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PRE-CLINICAL RESEARCH

Telomerase Inhibition by Everolimus Suppresses Smooth Muscle Cell Proliferation and Neointima Formation Through Epigenetic Gene Silencing

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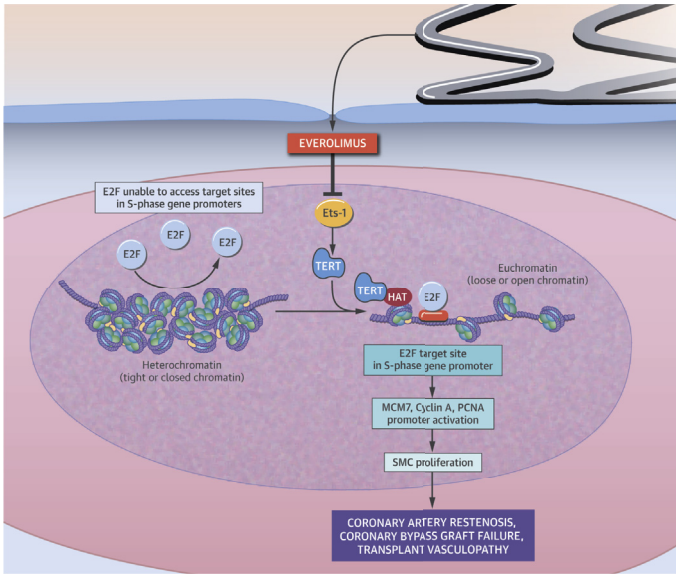
VISUAL ABSTRACT



Aono, J. et al. J Am Coll Cardiol Basic Trans Sci. 2016; 1(1-2):49-60.

HIGHLIGHTS

- The proliferative capacity of smooth muscle cells (SMC) during neointima formation is prevented by everolimus-coated drug-eluting stents.
- Everolimus failed to inhibit neointima formation by in mice overexpressing telomerase reverse transcriptase (TERT).
- Everolimus reduced TERT-dependent SMC proliferation through inhibition of Ets-1-dependent promoter activation.
- The inhibition of TERT-dependent SMC proliferation by everolimus occurred as a result of a G1→S-phase arrest, rather than telomerase shortening.
- Chromatin immunoprecipitation assays demonstrated that TERT induced E2F binding to S-phase gene promoters and supported histone acetylation.
- These studies identify a novel mitogenic pathway in SMC that depends on the epigenetic activation of S-phase gene promoters by TERT.

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SUMMARY

Proliferation of smooth muscle cells (SMCs) during neointima formation is prevented by drug-eluting stents. The replicative capacity of mammalian cells is enhanced by telomerase expression; however, the contribution of telomerase to the proliferative response underlying neointima formation and its potential role as a pharmacological target are unknown. The present study investigated the mechanisms underlying the mitogenic function of telomerase, and tested the hypothesis that everolimus, which is commonly used on drug-eluting stents, suppresses SMC proliferation by targeting telomerase. Inhibition of neointima formation by everolimus was lost in mice overexpressing telomerase reverse transcriptase (TERT), indicating that repression of telomerase confers the anti-proliferative efficacy of everolimus. Everolimus reduced TERT expression in SMC through an Ets-1-dependent inhibition of promoter activation. The inhibition of TERT-dependent SMC proliferation by everolimus occurred in the absence of telomere shortening but rather as a result of a G1→S-phase arrest. Although everolimus failed to inhibit phosphorylation of the retinoblastoma protein as the gatekeeper of S-phase entry, it potently repressed downstream target genes. Chromatin immunoprecipitation assays demonstrated that TERT induced E2F binding to S-phase gene promoters and supported histone acetylation. These effects were sensitive to inhibition by everolimus. These results characterize telomerase as a previously unrecognized target for the antiproliferative activity of everolimus, and further identify a novel mitogenic pathway in SMC that depends on the epigenetic activation of S-phase gene promoters by TERT. (J Am Coll Cardiol Basic Trans Sci 2016;1:49–60)

In addition to endothelial dysfunction and inflammation, proliferation of vascular smooth muscle cell (SMC) constitutes an essential component for atherosclerosis formation and neointimal remodeling (1). Once luminal obstruction in the course of atherosclerotic remodeling occurs, revascularization procedures represent primary treatment strategies (2). However, the clinical success of these procedures is limited by post-angioplasty restenosis, coronary artery bypass graft failure, and transplant vasculopathy. Considering the critical role of SMC proliferation in the pathophysiology of these treatment failures, the implantation of drug-eluting stents has become standard-of-care to prevent restenosis after angioplasty (3). Among the widely employed mammalian target of rapamycin (mTOR) inhibitors, recent clinical evidence has indicated that particularly second-generation, everolimus-eluting stents provide improved revascularization outcomes (4,5). Although everolimus potently inhibits neointima formation (6) and SMC proliferation (7), the detailed molecular mechanisms by which mTOR inhibitors elicit their antiproliferative efficacy and prevent cell cycle progression in SMC remain controversial. Early studies indicated that mTOR inhibitors induce G1 cell cycle arrest by targeting the retinoblastoma protein (RB), the key gatekeeper of the cell cycle, which represses S-phase gene expression induced by the transcription factor E2F (8). However, more recent evidence in SMC that are deficient for the RB protein have indicated that mTOR inhibitors may repress cell cycle progression independently of RB protein phosphorylation (9). Considering this controversy, the precise mechanisms by which commonly implanted everolimus-eluting stents prevent in-stent restenosis remain elusive.

By maintaining the stability of telomeres, repetitive deoxyribonucleic acid (DNA)-protein complexes that protect the ends of chromosomes, telomerase is rate limiting for cell proliferation and tissue renewal (10). Overexpression of the catalytic subunit telomerase reverse transcriptase (TERT) confers a virtually unlimited replicative capacity (11). In contrast, most somatic cells are thought to repress TERT, resulting in telomere shortening and limited replicative potential (12). However, accumulating evidence revealed that TERT is inducible in response to various environmental cues (13), which enhance proliferative responses during tissue renewal (10). Similarly as in other somatic tissues, we (14–16) and others (17) have previously reported that TERT expression is induced in proliferating SMC and in response to neointima and atherosclerosis formation. However, whether TERT expression in the vascular wall causes neointima formation and the transcriptional mechanisms by which TERT induces aberrant SMC proliferation remain unknown. Moreover, the possibility that TERT constitutes an alternative molecular target for the RB-independent antiproliferative efficacy of mTOR inhibitors on drug-eluting stents has previously not been investigated. Here, we demonstrate that TERT

ABBREVIATIONS AND ACRONYMS

ChIP = chromatin immunoprecipitation

MCM7 = minichromosome maintenance protein 7

mTOR = mammalian target of rapamycin

PCNA = proliferating cell nuclear antigen

RB = retinoblastoma protein

SMC = vascular smooth muscle cells

TBP = TATA binding protein

TERT = telomerase reverse transcriptase

TERTtg = telomerase reverse transcriptase-overexpressing transgenic

WT = wild type