New Targets to Inhibit the Growth of Vascular Smooth Muscle Cells

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Excessive proliferation of vascular smooth muscle cells (VSMCs) contributes to the pathogenesis of many cardiovascular diseases, including atherosclerosis and pulmonary arterial hypertension (PAH). VSMC proliferation also underlies the failure of many therapies, notable examples being restenosis following coronary angioplasty, vein graft failure in patients with coronary artery bypass grafts, and transplant vasculopathy. Few therapies directly target excess VSMC proliferation, in part, because the underlying pathways have been unknown. Recently, several pathways of VSMC proliferation have been defined, and new therapeutic targets have emerged.

An example of the power of preventing VSMC proliferation in reducing human cardiovascular disease is the rapamycin (sirolimus)-coated coronary stent. After dozens of agents failed to prevent the 30% restenosis rate postangioplasty, this VSMC proliferation inhibitor reduced the number to \( \approx 6\% \). However, rapamycin has toxicities, limiting its systemic use. Although growth factors are necessary to initiate cell cycle, the underlying pathologic conditions in human disease are the intersections of this work with several newly identified antiproliferative drug targets.

Members of this group expand on their previous work showing that peroxisome proliferator-activated receptor (PPAR) activation suppresses G1 \( \rightarrow \) S cell cycle progression by increasing the expression of the cyclin dependent kinase (CDK) inhibitor p16\(^{INK4a}\). The present study is built on a solid body of knowledge of the CDK–cyclin complexes phosphorylate Rb proteins, allowing release of E2F and transcription commences. However, another group of molecules, the CDK inhibitors (p16\(^{INK4a}\), p21\(^{WAF1}\), and p27\(^{KIP1}\)) can block this transcription of S-phase genes, offering further opportunity for inhibiting proliferation.

Gizard et al provide a new understanding of the transcriptional regulation underlying proliferation, demonstrating the cooperation of the CDK pathway and telomerase. Telomeres are the DNA TTAGGG repeat sequences that cap and stabilize chromosomes. Traditionally thought to fend off senescence, it appears increasingly likely that they also regulate cell proliferation. Telomerase reverse transcriptase (TERT) is the catalytic factor that leads to telomerase activation. Relevant to its role in vascular disease, TERT is activated by mitogens, which are upregulated in diseases characterized by VSMC proliferation. There is emerging evidence that inhibiting telomerase in vivo may reduce vascular disease.

In this context, Gizard et al define the mechanism by which PPAR\(\alpha\) impairs cell proliferation in human coronary VSMC. They demonstrate that PPAR\(\alpha\) activation inhibits mitogen-induced telomerase activity by transcriptionally repressing TERT. Their methodical approach revealed that the effect of PPAR\(\alpha\) was indirect and related to inhibition of E2F binding sites in the TERT promoter. These sites had been described previously but not in vascular cells. Gizard et al found that PPAR\(\alpha\) activation inhibits TERT transcription by blocking the binding of E2F-1 to its binding sites in the proximal TERT promoter. In addition, p16 mediates some of the repression of TERT by recruiting p107 and p130 to the proximal E2F-1 site, further blocking TERT transcription. PPAR\(\alpha\) activation by fibrates inhibits telomerase activity by inducing p16, resulting in inhibition of E2F-dependent transcriptional activation of the TERT promoter. The authors present convincing loss- and gain-of-function studies to support a key role of E2F in the regulation of telomerase activity and prove that E2F is required for the repression of TERT promoter activity by PPAR\(\alpha\). Moreover, their demonstration that fenofibric acid and gemfibrozil inhibit telomerase activation in a femoral artery endothelial-denudation model suggests this as a potential therapy in humans. This is feasible, because these drugs are widely used to increase high-density lipoprotein in humans, also through a PPAR\(\alpha\)-dependent mechanism. Thus, it appears that the antiproliferative effects of PPAR\(\alpha\) are caused by impingement on the p16/Rb/E2F transcriptional cascade and are ultimately mediated by suppression of telomerase (in vitro and in vivo). What are the intersections of this work with several newly identified pathways of VSMC proliferation (Figure)?

**Regulation of VSMC Proliferation**

Although it is not possible to discuss all molecules involved in controlling VSMC proliferation, some are noteworthy because...
they are recently recognized or are targeted by drugs that are either in clinical use or preclinical development. These are the low-hanging fruit that may be harvested in the attempts to selectively decrease VSMC proliferation in vascular disease.

Mammalian Target of Rapamycin

Mammalian target of rapamycin (mTOR) (Figure) is an ubiquitously expressed kinase that integrates cellular energy and nutrient status with external mitotic signals. When sufficient nutrients and appropriate mitogens are present, mTOR will lead to proliferation by the expression of several proteins including cyclin D1. Mitochondrial dysfunction, energy depletion, and amino acid deprivation all lead to mTOR inactivation, effectively blocking proliferation. The interaction of mTOR with mitochondria raises the possibility that changes in mitochondrial function would alter VSMC proliferation. Impaired mitochondrial function, and fusion has been proposed to be responsible for the development of certain forms of pulmonary arterial hypertension. In a potential link between this pathway and the present study, rapamycin upregulates CDK inhibitors p21WAF1 and p27KIP1 in VSMCs.

PPARα and PPARγ Agonists

Thiazolidinediones, such as rosiglitazone, are PPARγ agonists (Figure) developed for the therapy of type 2 diabetes mellitus. They decrease VSMC proliferation by inhibiting mitogen-induced degradation of the CDK inhibitors p21WAF1 and p27KIP1. PPARγ agonists might have a role in preventing transplant vasculopathy and neointima formation in PAH. For example, rosiglitazone reduces both pulmonary hypertension and vascular remodeling in pulmonary hypertensive apolipoprotein E–knockout mice fed a high-fat diet.

Survivin

Survivin (Figure), a known apoptosis inhibitor, was traditionally thought to be exclusively expressed in cancers but more recently has been found in vascular injury and PAH. However, survivin not only impairs apoptosis but also increases proliferation by initiating cell cycle entry. When survivin is translocated to the nucleus, it competes with cyclin/CDK for interaction with p16INK4a, a potential link to the pathway described by Gizard et al. Inhibition of survivin can be therapeutically exploited to prevent neointima formation and reverses pulmonary hypertension and vascular obstruction in experimental models.

Pre–B-cell Colony-Enhancing Factor and Histone Deacetylase

The pre–B-cell colony-enhancing factor (PBEF) is a regulator of SMC proliferation that increases nicotinamide phosphoribosyltransferase activity, upregulating supplies of nicotinamide adenine dinucleotide (NAD+) for the SIRT transcription regulators. SIRT family members regulate transcription and apoptosis through their ability to deacetylate histones and nonhistone proteins (eg, p53 and TERT). PBEF is able to shift VSMC from a proliferative to a contractile phenotype. PBEF overexpression enhances cell survival whereas PBEF knockdown increases SMC apoptosis. Through regulation of histone deacetylase (HDAC) activity, PBEF modulates VSMC proliferation and survival. There is a potential interaction between the cyclin-CDK-telomerase pathway and HDAC. The HDAC inhibitor trichostatin A results in histone hypermethylation of the p21WAF1 promoter, leading to increased p21WAF1 expression and cell cycle arrest in a variety of cells, including VSMCs.

Bone Morphogenetic Protein Receptor Type 2

Loss of function mutations in bone morphogenetic protein receptor type 2 (BMPR2) (Figure) are common in familial PAH, a disease characterized by excessive VSMC proliferation, particularly in response to transforming growth factor (TGF)β. Signaling from the BMPR2 receptor involves the
SMAD signaling cascade. The connection of BMPR2 signaling to the cyclin–CDK–telomerase pathway is unknown; however, BMPR2 inactivation, which decreases VSMC differentiation, does lower p27KIP1 expression.21

Future Studies

There are several areas which were not addressed by Gizard et al that merit further study.

First, TERT activity can also be regulated at the posttranslational level by phosphorylation. For example, hypoxia (a well-established cause of pulmonary hypertension and excessive PASMC proliferation) increases TERT phosphorylation, which increases SMC proliferation.22 Thus, both activity and expression of TERT modulate telomerase activity and the role of “activity” deserves further study.

Second, there are many parallels between proliferation of VSMC and cancer cells.23 A recent publication demonstrated reduced TERT expression in cancer cells exposed to TGF-VSMC and cancer cells.23 A recent publication demonstrated the role of “activity” deserves further study.

None.

Many signaling pathways, including PPAR-PPARα, converge in having effects of the CDK inhibitors, including p16INK4a, p21WAF1, and p27KIP1. Third, we now have another potential common point at which to attack proliferation-TERT.

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References


17. van der Veer E, Nong Z, Q’Neil C, Urrahet B, Freeman D, Pickering JG. Sources of Funding


30. Desai BN, Myers BR, Schreiber SL. FKBP12-rapamycin-associated protein associates with mitochon-
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