Unexpected Proatherogenic Properties of p21
Beyond Cell Cycle Control?
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Atherosclerosis is an inflammatory disease that involves the interplay between endothelial cells, smooth muscle cells (SMCs), and immune cells. Various atherogenic stimuli lead to endothelial damage, which in turn triggers the adhesion and extravasation of circulating monocytes and lymphocytes into the artery wall. Resident immune cells produce a plethora of inflammatory mediators that exacerbate leukocyte recruitment and proliferation and promote SMC growth and migration from the underlying media toward the developing atheroma. Abnormal cell proliferation and migration are also a hallmark of neointimal thickening during postangioplasty restenosis, transplant vasculopathy, and graft atherosclerosis.

Role of Cell Cycle–Regulatory Factors in Neointimal Thickening: Lessons Learned From Animal Models
There is compelling evidence from animal studies that cell cycle–regulatory factors are key modulators of neointimal hyperplasia. Consistent with this notion, gene transfer of p21, p27, p57Kip2, and p53, a tumor suppressor, in mice doubly deficient for p21 and apoE (p21−/−/apoE−/−) mice disclosed a significant increase in arterial cell proliferation with no significant changes in apoptosis. Abnormal cell proliferation and migration are also a hallmark of neointimal thickening during postangioplasty restenosis, transplant vasculopathy, and graft atherosclerosis.

Absent of p21 Is Now Shown to Protect Against Atherosclerosis in ApoE-Null Mice
The aforementioned studies highlight the role of CKIs and p53 as negative regulators of neointimal thickening. In this issue of Circulation, Merched and Chan report, surprisingly, that mice doubly deficient for p21 and apoE (p21−/−/apoE−/−) maintained on a regular chow disclosed a significant increase in arterial cell proliferation with no significant changes in apoptosis. Abnormal cell proliferation and migration are also a hallmark of neointimal thickening during postangioplasty restenosis, transplant vasculopathy, and graft atherosclerosis.
apoE-null mice examined at different ages and under different nutritional regimens. Because transplantation of p21-null bone marrow cells into irradiated apoE-null mice reproduced most of the protective effect of whole-body p21 inactivation, including a thicker and better-formed fibrous cap, it was concluded that the absence of p21 expression in the neointimal macrophages is important to the beneficial effect against atherosclerosis.

Why p21 Disruption Protects ApoE-Null Mice From Atherosclerosis
Merched and Chan\(^{12}\) performed a series of experiments that provide mechanistic insight into the unexpected atheroprotective role of the absence of p21 in apoE-null mice: (1) Atherosclerotic lesions in the absence of p21 expression exhibited increased apoptosis, a higher level of scavenger receptor type B-I (SR-BI) expression, and reduced inflammatory vascular cell adhesion molecule-1 expression; (2) thioglycolate-elicited peritoneal macrophages obtained from p21-null mice disclosed higher expression of putative atheroprotective factors (ie, SR-BI, macrophage scavenger receptor A, and LDL receptor–related protein) and lower levels of proatherogenic molecules (ie, macrophage inflammatory proteins 1 and 2, interleukin-1\(\beta\), and LDL receptor–related protein) and lower levels of protective factors (ie, SR-BI, macrophage scavenger receptor type B-I (SR-BI) expression, and reduced inflammatory response that may contribute to the atheroprotective role of p21 expression in apoE-null mice.

Preservation of a quiescent, multipotential stem cell pool capable of intermittently giving rise to highly proliferative progenitors is essential for maintaining blood homeostasis. Studies using knockout mice suggest that p27 and p21 play distinct roles in regulating these events. Unlike p27, which appears to govern stem cell repopulation efficiency but not pool size,\(^{13}\) p21 has been proposed as the molecular switch governing the entry of stem cells into the cell cycle, and increased cell cycling in the absence of p21 leads to stem cell exhaustion.\(^{14}\) Thus, determining whether stem cell deficiency might contribute to reduced atherosclerosis in p21\(^{-/-}\)apoE\(^{-/-}\) mice warrants examination. It is also noteworthy that specific inhibition of p21 by transfer of antisense oligonucleotides does not abolish the growth-inhibitory effect of transforming growth factor-\(\beta\) in cultured SMCs, yet it markedly reduces both the synthesis and secretion of laminin and fibronectin.\(^{15}\) Thus, diminished matrix protein deposition may attenuate atherosclerosis in p21\(^{-/-}\)apoE\(^{-/-}\) mice.

Because excessive cell growth is thought to contribute to atheroma development\(^{16,17}\) and p21 is generally considered a growth suppressor,\(^{3}\) one must also reflect on cellular proliferation when considering the provocative study by Merched and Chan.\(^ {12}\) The authors conducted bromodeoxyuridine (BrdU) incorporation studies in 20-week-old mice fed a normal chow diet and found no differences in plaque cell proliferation when comparing p21\(^{-/-}\)apoE\(^{-/-}\) and p21\(^{-/-}\)apoE\(^{+/+}\) mice. However, additional BrdU incorporation experiments at different ages and different time points after the onset of the atherogenic diet are warranted to precisely establish the consequences of p21 inactivation on arterial cell growth in this murine model. These supplementary studies may reveal increased proliferation in the absence of p21 (ie, at the very onset of atheroma development). Conversely, if no differences in proliferation are confirmed in vivo, analysis of cultured cells should provide significant mechanistic insight. For example, if cultured p21-null SMCs and/or macrophages disclose higher proliferative capacity than wild-type cells, one may speculate that reduced inflammatory response as a result of macrophage p21 deficiency (see above) might compensate in vivo for the intrinsic higher proliferative capacity of p21-deficient cells, thus resulting in similar BrdU incorporation rates within the atheroma of p21\(^{-/-}\)apoE\(^{-/-}\) and p21\(^{-/-}\)apoE\(^{+/+}\) mice. Given that activation of cyclin D/CDK holoenzymes is actually facilitated by their interactions with p21 and p27,\(^{16}\) it is also plausible that p21 disruption results in impaired cell growth. Thus, lack of p21 might contribute to limited atherosclerosis in p21\(^{-/-}\)apoE\(^{-/-}\) mice by inhibiting cell proliferation as a result of reduced cyclin D–associated CDK activity.

The balance between cell proliferation and apoptotic cell death is a chief determinant of neointimal thickening and stability.\(^{17}\) p53 and p21 are important molecules in both cell proliferation and apoptosis. As discussed below, studies in different genetic murine models have yielded confounding results about the specific role of p53 and p21, as well as their interplay, in the regulation of neointimal hyperplastic growth and apoptotic cell death.\(^{7,9,18}\) Not only does p53 inactivation accelerate aortic atherosclerosis, it also leads to vulnerable-appearing plaques. Remarkably, similar to the effect of p53 disruption, adenovirus-mediated overexpression of p53 in preexisting carotid atherosclerotic lesions of apoE-null mice induces features of vulnerable plaque, and this correlates with an increase in fibrous cap cell apoptosis.\(^ {19}\) Likewise, adenovirus-dependent p53 gene transfer in isolated segments of balloon-injured rat carotid arteries increased apoptosis.\(^ {20}\) In the Merched and Chan study,\(^ {12}\) p21 deficiency resulted in an \(~2\) -fold increase in apoptosis within the lesion, affecting primarily the macrophage population (although the frequency of TUNEL-positive plaque cells was very low in both groups of mice), yet analysis of similar-sized lesions revealed thicker and better-formed fibrous caps and lower expression of indicators of ruptured plaques in p21\(^{-/-}\)apoE\(^{-/-}\) compared with p21\(^{+/+}\)apoE\(^{+/+}\) counterparts. As pointed out by the authors, the proliferative and apoptotic responses are probably uncoupled in p21\(^{-/-}\)apoE\(^{-/-}\) mice. Moreover, the authors considered the possibility that a redundancy of factors might take over some of the functions of p21. Indeed, they found that transcripts for p16, pRB, and p53 were significantly elevated in p21-null macrophages, suggesting that upregulation of these growth suppressors might compensate for the absence of the inhibitory effect of p21.

Conclusions and Future Perspective
The evidence accumulated over the last years lent support to the notion that p21, p53, p57, and p27 can limit neointimal thickening in animal models of atherosclerosis and angioplasty (Table).\(^ {7,12,19,26-40}\) On the basis of immunohistopathological studies,\(^ {21-25}\) these factors have also been considered as
negative regulators of cell proliferation in human atherosclerotic and restenotic lesions, and their overexpression has been generally proposed as a promising approach to treat or prevent these diseases.\(^5,6\) However, the common perception of p21 as an atheroprotective factor is now challenged by the unexpected acceleration of atherosclerosis in p21 null mice.\(^12\) It is now evident that p21 may control more than the cell cycle, because major differences in inflammatory marker gene expression exist between macrophages isolated from p21-null and those isolated from wild-type animals. This novel “inflammatory” aspect of p21 function may contribute to its proatherogenic role in hypercholesterolemic mice. Bearing in mind these new findings, it is tempting to speculate that arterial expression of a subset of growth factors (ie, p21) may facilitate lesion development and/or vulnerability to rupture. This question is certainly difficult to address, particularly in relation to human disease; however, there is still much to be learned from experimental animals. Because lack of p21 expression is not likely to prevent these diseases.\(^5,6\) However, the common perception of p21 as an atheroprotective factor is now challenged by the unexpected acceleration of atherosclerosis in p21 null mice.\(^12\) It is now evident that p21 may control more than the cell cycle, because major differences in inflammatory marker gene expression exist between macrophages isolated from p21-null and those isolated from wild-type animals. This novel “inflammatory” aspect of p21 function may contribute to its proatherogenic role in hypercholesterolemic mice. Bearing in mind these new findings, it is tempting to speculate that arterial expression of a subset of growth suppressors (ie, p53 and p27) may be beneficial, whereas other factors (ie, p21) may facilitate lesion development and/or vulnerability to rupture. This question is certainly difficult to address, particularly in relation to human disease; however, there is still much to be learned from experimental animals. Because lack of p21 expression is not likely to represent the usual scenario in human atheromas, it would be of interest to investigate atherosclerosis development in the presence of varying levels of p21 expression. This can be achieved by comparing, in different genetic and dietary models, the phenotype of p21 transgenic and p21\(^{-/-}\) mice versus that of controls with normal p21 gene dosage. Another important consideration is that whole-body inactivation of p21 is unlikely to occur in patients, and therefore, future studies on the role of p21, and other cell cycle–regulatory factors such as p27 and p53, should include the analysis of mice with genetic manipulation restricted to specific cell types involved in atherosclerosis (ie, endothelial cells, SMCs, lymphocytes, and monocytes/macrophages). In addition, despite the obvious limitations of descriptive studies, analysis of a large number of human atherosclerotic and restenotic specimens should help clarify the roles of p21, p27, and p53 in various aspects of human neointimal thickening, including the inflammatory response elicited by immune cells.

**References**


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