Decorin Mimic Inhibits PDGF-Stimulated Smooth Muscle Cell Proliferation and Migration

Rebecca A. Scott, Alyssa Panitch.
Weldon School of Biomedical Engineering, Purdue University, West Lafayette, IN

Statement of Purpose: Over the past 10 years, the number of percutaneous coronary interventions (PCI) performed in the United States has increased by 33%, thrombosis, neointimal hyperplasia, and restenosis remain complications of this procedure and inhibit complete functional recovery of the vessel wall. While a wide range of anti-restenotic therapeutics has been developed, many elicit non-specific effects that compromise vessel healing. Towards this effort, our lab has developed a mimic of the natural proteoglycan decorin, termed DS-SILY, which can mask exposed collagen and thereby effectively decrease platelet activation and neointimal hyperplasia by 60% in Ossabaw swine. [1-2] Furthermore, we also recently demonstrated that DS-SILY decreased smooth muscle cell (SMC) proliferation, migration, protein synthesis, and pro-inflammatory cytokine secretion *in vitro* in a concentration dependent manner [2]. However, to better examine the effect of DS-SILY on SMC behavior *in vitro,* the interactions between growth factors, such as platelet derived growth factor (PDGF), present at the site of vessel injury and SMCs should be investigated. Here, we investigate the interactions of DS-SILY and PDGF, as well as their effect on SMC behavior, in order to better understand the potential effect of the decorin mimic as a therapeutic for the treatment of restenosis.

Methods: DS-SILY was fabricated by coupling the type I collagen-binding peptide sequence RRANAALKAGELYKSILYGC (noted as SILY) to the backbone of oxidized dermatan sulfate (DS) via a heterobifunctional crosslinker. The affinity of PDGF for DS-SILY was assessed via solid-phase binding. For cell studies, primary human coronary artery smooth muscle cells (SMCs) were initially seeded at 5×10^4 cells/cm^2 in Ibidi angiogenesis μ-slide. To examine the effects of DS-SILY on both proliferative and quiescent SMC cultures, either proliferative media, containing high serum, or contractile media, supplemented with low serum and 30 μg/mL heparin, was added for 24 hrs. Cultures were then treated with 0, 0.01, 0.1, 1, or 10 μM DS-SILY, in the presence and absence of 10 ng/mL PDGF, prior to analysis. Post-treatment, SMC proliferation was measured by determining the number of SMC nuclei per volume; cytokine production and intracellular mitogen-activated protein kinases (MAPK) signaling was examined via multi-spot cytokine assay using MSD Sector Imager (Mesoscale Discovery, Rockville, MD). Protein synthesis was assessed as described elsewhere. [3] SMC migration was examined via a modified Boyden chamber. ANOVA was utilized ($p<0.05$) for statistical significance.

Results: Using solid-phase binding assay, PDGF was found to bind to DS-SILY with a dissociation constant of (K_d) of 29.9 ± 5.7 nM. The addition of PDGF to SMCs enhanced proliferation, migration, and protein synthesis in SMCs exhibited either a proliferative or quiescent phenotype compared to controls. A dose dependent decrease in SMC proliferation

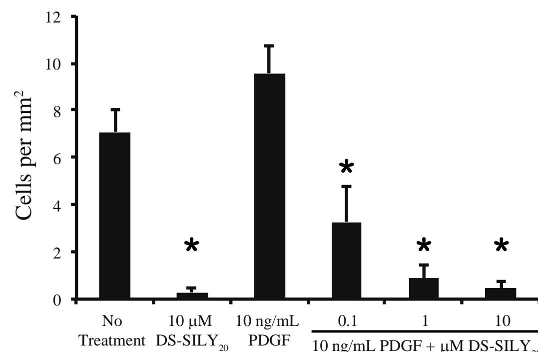

Figure 1. SMC migration in response to DS-SILY and PDGF treatments. * represents significance from no treatment and 10 ng/mL PDGF cultures.

and protein synthesis was observed as the DS-SILY concentration increased in PDGF-stimulated SMC cultures. Moreover, the addition of DS-SILY to *in vitro* PDGF-stimulated SMC cultures decreased migration by 94% and 86% in proliferative (Figure 1) and quiescent cultures, respectively. PDGF significantly increased IL-1β, IL-6, and TNF-α production in SMC cultures; however, inflammatory cytokine production decreased as the concentration of DS-SILY$_{20}$ increased in PDGF-stimulated cultures. Following stimulation with PDGF, the relative ratio of phosphorylated ERK/total ERK (pERK/ERK) and phosphorylated p38/total p38 (pp38/p38) increased in SMC cultures. However, a decrease in both pERK/ERK and pp38/p38 was exhibited as the concentration of DS-SILY increased in cultures.

Conclusions: Due to the highly negative charge associated with the DS backbone of our decorin mimic, DS-SILY, PDGF is able to bind to DS-SILY with high affinity, as the DS likely immobilizes the growth factor, keeping it from reaching exposed SMCs and stimulating them into a synthetic phenotype. As demonstrated in this work, the addition of PDGF to *in vitro* cell cultures increased SMC proliferation, migration, protein synthesis, and inflammatory cytokine production. However, the effect of PDGF on SMC behavior was mitigated by DS-SILY, where as the concentration of DS-SILY increased significant decreases in proliferation, migration, protein synthesis, and cytokine production were exhibited. The trends demonstrated here correlated to patterns seen intracellularly, where intracellular phosphorylation of the MAPKs ERK and p38 decreased with increased concentration of DS-SILY. As these results indicate that DS-SILY is able to mitigate inflammatory SMC behavior stimulated by growth factors, its use as an antithrombotic therapeutic during PCI may aid in the functional healing of the injured vessel wall and further exploration is warranted.

References: [1] Paderi JE. Biomaterials. 2010;32:2516-2523. [2] Scott RA. PLOS ONE. *In Press*. [3] Beatty KE. et al. Angew Chem Int Ed. 2006;45:7364-7367.

©2014 Society for Biomaterials