A terial injury initiates a complex series of events including proliferation of smooth muscle cells (SMCs) that culminates in the formation of the neointima. Neointimal formation can be a clinically problematic event, significantly narrowing the vessel lumen after angioplasty, bypass vein grafting, and transplant. Numerous growth factors and cytokines trigger the complex and redundant signaling pathways that lead to cell cycle entry.1–6 Because of the redundancy of these signaling pathways, targeting individual growth factors and cytokines has failed to affect neointimal proliferation and has obviated the need to target the “final common pathway” of events.1–6

Growth factor–stimulated proliferation is mediated by an early upregulation in transcription of the proto-oncogenes c-fos, c-myc, c-myb, B-myb, and ras.1,7–10 The gene products then act as transcription factors that increase expression of cell cycle regulatory genes, including the cyclins, that when complexed with cyclin-dependent kinases (CDKs), coordinate cell cycle progression.7,11–14 Certain proto-oncogene gene products also have the ability to augment cyclin-associated kinase activity and to couple growth regulatory signals to second messenger pathways.15,16 Early upregulation of proliferating cell nuclear antigen (PCNA) occurs as well, stimulating DNA-polymerase-β activity. In opposition to cell cycle progression are the cyclin-dependent kinase inhibitors (CKIs), such as p27kip1 and p21cip1. Transcription factors, such as p53, GAX, GATA-6, E2A, and Id, are expressed in the developing neointima after vascular injury and regulate the expression of the CKIs.17–20 The final common pathway, therefore, involves regulation of the cell cycle through transcription and translation of cell cycle proteins. In addition, regulation of cell cycle protein function through posttranslational modulations, such as phosphorylation, is also important.

The study by Zhang et al21 in this issue of *Arteriosclerosis, Thrombosis, and Vascular Biology* describes the role of Grb2 in vascular neointimal formation and provides further evidence underscoring the rationale of targeting the final common pathway. The authors focus mainly on inhibiting downstream results of ras activation including activation of the Raf-MEK-ERK MAPK cascade, Pl3 kinase-PDK1-Akt cascade, ral cascade, JNK, and p38 MAPKs all of which culminate in affecting gene expression, cytoskeletal regulation, metabolism, and cell cycle progression (Figure). In order to accomplish this, they focus on the protein Grb2, which facilitates ras activation in response to activation of several upstream receptors. Clearly Grb2 is important to normal development in that the knockout mice do not survive embryogenesis due to defective endoderm and inability to develop epiblast. The authors demonstrate that Grb2 is, indeed, important to SMC proliferation and neointimal development following injury. Through use of morpholino antisense oligodeoxyynucleotides (ODNs) directed against Grb2, it is clearly shown that reducing Grb2 levels results in decreased SMC growth in culture. They further demonstrate that Grb+/− animals develop less neointima in response to injury. They nicely show that this likely occurs through decreased activation of p38, ERK and JNK, thus creating a break in the series of events involved in the final pathway leading to cellular proliferation. Grb2, therefore, represents a possibly useful target for in vivo oligonucleotide or pharmacologic therapy as it appears to have multiple effects on downstream participants in the final common pathway of events.

Manipulation of the expression and function of these final common pathway proteins does indeed lead to reduction in neointimal formation after injury. Inhibition of membrane adherence of the small G protein p21ras with a farnesyl transferase inhibitor inhibited activation of MAPK, thus decreasing neointimal size in injured porcine coronary arteries.22 Growth factor receptor tyrosine kinases have also been shown to be useful targets as inhibitors prevent the initial phosphorylation event necessary for recruiting downstream cell cycle regulators thus decreasing cell growth.23 Cdk2 inhibitors have also been successful in inhibiting neointimal formation through their ability to block induction of cyclin D1, PCNA, and hyperphosphorylation of the retinoblastoma protein.24,25 Antisense ODNs have been used to inhibit the cell cycle regulatory genes c-myb, PCNA, c-myc, AP-I, cdc2, and cdk2, resulting in a decrease in neointimal formation after injury in rat carotid models.26–30 Trans-catheter delivery of c-myc antisense ODNs in a porcine coronary model was also effective as was the treatment of vein grafts with antisense ODN against PCNA and Cdk1.31–33 Further, multigene strategies have been shown to be more effective than targeting a single gene.26,28 ODN decoys have also been developed to prevent interaction between the transcription factor and its targeted promoter region and have been used successfully to bind the factors E2F and AP-1 in arterial balloon injury models.34–36 Conditional expression of a dominant-negative c-myb in transgenic mice, as well, provided means for
decreased neointimal formation.\textsuperscript{37} Ribozymes have been used to cleave target mRNA of Cdk1 and PCNA decreasing neointimal formation in rat carotid injury models.\textsuperscript{3} Overexpression of inhibitory molecules has also been implemented via adenoviral vectors and liposome-mediated gene transfer. Overexpression of the inhibitory molecules p21\textsuperscript{Waf1}, p27\textsuperscript{Kip1}, GAX, GATA-6, and p53 have all resulted in decreased neointimal formation in animal arterial injury models.\textsuperscript{1,17,38–42}

In addition to SMC proliferation, multiple other processes are involved in neointimal formation after vascular injury such as inflammation, matrix formation, migration, and loss of vasoactive responses.\textsuperscript{2,4} Indeed, similar decreases in neointimal size in vivo have been attained targeting these aspects of neointimal formation.\textsuperscript{43–49} Thus, advances in understanding the molecular mechanisms involved in neointimal formation have given us many potential targets for limiting neointimal formation in humans. Yet few of these strategies have been successfully brought to the clinical arena.

The current armamentarium for targeting the neointimal formation in humans includes pharmacologic agents and brachytherapy. Pharmacologic agents have been studied in relation to various components of the pathway. Rapamycin has been shown to inhibit down regulation of p27\textsuperscript{Kip1} and block enzymatic activation of cyclin-dependent kinases and phosphorylation of the retinoblastoma gene product thus inhibiting proliferation.\textsuperscript{30–33} Paclitaxel stabilizes microtubules and indirectly upregulates p21\textsuperscript{Waf1}.\textsuperscript{54,55} Rapamycin- and paclitaxel-coated stents have been used successfully as directly delivered pharmacologic therapy in humans, effectively decreasing neointimal formation after stent placement.\textsuperscript{56–59} However, pharmacologic stents have failed to obliterate restenosis as initially suggested and require longer periods of anticoagulation.\textsuperscript{50,65} Brachytherapy employs beta and gamma radiation to create breaks in double stranded DNA halting cell division and successfully decreasing rates of restenosis.\textsuperscript{52–54} Edge stenosis and late total occlusion have complicated its use.\textsuperscript{65,66} Although these therapies have greatly impacted restenosis, they have clinically problematic limitations and do not address the issues of vein graft occlusion and transplant arteriopathy. Local delivery of oligonucleotides that bind the cell cycle regulatory factor E2F has successfully prevented vein graft failure in peripheral and coronary vein graft bypasses in human trials representing a unique class of therapy that may prove useful in multiple cardiovascular disease processes.\textsuperscript{67,68}

It is, therefore, of extreme importance that we continue, as these authors have, to search for new mechanisms that regulate neointimal proliferation, new targets that may limit this process, and new mechanisms of therapy. Both our wealth and lack of knowledge present us with the difficult task of somehow translating this knowledge into clinically relevant and useful strategies while we still strive to further elucidate the final common pathway.

References


Regulation of Vascular Smooth Muscle Cell Growth: Targeting the Final Common Pathway
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