Smooth muscle FGF/TGFβ cross-talk regulates atherosclerosis progression

P-Y. Chen et al., Appendix

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Appendix Figure S1 and figure legend

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Appendix Table S1
Appendix Figure S1. FRS2α knockdown inhibits proliferation of human aortic smooth muscle cells (HASMCs).

(A) Control and FRS2α knockdown HASMCs were cultured in the growth medium (M231 + SMGS). Cell proliferation was analyzed using real-time cell analysis (xCELLigence). Cell proliferation curves are representative of three independent experiments (**p<0.001 compared to control; unpaired two-tailed Student’s t test).

(B) Left: Control and FRS2α knockdown HASMCs were cultured in the growth medium (M231 + SMGS). Immunoblot analysis of cell cycle regulators Cyclin D1, p21, and p27 in control and FRS2α knockdown HASMCs. Blots are representative of four independent experiments. Right: Band intensities of Cyclin D1, p21, and p27 were normalized to GAPDH and expressed as a fraction of a control value. Results are expressed as means ± SD (**p<0.01; ***p<0.001 compared to control; unpaired two-tailed Student’s t test).

(C) Control and FRS2α knockdown HASMCs were cultured in the growth medium (M231 + SMGS). Flow cytometry analysis with propidium iodide (PI) staining was used to evaluate the percentage of cellular DNA content in control and FRS2α knockdown HASMCs. Histogram of cell cycle distribution results are representative of three independent experiments.

Source data are available online for this figure.
Appendix Figure S2. FGFR1 knockdown activates TGFβ signaling and induces smooth muscle marker gene expression in primary human aortic smooth muscle cells (HASMCs).

(A) qRT-PCR analysis of FGFRs, FRS2α, and Klotho family gene expression in primary human aortic smooth muscle cells (HASMCs). Data are presented as mean ± SD. β-actin was used for sample loading normalization. Histogram of qRT-PCR results are representative of four independent experiments.

(B-C) qRT-PCR analysis of TGFβ ligands, TGFβ receptors, and downstream target genes in control and FGFR1 knockdown HASMCs. (*p<0.05; **p<0.01; ***p<0.001 compared to control; unpaired two-tailed Student’s t test). β-actin was used for sample loading normalization. Histogram of qRT-PCR results are representative of three independent experiments.

(D) qRT-PCR analysis of smooth muscle cell transcription factors and smooth muscle marker gene expression in control and FGFR1 knockdown HASMCs. (*p<0.05; **p<0.01; ***p<0.001 compared to control; unpaired two-tailed Student’s t test). β-actin was used for sample loading normalization. Histogram of qRT-PCR results are representative of four independent experiments.
compared to control; unpaired two-tailed Student’s t test. N=3). β-actin was used for sample loading normalization.

(E) Left: Immunoblot analysis of TGFβ signaling, TGFβ downstream targets, and smooth muscle markers in control and FGFR1 knockdown HASMCs. Blots are representative of four independent experiments. Right: Band intensities of p-Smad2, p-Smad3, TGFβR1, and SM α-actin, SM22α, and SM-calponin were normalized to Smad2/3 or HSP90 and expressed as a fraction of a control value. Results are expressed as means ± SD (*p<0.05; **p<0.01 compared to control; unpaired two-tailed Student’s t test).

Source data are available online for this figure.
### Appendix Table S1

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