S100A4 and PDGF-BB induce smooth muscle cell phenotypic activation: it also takes two to waltz

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During atherosclerosis, smooth muscle cells (SMCs) accumulate into the intima and switch from a contractile to a synthetic phenotype. We have previously isolated two distinct SMC populations from the porcine coronary artery, spindle-shaped (S) and rhomboid (R) SMCs. R-SMCs display the features of synthetic SMCs. S100A4, a calcium-binding protein, was identified as being a marker of R-SMCs in vitro and of intimal SMCs, in both pig and man. Recently, we have shown that the extracellular form of S100A4 is essential for the establishment of the R-phenotype. By treating S-SMCs with S100A4-rich conditioned medium, we observed the phenotypic transition towards R-phenotype that was associated with acquisition of pro-inflammatory properties.

To further study the pivotal role of extracellular S100A4 on SMC phenotypic transition, S-SMCs were treated with dimeric and multimeric form of recombinant S100A4. Dimeric S100A4 had no effect on SMC phenotypic transition nor activation. Treatment with multimeric S100A4 was associated with a partial transition from S- to R-phenotype, NFκB translocation into the nucleus, increased expression of S100A4 and decreased expression of α-smooth muscle actin (α-SMA). Remarkably, treatment of S-SMCs with multimeric S100A4 and platelet-derived growth factor-BB (PDGF-BB) together induced a complete SMC transition toward a R-phenotype, associated with enhanced proliferation, increased S100A4 expression, decreased expression of α-SMA and NFκB activation compared with multimeric S100A4 or PDGF-BB alone (Figure). Multimeric S100A4 and PDGF-BB together induced a pro-inflammatory profile as shown by real-time PCR of matrix metalloproteinase-3 (fold change versus control 100.8±10.1) and granulocyte-macrophage colony-stimulating factor (fold change versus control 184.5±15.1). Moreover RNA sequencing showed that 67 genes were strongly upregulated exclusively when cells were treated with multimeric S100A4 and PDGF-BB together compared to treatments with multimeric S100A4 or PDGF-BB alone. High majority of these genes are involved in pathways related to inflammation. Furthermore, multimeric S100A4 and PDGF-BB-induced SMC phenotypic transition as well as activation were prevented by silencing of toll-like receptor 4 (TLR4).

Our results indicate that extracellular multimeric S100A4 and PDGF-BB act in synergy to promote a pro-inflammatory-like SMC phenotype likely through TLR4. Pro-inflammatory-like SMCs could be involved in plaque rupture and monocyte activation. Further studies will be focused on signaling pathways activated by extracellular multimeric S100A4 and/or PDGF-BB and on in vivo studies. We have preliminary shown that intimal SMCs in this mouse model co-expressed S100A4 and α-SMA while in the media S100A4 was absent, thereby confirming that S100A4 is a marker of intimal SMCs. The effect of S100A4 neutralization in balloon catheter-induced intimal thickening in mice will be further examined.
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