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Smooth muscle-specific drug targets for next generation Drug-eluting stent

Rui Tang¹ and Shiyu Chen^{1,*}

¹Department of Physiology and Pharmacology, University of Georgia, Athens, GA 30602

Abstract

The occurrence of stent thrombosis is one of the major obstacles limiting the long-term clinical efficacy of percutaneous coronary intervention. The anti-smooth muscle proliferation drugs coated on drug-eluting stents (DES) often indistinguishably block re-endothelialization, an essential step toward successful vascular repair, due to their non-specific effect on endothelial cells (EC). Therefore, identification of therapeutic targets that differentially regulate vascular smooth muscle cell (VSMC) and EC proliferation may lead to the development of ideal drugs for next generation DES. Our recent studies have shown that CTP synthase 1 (CTPS1) differentially regulates the proliferation of VSMC and EC following vascular injury. Therefore, CTPS1 inhibitors are promising agents for DES. In addition to CTPS1, other factors have also shown cell-specific effects on VSMC and/or EC proliferation and thus may become potential molecular targets for developing drugs to coat stents.

Keywords

vascular remodeling; smooth muscle cell; endothelial cell; cell proliferation; neointima formation; re-endothelialization; drug eluting stent

The occurrence of stent thrombosis (ST) is one of the major obstacles limiting the long-term clinical efficiency of percutaneous coronary intervention (1). Drug-eluting stents (DES) are peripheral or coronary stents that slowly release a drug to block cell proliferation and thus prevents ST (2). Three fundamental components in the stents may be further improved for the safety and efficacy of DES: the stent platform, the polymer and the drug. Developing an eluting drug with anti-proliferation, anti-inflammation, and anti-clog properties is a huge challenge while it is also an exciting adventure for biomedical researchers. It is established that media layer vascular smooth muscle cell (VSMC) proliferation and migration in response to the injury are essential events leading to subsequent neointimal thickening (3), which eventually causes vessel narrowing and ST. Therefore, the first and second generation of DES is designed to block SMC proliferation. The most popular first-generation DES uses sirolimus and paclitaxel, and the second generation of stents uses zotarolimus and everolimus. Although blocking VSMC proliferation is important to hindering intimal

*To whom correspondence should be addressed: Shi-You Chen, PhD, Department of Physiology & Pharmacology, The University of Georgia, 501 D.W. Brooks Drive, Athens, GA 30602, Tel: 706-542-8284, Fax: 706-542-3015, sc229@uga.edu.

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hyperplasia, re-endothelialization/endothelial cell (EC) growth is essential for successful vascular repair (4). First and second generation drugs indiscriminately targeting both EC and VSMC proliferation often leads to severe side effects because of impaired re-endothelialization, which increases the risk of late thrombosis (5). It will be ideal if SMC-specific anti-proliferative drugs can be identified for the next generation drug-eluting stents, with a hope to preserve re-endothelialization while blocking neointima formation. Based on recent discoveries, SMC-specific drugs may be achieved through five different approaches: 1) identifying intracellular protein targets that differentially regulates EC and SMC proliferation; 2) screening non-coding RNA targets that differentially regulates EC and SMC proliferation; 3) identifying growth factor/hormones that have differential cellular effects on EC and SMC; 4) study previously-discovered anti-neointima molecules to observe if they have a protective role in endothelial progenitor cells (EPCs) function; 5) combined usage of multiple drugs to achieve distinct functions in EC and SMC.

The notion of identifying SMC-specific targets has been implemented by our laboratory and other groups. Recently, we uncovered a fundamental difference between SMCs and ECs in CTP biosynthesis during vascular remodeling, which has provided a novel strategy by using cyclopentenyl cytosine (CPEC) or other CTP synthase (CTPS) inhibitors to selectively block neointima formation without disturbing re-endothelialization for effective vascular repair (6). CTPS is a metabolic enzyme that catalyzes CTP biosynthesis from UTP, ATP and glutamine, an essential event for DNA and RNA synthesis during cell proliferation (7). CTPS was induced in proliferative SMCs in vitro and neointima SMCs in vivo. Knockdown of CTPS or inhibition of CTPS activity suppresses SMC proliferation and neointima formation. Importantly, blockade of CTPS activity or expression has much less inhibitory effect on EC proliferation and migration in vitro and does not block re-endothelialization in vivo due to the induction of CTPS salvage pathway enzymes non-metastatic cells protein 1 and 2 (NME1 and NME2) in ECs, but not SMCs. NME preserves EC proliferation via utilization of extracellular cytidine to synthesize CTP. Our findings provide a basis for developing SMC-sensitive drugs for next generation DES.

In addition to our studies, similar mechanisms have been identified by other groups. Daniele Torella et. al. have reported that protein kinase A (PKA) induces phosphatidylinositol 3-kinase regulatory subunit (p85 α) activation leading to differential cellular response in SMC and EC (8). In SMC, PKA-activated p85 α binds p21ras, reduces ERK1/2 activation, and suppresses cell proliferation. In contrast, EC proliferation inhibited by cAMP is independent of PKA modification of p85 α and ERK1/2 inhibition. Ji Won Yoon et. al. have also shown that treatment with a fatty acid oxidation inhibitor, trimetazidine (TMZ), decreases proliferation and migration of SMC while increasing proliferation and decreasing apoptosis of EC (9). Moreover, K. Hamesch et. al. demonstrates that inhibition of SMC proliferation and neointima formation by CXCR4 antagonist POL5551 is equally effective as sirolimus, but POL5551 is more beneficial in vascular repair because POL5551 treatment results in a more stable lesion phenotype because it does not impair re-endothelialization (10). However, molecular metabolic mechanisms underlying the different effects of TMZ and POL5551 on EC and SMC remain to be determined.

In addition to protein targets, non-coding RNAs have also been shown to differentially regulate EC and SMC proliferation. Using gain and loss of function studies, Claudio Iaconetti et. al. have found that inhibition of miR-92a results in an increased EC proliferation and migration through regulation of Kruppel-like factor 4 (KLF4) and MAP kinase kinase 4 (MKK4) expression. However, miR-92a has no effect on SMC proliferation or migration in vitro. In vivo suppression of miR-92a enhances re-endothelialization in injured carotid arteries and reduces neointimal formation after balloon injury or arterial stenting (11).

Extracellular proteins have also exhibited different mitotic effects on EC and SMC and thus may be considered as candidates for eluting drugs. Khambata et. al. have reported that an endothelium-derived vasorelaxant, C-type natriuretic peptide (CNP), has disparate regulatory effect on EC and SMC proliferation through different downstream pathways of Gi-coupled natriuretic peptide receptor 3 (NPR3)-induced ERK 1/2 activation (12). In EC, ERK 1/2 activation enhances the expression of the cell cycle regulator cyclin D1, which promotes cell proliferation; whereas in SMC, ERK 1/2 activation increases the expression of the cell cycle inhibitors p21^{waf1/cip1} and p27^{kip1}. Statins are known to inhibit the proliferation of VSMC and promote vascular healing. By analyzing mitotic effects of six marketed statins in porcine coronary artery model, Tsukie et. al. have shown that pitavastatin (Pitava) eluting stents attenuates in-stent stenosis as effectively as sirolimus-eluting stents (SES) without the delayed endothelial healing effects (13).

Although recent study indicates that re-endothelialization is mainly driven by the migration and proliferation of resident ECs adjacent to the injury site (4), EPCs are also reported to be critical for re-endothelialization (14). Rie Kawabe-Yako et. al. have found that anti-platelet drug cilostazol (CLZ) inhibits VSMC proliferation and reduces neointima formation following arterial injury. More interestingly, CLZ accelerates re-endothelialization via enhancing EPC mobilization from BM and recruiting EPC to neoendothelium through a stromal-derived factor-1 (SDF-1) (SDF-1 α)/CXCR4 axis in injured arteries (15).

Combined usage of different drugs to achieve multiple functions for different cells is another way to improve DES. Tacrolimus has positive effects on re-endothelialization via stimulating SMC and EC in production of endoglin, a pro-angiogenic factor that plays an important role in repair of endothelial injury. However, clinical trial for tacrolimus-coated DES is failed because it has unexpected pro-proliferative effect on VSMC. Interestingly, Atorvastatin, one of the most effective drugs for treating cardiovascular diseases, suppresses tacrolimus-stimulated VSMC proliferation via down-regulation of β -catenin, ERK1/2, and cyclin B. Moreover, the combined treatment of atorvastatin and tacrolimus enhances EC proliferation and re-endothelialization because both agents stimulate endoglin production in EC (16). Another example comes from Claudia Tersteeg and colleagues' work on tropoelastin. Elastin is the most abundant extracellular matrix protein in vascular wall. Tropoelastin is a major component of elastin and are secreted by VSMCs. Tropoelastin is shown to regulate VSMC phenotypic switch and inhibit VSMC proliferation and migration. However, treating EC with tropoelastin in vitro often leads to increased inflammatory response, which hinders vessel repair. By comparing the cellular effects of stent coatings including fibronectin, fibrinogen, and tropoelastin on human umbilical vein endothelial cell

(HUVEC) and VSMC. Claudia Tersteeg et. al. have found that tropoelastin treatment only inhibits VSMC migration but leads to increased inflammatory response in EC. However, combined treatment with fibronectin, fibrinogen and tropoelastin inhibits VSMCs while reducing the inflammatory effects on EC (17). Therefore, coating a mixture of fibronectin/fibrinogen/tropoelastin on a stent may promote re-endothelialization and induce a better vascular repair following vascular intervention.

In summary, identification of SMC-specific anti-proliferative agents is a promising approach in designing drugs for the next generation DES. These drugs shall effectively suppress neointima formation while preserving or promoting re-endothelialization, and thus eliminate the potential side effects caused by drugs coated on DES that are currently used in cardiovascular intervention.

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References

1. Degertekin M, Serruys PW, Foley DP, Tanabe K, Regar E, et al. *Circulation*. 2002; 106:1610–3. [PubMed: 12270850]
2. Woods TC, Marks AR. *Annu Rev Med*. 2004; 55:169–78. [PubMed: 14746515]
3. Clowes AW, Schwartz SM. *Circulation research*. 1985; 56:139–45. [PubMed: 3967343]
4. Hagensen MK, Raarup MK, Mortensen MB, Thim T, Nyengaard JR, et al. *Cardiovascular research*. 2012; 93:223–31. [PubMed: 22012957]
5. Finn AV, Joner M, Nakazawa G, Kolodgie F, Newell J, et al. *Circulation*. 2007; 115:2435–41. [PubMed: 17438147]
6. Tang R, Cui XB, Wang JN, Chen SY. *Arteriosclerosis, thrombosis, and vascular biology*. 2013; 33:2336–44.
7. Long CW, Levitzki A, Koshland D Jr. *Journal of Biological Chemistry*. 1970; 245:80–7. [PubMed: 5411547]
8. Torella D, Gasparri C, Ellison GM, Curcio A, Leone A, et al. *American Journal of Physiology-Heart and Circulatory Physiology*. 2009; 297:H2015–H25. [PubMed: 19783773]
9. Yoon JW, Cho BJ, Park HS, Kang SM, Choi SH, et al. *International journal of cardiology*. 2012
10. Hamesch K, Subramanian P, Li X, Dembowski K, Chevalier E, et al. *Thrombosis and haemostasis*. 2012; 107:356. [PubMed: 22234341]
11. Iaconetti C, Polimeni A, Sorrentino S, Sabatino J, Pironi G, et al. *Basic research in cardiology*. 2012; 107:1–14.
12. Khambata RS, Panayiotou CM, Hobbs AJ. *British journal of pharmacology*. 2011; 164:584–97. [PubMed: 21457229]
13. Tsukie N, Nakano K, Matoba T, Masuda S, Iwata E, et al. *Journal of atherosclerosis and thrombosis*. 2012
14. Urbich C, Dimmeler S. *Circulation research*. 2004; 95:343–53. [PubMed: 15321944]
15. Kawabe-Yako R, Masaaki I, Masuo O, Asahara T, Itakura T. *PloS one*. 2011; 6:e24646. [PubMed: 21931795]
16. Giordano A, Romano S, Monaco M, Sorrentino A, Corcione N, et al. *American Journal of Physiology-Heart and Circulatory Physiology*. 2012; 302:H135–H42. [PubMed: 22058159]
17. Tersteeg C, Roest M, Mak-Nienhuis EM, Ligtenberg E, Hoefer IE, et al. *Journal of Cellular and Molecular Medicine*. 2012; 16:2117–26. [PubMed: 22225866]