Editorials

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The Balance of Power: The Law of Yin and Yang in Smooth Muscle Cell Fate

Is YY1 a Vascular Protector?

Masanori Aikawa

Once upon a time in China, the Han philosophers introduced a syncretic intellectual framework to unite various thoughts using a new theory: the law of Yin and Yang. The basic concept of the theory is that the balance of two opposite principles regulates the universe and thus all phenomena, including human health. Yin represents the moon, cold, darkness, night, black, and quiescence, whereas Yang is sun, heat, brightness, day, white, and activity. Notably, the concept underscores the interplay of two opposite forces, but not conflicts between the two. In other words, they are not simply black and white, rather, two principles interrelating, complementing and harmonizing each other. Ancient philosophers already knew that health is life in balance, but underlying mechanisms are complex. More than a millennium later, we are still attempting to answer the same questions.

An exciting and provocative study by Santiago et al, published in this issue of Circulation Research, used comprehensive in vitro and in vivo approaches to demonstrate that Yin Yang 1 (YY1), a ubiquitous and dual-functional GLI-Krüppel zinc finger transcription factor, suppresses smooth muscle cell (SMC) growth. The authors suggest YY1 may serve as a therapeutic tool, combating neointima formation following vascular injury. Despite a relatively new face in vascular biology, investigators in other fields of medical sciences, particularly cancer biology, have extensively studied this mysterious molecule since its initial characterizations in the late 1980s to early 1990s. YY1 was originally discovered as a transcriptional regulator that interacts with E1A gene products, oncoproteins that activate the adenovirus (AAV) P5 promoter. When E1A is present, YY1 induces transcription; its absence converts YY1 into a repressor. The YY1 gene encodes a 414 amino-acid protein with an estimated weight of 44kD. Alternative splicing generates at least eight isoforms with unknown functional differences. YY1 also contributes to various normal biological processes, including development, differentiation, and cellular proliferation. YY1 regulates target genes by either activating or repressing their transcription directly or indirectly. Several proposed models of YY1-mediated transcriptional regulation include: 1) direct binding to the promoter as an activator; 2) repression of an activator; 3) activation by recruitment of a coactivator; and 4) indirect repression of an activator via formation of a repressor complex in collaboration with a corepressor. The multifunctional properties of YY1 also appear to depend on context and cell type, but the mechanisms that regulate its diverse bidirectional actions remain unclear.

Previous studies have suggested that in various types of tumors, YY1 promotes cell proliferation and counteracts apoptotic signals, leading to initiation and progression of tumor growth. Investigators in cancer biology therefore find YY1 useful as a tumor marker and therapeutic target. Proposed mechanisms of action for YY1 on tumorigenesis, through imbalance involving uncontrolled cell cycle progression versus impaired cell death, include activation of the proto-oncogene c-myc, interaction with cyclin D1, suppressed activity of the tumor suppressor gene product p53, negative regulation of cell death genes such as Fas, and multiple levels of interaction with NF-κB. Accumulating evidence also suggests the role of YY1 in immune cells. YY1 induces COX-2 expression in macrophages, and it regulates transcription of CXCR4, a chemokine receptor, in macrophages and T lymphocytes.

Imbalance of various pairs of counteractions also causes vascular diseases. For example, paucity of intimal collagens may promote atherosclerotic plaque instability and acute thrombotic complications whereas excessive collagen accumulation may increase arterial stiffness and dysfunction. In apparently healthy human arteries, where the tunica intima normally exists, principles in SMC biology are in equilibrium. However, dysregulated counterbalance may trigger vascular disease. Accumulating evidence has established that SMC proliferation plays a role in the pathogenesis of chronic atherosclerosis and arterial remodeling following injury. In contrast, other lines of evidence have suggested the protective role of arterial SMC. Consequently, SMC loss in the fibrous cap because of apoptosis may promote paucity of collagen, physical disruption and subsequent thrombosis. Arterial injury induces not only SMC proliferation, but also apoptosis, suggesting active turnover. Thus, understanding the mechanisms that regulate molecular switching between SMC proliferation and apoptosis deserves extensive research efforts.

Santiago et al demonstrated that mechanical vascular injury promotes YY1 expression in vascular SMC in a rat
model. The study further showed in vitro that forced YY1 expression stifled SMC replication, which conflicted with previous studies demonstrating that this factor promotes cell growth. The more recent study by the same group, published in this issue of *Circulation Research*, used extensive in vitro and in vivo experiments to establish further the unambiguous evidence for YY1’s suppressive role in SMC growth. Overexpression of YY1 suppressed proliferation of human, rabbit, and rat SMC in vitro. The study also used rabbit and rat carotid artery injury models to demonstrate that YY1 inhibits neointima formation in vivo. This piece of information disagrees with a previous study by Favot et al, which reported that silencing YY1 with siRNA in C2C12 cells inhibited proliferation. To provide a mechanistic link, Santiago et al implemented gain-of-function and loss-of-function approaches to illustrate that YY1 reduced transcription of the p21^WAF1/Cip1 gene and inhibited formation of the cyclin D1-cdk4-p21^WAF1/Cip1 complex. These findings also challenge the conventional recognition of p21^WAF1/Cip1 as a negative cell cycle regulator. YY1 further induced p53 ubiquitination and proteosomal degradation. Notably, the study indicates that YY1 treatment did not affect endothelial proliferation or apoptosis.

A number of studies have addressed the role of altered smooth muscle differentiation in vascular pathobiology. Expression levels of SMC specific genes such as myosin heavy chain isoforms and SM22α reflect the differentiated state and serve as molecular markers of SMC differentiation. SMC maintain high levels of plasticity, unlike skeletal and cardiac muscles, and can reduce their differentiation state in response to a proinflammatory microenvironment. This unique plasticity enables SMC to regain a mature phenotype in response to disappearing pathological stimuli. SMC differentiation state also often, albeit not always, inversely associates with proliferative activity: expression of SMC-specific genes usually diminishes in proliferating SMC. Therefore, tremendous efforts have sought to elucidate the molecular mechanisms regulating SMC differentiation. Recent evidence suggests that serum responsive factor (SRF) and its cofactors interact with CArG box sequences in the promoters of SMC-specific genes, and activate their transcription. Several studies suggest potential interplay between YY1 and SRF in transcriptional regulation of smooth muscle myosin heavy chain and SM22α genes. Itoh et al demonstrated that YY1 suppresses nitric oxide (NO)–mediated activation of the myosin heavy chain gene by competing with SRF for the overlapping CArG element. Accordingly, YY1 may interfere with NO’s protective effects on SMC. Although undifferentiated or dedifferentiated SMC do not always proliferate, particularly in vivo, these lines of previous evidence for the negative actions of YY1 on SMC-specific gene expression do not concur with Santiago’s study on its inhibitory role in SMC growth. Further studies are needed to clarify the role of YY1 in various aspects of SMC biology. Other studies suggest crosstalk between YY1 and a few fundamental cell-signaling pathways. YY1 interacts with the TGF-β/Smad pathway that regulate fibroblast differentiation into myofibroblasts or osteoblast-like cells. YY1 thus may play a role in cardiovascular calcification or valvular dysfunction. Notch signaling may mediate vascular activation and inflammation. YY1 appears to interact with the intracellular domain of Notch1, a receptor of this signaling pathway. The potential interplay between YY1 and signaling mechanisms regulating SMC biology warrants extensive investigation.

The recent and present studies by Santiago et al present a strong case for a suppressive role of YY1 in vascular diseases involving SMC growth. Gain-of-function and loss-of-function experiments both in vitro and in vivo effectively provide complementary results and support the authors’ central hypothesis. Disagreements with previous studies using different cell types emphasize the context-dependent nature of this dual-functional transcription factor. Genetic manipulation of the YY1 gene, eg, SMC-selective deletion in mice, may address its more specific and precise roles in SMC biology. Human genetics studies may also expose YY1’s role in cardiovascular diseases. Establishing YY1 as a clinically translatable vascular protector will require scrutiny of this ubiquitous transcription factor’s effects not only on SMC function, but also on other aspects of vascular biology, such as angiogenesis and inflammation. Better understanding of the overall balance of the Yin and Yang sides of YY1 should provide new insights into the biology and clinical translation of this mysterious transcription factor.

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