Response Gene to Complement-32 mediates aortic smooth muscle cells differentiation in human atherosclerotic lesions

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Introduction

The shift from the differentiated contractile smooth muscle cell (SMC) phenotype to a less differentiated, proliferative state and excessive extracellular matrix (ECM) production, both TGF- β regulated events, are key alterations in atherogenesis.¹ The complement system takes part in the initiation and progression of atherosclerosis. First described as a cell cycle regulator, Response Gene to Complement (RGC)-32 is also implicated in tumorigenesis, immune system regulation, scar tissue formation, regulation of lipid and glucose metabolism and atherogenesis. We have shown that RGC-32 modulates C5b-9-induced endothelial cell (EC) proliferation and migration and is involved in EC cytoskeletal organization and cell adhesion.² Additionally, RGC-32 promotes vascular SMC proliferation.³ RGC-32 also mediates some of TGF- β -induced profibrotic effects in a variety of tissues.^{4, 5}

Methods

We examined the expression of RGC-32, alpha-smooth muscle actin (α -SMA - as a marker of aortic SMC differentiation), collagen type I to V and fibronectin (as ECM components) in 9 human aortic atherosclerotic lesions (5 fibrous plaques and 4 intimal thickenings) by means of the indirect immunoperoxidase method. Rabbit IgG anti-RGC-32 (Bioss Inc), rabbit IgG anti-collagen I, III, IV and V (Santa Cruz Biotech) and rabbit polyclonal alpha smooth muscle Actin antibody (Abcam) along with the RTU Vecastain kit (Vector Labs) were used. Human aortic SMC (ASMC) from Lonza (Walkersville, MD) were grown 3–5 passages in SMC basal medium, containing supplements of 5% FBS, 10 ng/ml human EGF, 2 ng/ml human FGF and 5 μ g/ml insulin. ASMC were then exposed to TGF- β (10ng/ml) for 1, 3, 6 or 18 hours. Expression of fibronectin, pro-collagen type I, IV, V and α -SMA was determined by real-time PCR. The impact of RGC-32 in mediating ECM expression in ASMC was then explored via silencing RGC-32 expression in ASMC, which were transfected with siRGC-32 (Santa Cruz Biotech) or siCTR using Lipofectamine 2000. After 48 h, cells were stimulated with TGF- β (10ng/ml) and total mRNA was purified and analyzed for the mRNA expression of pro-collagen I, IV, V, fibronectin and α -SMA.

Results

Figure 1. Immunohistochemical localization of RGC-32 in human intimal thickenings. RGC-32 was found to be expressed by endothelial cells (A), monocytes infiltrating the intima (A, B) and less by smooth muscle cells in the media (C). Controls of immunoperoxidase reaction were negative (D).

Figure 2. Immunohistochemical localization of RGC-32 in human fibrous plaques. RGC-32 was found to be expressed by endothelial cells (A), monocytes infiltrating the intima (A, B), ASMC migrated in the intima and ASMC in the media (C). Controls of immunoperoxidase reaction were negative (D).

Figure 3. Immunohistochemical localization of collagen I and III in human fibrous plaques. Collagen I and III were found to be expressed by endothelial cells (A), monocytes infiltrating the intima (A, B), ASMC migrated in the intima (A, B) and ASMC in the media (C). Controls of immunoperoxidase reaction were negative (D).

Figure 4. TGF- β -induces expression of pro-collagen I, IV, V and fibronectin. The expression of the mRNA at the beginning of the experiment (CTR) was considered to be 100%.

Figure 5. Effect of RGC-32 silencing on pro-collagen I, IV, V and fibronectin. RGC-32 silencing significantly reduced the mRNA expression of pro-collagens I ($p < 0.02$), IV ($p < 0.03$), V and fibronectin ($p < 0.05$) (A) when compared with siCTR.

Figure 6. Effect of RGC-32 silencing on α -smooth muscle actin. RGC-32 silencing in ASMC led to a significant reduction in TGF- β induced α -SMA ($p < 0.02$).

Conclusions

Our data infers that RGC-32 is a vital player in TGF- β -mediated differentiation and ECM production by ASMC, rendering it an attractive target for therapeutic intervention in atherosclerosis.

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