon injury, the expression of p\textsuperscript{2\textsubscript{7}Kip\textsubscript{1}} is rapidly downregulated, whereas both p\textsuperscript{2\textsubscript{1}Cip\textsubscript{1}} and p\textsuperscript{53} levels are increased in the neointima within 7 days.\textsuperscript{153–155} p\textsuperscript{2\textsubscript{1}Cip\textsubscript{1}} is believed to provide a counterbalance to the increased accumulation and enzymatic activity of cyclin/cdk complexes.\textsuperscript{156} Balloon angioplasty also induces upregulation of PCNA, cdk2, cyclin E, and cyclin A within 48 hours.\textsuperscript{156–159} The expression of these molecules is increased in the media at 36 to 60 hours and in the neointima within 2 weeks.\textsuperscript{159}

In light of multiple potential origins of neointimal cells, the extent of contribution of SMC proliferation in the subendothelial layer to neointimal formation is not clear. Atherectomy tissues from human restenosis lesions contain a small number of proliferating cells, and the number of SMCs decreases over time as extracellular matrix increases.\textsuperscript{159–164} However, these tissues are mostly obtained at the chronic phase, and only fuggacious proliferation immediately after injury may generate a sufficient number of cells to produce the extracellular matrix of neointimal lesions. In addition, approaches aimed at cell-cycle inhibition have been successful in preventing neointimal formation in various animal models. These include the rapamycin (sirolimus)-eluting stent (Fig. 25-3A) (see the section on prevention of restenosis), antisense ODN-mediated inhibition of the positive regulators (PCNA, cdk1, cdk2, cyclin B1), viral vector-mediated overexpression of the negative regulators (RB, RB2/p130, p\textsuperscript{2\textsubscript{7}Kip\textsubscript{1}}, p\textsuperscript{2\textsubscript{1}Cip\textsubscript{1}}, p\textsuperscript{53}, \textit{Gax}), and E2F-decoy ODN.\textsuperscript{160,157,165–173} The efficacy of E2F-decoy ODN, which binds to and inactivates E2F, was evaluated in a clinical trial, and fewer graft occlusions, revisions, or critical stenoses were observed in the E2F-decoy group than in the untreated group\textsuperscript{172} (Fig. 25-3B). Furthermore, the antiproliferation approaches used in cancer therapy have also been shown to be effective. Overexpression of cdc2, DNA, thymidine kinase, or Fas ligand in combination with parental drugs are the classic examples, and all of them significantly reduce neointimal formation.\textsuperscript{174–177} Therefore, SMC proliferation still appears to be one of the major components of neointimal formation, regardless of the origin of such cells.

Extracellular Matrix

The architectural integrity of the vascular layers is largely dependent on the equilibrium between MMPs and TIMPs. MMPs degrade extracellular matrix components and facilitate cell migration across the layers. The expressions of MMPs and TIMPs are very low in uninjured vessels but increase 2 hours after balloon injury. By 3 days after injury, gelatinases (MMP2 and MMP9) are highly expressed in the adventitial myofibroblasts surrounding the injury site, which are subsequently localized to the developing neointima. Expression of MMP2 and MMP9 is more intense and sustained in stent implantation than in balloon angioplasty. MMP inhibitors dramatically suppress SMC migration into the intima, but resultant neointimal formation is not affected, mainly because MMP inhibitors result in accelerated proliferation of SMCs that contributes to compensatory growth of the neointima. Adenoviral gene transfer of TIMP-1 reduced SMC migration after balloon injury in rats. Tenascin is another extracellular matrix protein associated with cell migration and the breaking of focal adhesions holding cells in place. Tenascin is upregulated early after vascular injury by angiotensin II (AngII) and PDGF-BB and mainly expressed by adventitial myofibroblasts. Tenascin expression shifts toward the luminal surface and reaches the developing neointima by 1 week, implying active migration from the adventitia to the neointima. In addition to active breakdown of extracellular components mediated by MMPs, balloon injury induces significant increase in synthesis of extracellular matrix components, including collagen, elastin, and proteoglycan, suggesting that there is a compensatory mechanism to maintain vascular structure.

Protooncogenes

Growth factors and cytokines appear to regulate the cell cycle through activation of proto-oncogenes in response to various stimuli induced by vascular injury. Within 30 minutes to 2 hours after balloon injury, growth factors induce a series of protooncogene expressions in SMCs, such as c-myc, c-fos, c-jun, and thrombospondin. The proto-oncogene c-myc is crucial for the progression from G0 to G1 in the cell cycle, which promptly activates cyclin E/cdk2 and cyclin D/cdk4 complexes in quiescent cells. Disruption of the c-myc gene delays the expression of cyclin E and cyclin A, but the expression of cyclin Ds and cdk2 is unaffected. The c-myc or c-myb antisense ODNs reduces neointimal formation in animal models of artery injury, but the recently completed ITALICS trial failed to prove any benefit in humans. The upregulation of c-myc and c-fos in response to balloon injury mirrors the downregulation of gax. The protooncogene products induce the expression of cell-cycle regulator genes (cyclin Ds, cdk4, cyclin E) and growth factors (PDGF-A, TGFB1, bFGF receptor), resulting in SMC proliferation in an autocrine fashion. A number of signal transduction cascades induced by growth regulatory signals (EGF, PDGF, bFGF and IGF-1) converge on a membrane-associated GTPase ras, which activates cytoplasmic second-messenger pathways leading to cell prolife-ration. The cooperative action of ras and myc controls the activation of cyclin E/cdk2 and E2F. Overexpression of ras protein increases cyclin D1 and shortens the G1 phase, resulting in cellular transformation, whereas antibodies against ras inhibit entry of cells into the S phase. Adenoviral gene transfer of a dominant negative form of ras in animal angioplasty models results in a marked reduction in neointimal formation, whereas a constitutively active form results in significant augmentation of neointimal formation.

Mitogen-Activated Protein Kinases

Through phosphorylation cascades, ras regulates the downstream mitogen-activated protein kinases (MAPKs), such as c-jun N-terminal kinases (JNKs) extracellular signal-regulated kinases (ERKs), and p38. JNKs appear to contribute to SMC hypertrophy and hyperplasia. ERKs regulate SMC proliferation in response to various growth stimuli. Balloon injury of the rat carotid artery dramatically enhances JNK and ERK activities, which are followed by an increase in the DNA binding activity of transcription factor activator protein-1 (AP-1) that contains Jun and Fos proteins. JNK activation is remarkably suppressed by ACE inhibitors and AngII type 1 receptor (AT1) antagonists. AT1 receptor antagonists also prevent activation of ERKs by suppressing their tyrosine phosphorylation, although ACE inhibitors fail to prevent such activation. The increased AP-1 DNA binding activity was significantly inhibited by both ACE inhibitors and AT1 receptor antagonists. Activated by growth factors (PDGF, TGF-β, bFGF) and cytokines (TNF-α, and IL-1) that are increased in balloon-injured vessels, the level of p38 increases as early as 15 minutes after balloon injury. In balloon-injured vessels, the distribution of p38 roughly corresponds to that of differentiated α-SMA positive cells, and inhibition of p38 reduces neointimal formation.

Angiotensin II

Arterial injury by angioplasty induces local expression of ACE, and ACE level is increased in the subsequently developed neointimal lesions. AngII enhances neointimal proliferation in vivo after vascular injury, whereas ACE inhibitors prevent this process by inhibiting PDGF-AB synthesis in SMCs. In addition, AT1 receptor antagonists reduce neointimal formation by inhibiting activation of PDGF α- and β-receptors and by downregulating PDGF-A and PDGF-B chains in injured arteries. AngII also appears to stimulate SMC proliferation through MAPK pathways. However, high-dose and long-term ACE inhibition did not prevent restenosis and did not favorably influence the overall clinical and angiographic outcome after coronary angioplasty in a large-scale clinical trial.

The ACE gene exhibits an insertion/deletion (I/D) polymorphism depending on the insertion (I) or deletion (D) of 287 base pairs in intron 16, and the DD phenotype is associated with high plasma and tissue ACE levels. Therefore, it has been speculated that the DD phenotype has a higher rate of neointimal formation.
However, the results from a number of clinical studies are inconsistent, and the causal relationship between the ACE I/D polymorphism and restenosis remains unproven.227

Nitric Oxide
Endothelial denudation by angioplasty also results in loss of NO, an endogenous proapoptotic substance. Overexpression of inducible NO synthase (iNOS) in SMCs leads to marked apoptosis, suggesting a counteractive role of NO against neointimal proliferation.228 NO inhibits platelet aggregation and SMC mitogenesis and proliferation via a cGMP-dependent pathway.229,230 Dietary administration of L-arginine, an NO precursor, reduces neointimal formation and substantially inhibits the accumulation of macrophages in the injured vessels of hypercholesterolemic rabbits.231 Overexpression of NOS, either endothelial (eNOS) or inducible (iNOS), has been shown to reduce neointimal formation in various animal models. In addition, endothelial progenitor cells isolated from peripheral blood, amplified ex vivo, and seeded on decellularized vessel grafts were found to produce NO and remain patent for 150 days, whereas the control grafts occluded within 15 days.130 These results indicate the significance of NO in neointimal formation and provide the basis for a novel therapy for restenosis.

Endothelin
Endothelin-1 (ET-1) augments neointimal formation in vivo, and its level is elevated in the human coronary sinus after coronary balloon angioplasty. Endothelin converting enzyme (ECE-1) is also increased in neointimal SMCs in both rat balloon-injured arteries and in human coronary atherosclerotic lesions. Blockade of ET receptor or ECE-1 reduces neointimal formation after balloon injury in experimental models.232,233

INFECTION AND RESTENOSIS
Over the past decade, infectious agents have attracted attention as a potential culprit of the pathogenesis of coronary restenosis. Approximately 33% of restenosis lesions contain sequences of cytomegalovirus (CMV) DNA, and SMCs grown from the restenosis lesions express CMV protein IE84 and high amounts of p53. Because IE84 binds to and inhibits p53, a hypothesis has been formed that CMV may enhance SMC proliferation via cell-cycle regulation.234 A prospective study of 75 patients undergoing directional atherectomy showed that CMV-seropositive patients had a greater reduction in the luminal diameter, resulting in a significantly higher rate of restenosis at 6 months than seronegative patients.235 Other epidemiologic studies have shown an increased prevalence of seropositivity of Chlamydia pneumoniae and Helicobacter pylori in patients with coronary artery disease. However, larger clinical studies failed to establish an association between the risk of restenosis and the serologic status of CMV, C. pneumoniae, or H. pylori.234,236–238 Therefore, it remains unclear whether these infectious agents contribute to pathogenesis of coronary restenosis.

PREVENTION OF RESTENOSIS
Although the need to overcome coronary restenosis resulted in an aggressive pursuit for novel therapies, more than 100 drugs and devices have failed to show any benefits.239–242 Recently, elucidation of molecular mechanism of neointimal formation has contributed to the emergence of potent antirestenosis therapies that yield consistently positive results.

Heparin-Coated Stents
Because subacute stent thrombosis was a major issue with early stent devices, various stent surface coatings were developed to reduce protein deposition and platelet adhesion. In early clinical studies, heparin-coated stents appeared to reduce subacute stent thrombosis in comparison with historical controls, but they did not significantly decrease the long-term restenosis rate.8,243 Heparin-coating of the stent was subsequently shown to have no impact on subacute stent thrombosis or on restenosis in direct comparison with bare metal stents.244,245

Intravascular Brachytherapy
Ionizing radiation nonspecifically destroys DNA double strands and, thus blocks cell proliferation.246 In response to DNA damage elicited by radiation, a tumor suppressor protein p53 upregulates p21Cip1 that directly inhibits PCNA, thereby allowing DNA repair and arresting cells in the G1 phase.247–251 In addition to inhibiting neointimal cell proliferation, intracoronary radiation therapy (brachytherapy) appears to prevent remodeling of the angioplasted vessel by inhibiting myofibroblast proliferation in the tunica adventitia, thereby attenuating adventitial scar formation and subsequent vascular remodeling.252,253 Two sources of radiation have been tested for brachytherapy: γ (photons) and β (electrons). γ-radiation penetrates beyond the vessel wall and requires extensive radiation protection of the operating personnel, whereas β-radiation is absorbed in living tissue by more than 99% within 5 mm from the source. Intracoronary brachytherapy with both γ and β radiation has been shown to be effective in preventing ISR in randomized clinical trials, reducing the rate of angiographic restenosis and repeat revascularization within the first year.254–257 In the SCRIPPS study, reduction of the rate of restenosis was still observed at 3-year follow-up, despite progressive decrease in lumen diameter between 6 months and 3 years.258,259

The initial excitement with intracoronary brachytherapy was dampened by several serious complications. Late stent thrombosis, manifesting as acute target vessel closure leading to acute myocardial infarction, occurs 1 to 9 months after brachytherapy in as many as 6% to 7% of the patients.260 The risk of late stent thrombosis appears low in the absence of new stent placement and
with extended antiplatelet therapy after intracoronary radiation. The FDA, therefore, recommends that antiplatelet therapy with aspirin and a thienopyridine derivative (clopidogrel or ticlopidine) be prescribed for a minimum of 6 months, and for at least 12 months if a new stent is implanted. The FDA also suggests that implantation of new stents be avoided after brachytherapy. Edge restenosis, or narrowing at the proximal and distal edges of the irradiated lesions, occurs often in patients who develop postbrachytherapy restenosis. Radioactive stent implantation is often associated with edge restenosis, or candy-wrapper effect. Therefore, radioactive stents perhaps delay but do not prevent neointimal proliferation. 281 Edge restenosis may be due to a progressive attenuation of radiation dose from the radiation source to the edges or from geographical miss resulting from technical issues or errors during the procedure. Development of coronary aneurysms within 6 months of brachytherapy has also been reported. 262, 263 The very long-term outcomes and complications of brachytherapy remain to be defined. 264

Drug-Eluting Stents

Antineoplastic or immunosuppressive drugs with antiproliferative activity have been shown to be effective in preventing neointimal formation in various animal models of vascular injury. However, these drugs have a high toxicity profile with systemic administration. Consequently, the previously developed surface-coating technology was applied to produce drug-eluting stents, which elute antiproliferative agents in the injured vessel. The advantage of drug-eluting stents is the achievement of highly concentrated drug delivery for a prolonged period of time with minimal systemic exposure. A variety of drug-eluting stents are currently under investigation, and some of them have yielded promising results.

Sirolimus (Rapamycin)

Sirolimus belongs to the class of macrocyclic immunosuppressive agents that bind to specific cytosolic proteins called immunophilins. 265 Cyclosporin A and tacrolimus (FK506) are two other members of this class. Although sirolimus and its analog tacrolimus share the same family of immunophilins (FKBP), sirolimus acts at a later stage in T-cell cycle progression by blocking cytokine-mediated signal transduction pathways. 265 FKBP, a sirolimus receptor, has been found to be up-regulated in neointimal SMCs in human atherectomy specimens from ISR lesions. 265 Sirolimus/FKBP complex ceases the cell cycle in transition from the G1 to S phase by inhibiting a specific cytosolic protein, the mammalian target of rapamycin (mTOR). mTOR is a key regulatory kinase that plays a major role in the mammalian cell cycle, and the inhibition of mTOR results in manifold effects to cease cell-cycle progression: downregulation of PCNA that is essential for DNA replication, inactivation of p70 S6 kinase and eukaryotic initiation factor 4E (eIF4E) that are crucial components of increasing protein synthesis in preparation for cell division, and inhibition of kinase activity of the cyclin D/cdk4 and cyclin E/cdk2 complexes via activation of p27Kip1 and RB (Fig. 25-3). 266 Sirolimus blocks both proliferation and migration of SMCs in vitro, 267, 268 inhibits arterial neointimal thickening, and reduces restenosis after angioplasty in various animal models of arterial injury. 269- 273

A small preliminary clinical trial demonstrated little neointimal formation at 12 month in patients with sirolimus-eluting stents. 274, 275 Subsequently, a multicenter, randomized clinical trial (RAVEL) reported significant reduction in angiographic restenosis in the sirolimus-eluting stent group compared with the bare metal stents group at 6 month (0% vs. 26.6%, P < 0.0001). There were no episodes of stent thrombosis, and the overall rate of major cardiac events was significantly lower in the sirolimus-stent group than in the standard-stent group during a follow-up period of up to 1 year (5.8% vs. 28.8%, P < 0.001). 276 Clinical benefits of sirolimus-eluting stents have consistently been demonstrated in larger double-blind clinical trials in patients with more challenging and complex lesions. 277, 278

Paclitaxel

Paclitaxel is an anticancer agent active against a broad range of cancers including breast, ovarian, and lung cancer. Paclitaxel promotes the polymerization of microtubules, which are extraordinarily stable and dysfunctional, thereby causing the death of the cell by disrupting the normal microtubule dynamics required for cell division and vital interphase processes. 279- 282 SMC proliferation and migration are inhibited by paclitaxel in both cell culture and a carotid artery balloon-injury model in rats. 281 In atherosclerotic rabbits, locally administered paclitaxel by a microporous balloon catheter prevented neointimal formation after balloon angioplasty. 282 In a pig coronary angioplasty model, paclitaxel-coated stents produced a significant dose-dependent inhibition of neointimal formation and luminal encroachment 28 days after implantation. 284

A randomized, double-blind, multicenter feasibility trial to evaluate the safety of the paclitaxel-coated stents (TAXUS I) demonstrated significant reductions in angiographic and intravascular ultrasound measures of restenosis for the paclitaxel-coated stents with no adverse events compared with bare metal stents. 285 Subsequently, the ASPECT trial reported a dose-dependent reduction in angiographic percent-diameter stenosis at 6 months. 286 and the TAXUS II trial demonstrated significant reductions in MACE at 12 months in patients treated with the paclitaxel-coated stents with focal de novo native coronary lesions. 287

Biodegradable Stents

Because coronary stents counteract elastic recoil and negative remodeling within a relatively short period of time, biodegradable stents may be ideal to minimize long-term complications. The Igaki-Tamai stent is made of a poly-l-lactic (PLLA) monofilament, which has been used in orthopedic surgery. The stent takes 18 to 24 months to completely degrade. A small clinical study to evaluate
safety and feasibility of the PLLA Igaki-Tamai stent has revealed no stent thrombosis and no major cardiac event within 6 months.\textsuperscript{288} Drug-eluting stents made of biodegradable materials are also under investigation. In animal studies, biodegradable stents coated with a tyrosine kinase inhibitor or recombinant polyethylene glycol (r-PEG)-hirudin and the prostacyclin analog iloprost significantly reduced angiographic restenosis.\textsuperscript{289,290}

FUTURE DIRECTIONS

Restenosis has been resistant to the technologic advances of PCIIs for the last 3 decades, frustrating both patients and cardiologists. As noted previously, the complexity of the molecular and cellular mechanisms leading to restenosis is beginning to be deciphered. Drug-eluting stents are one of the excellent examples of clinical application of basic cardiovascular science; elucidation of molecular and cellular pathogenesis at the bench has been brought back to the bedside in the form of a novel therapeutic modality. The emerging popularity of drug-eluting stents, no matter what pharmacologic agents are selected, is firmly supported by the promising data from clinical trials, the simple delivery system, and the minimal systemic toxicity profile. Nevertheless, the enthusiasm for a cure should not cloud critical and objective attitudes toward new technologies or limit other potential options. Whether a therapeutic modality turns out to be the answer to the longstanding predicament of restenosis remains to be clarified by long-term clinical trials.

REFERENCES

31. Inoue T, Sohna R, Miyazaki T, et al: Comparison of activation process of platelets and neutrophils after coro- 
25. Miller DD, Craig FE, Dressler FA, et al: Immunohistochemical characterization of immune cell composition and cytokine receptor expression in human coro- 
19. Fieldman IJ, Aguirre L, Ziol M, et al: Interleukin-10 inhibits intimal hyperplasia after angioplasty or stent implantation in hypercholes- 
16. Sirois MG, Simons M, Edelman ER: Antisense oligonucleotide inhi- 
10. Furic MB, McHugh DD: Migration of neutrophils across endothe- 
7. Yasukawa H, Imaizumi T, Matsuoka H, et al: Inhibition of intimal hyperplasia after balloon injury by antibodies to intercellular attachment molecule-1 and lymphocyte function-associated anti- 
5. Labinaz M, Hoffert C, Pels K, et al: Infusion of an antialpha4 inte- 


EDITOR'S CHOICE


Second generation stents on the horizon, likely to be a wide variety in the coming years, raises the prospects of head-to-head clinical evaluations down the road.