

FAK-RELATED NON-KINASE (FRNK) DISPLACES FOCAL ADHESION KINASE (FAK) FROM FOCAL ADHESION COMPLEXES IN VASCULAR SMOOTH MUSCLE CELLS

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INTRODUCTION: Myointimal hyperplasia (IH) is the most common cause of late failure after vascular interventions and operations. Vascular smooth muscle cell (SMC) migration from the vessel media to the intima precedes SMC proliferation and matrix deposition. Thus, inhibiting SMC migration may limit the progression of IH. Focal adhesion kinase (FAK) is a non-receptor tyrosine kinase that contributes to the regulation of cell migration through the promotion of integrin signalling cascades. The C-terminal homologue of FAK, FAK-related non-kinase (FRNK) displaces FAK from focal adhesion complexes in cardiac myocytes, (1) and since it lacks a kinase domain, it is thought to be a negative regulator of the integrin signalling cascade. Here we show that FRNK displaces FAK from focal adhesion complexes in vascular SMCs, and that the FRNK gene can be infected into SMCs that are suspended in a 3-D fibrin glue matrix.

METHODS: FRNK displacement of FAK was shown using canine carotid artery SMCs. SMCs were plated (10^6) on fibronectin-coated chamber slides overnight, then infected with adenovirus (Adv)-GFP or Adv-GFP-FRNK at 100 MOI for 24 hours. The slides were fixed, stained with a rhodamine tagged antibody for the kinase domain of FAK, and visualized under confocal microscopy. Uninfected canine carotid artery SMCs and canine jugular vein endothelial cells were inverted and suspended for 48 hours, and the subsequent cell pellets were entrapped in a fibrin glue matrix and suspended in 24 well plates with growth media. (2) 24 hours after gel suspension, Adv-GFP or Adv-GFP-FRNK was added to the media at 500 MOI, and efficiency of infection was determined at serial time points.

RESULTS: Infection of vascular smooth muscle cells suspended in a 3-dimensional co-culture angiogenesis system was pervasive by 7 days after infection. (Figure 1)

Canine carotid artery smooth muscle cells had 100% infection at 24 hours when plated on chamber slides. FRNK-infected SMCs had FAK displaced from focal adhesion complexes and replaced by FRNK, while GFP-infected cells showed the normal incorporation of FAK into focal adhesion complexes.

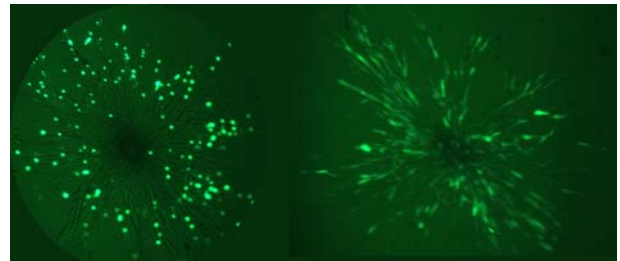


Fig. 1: Successful infection vascular smooth muscle cells in 3-D co-culture with endothelial cells. Adv-GFP-FRNK infected SMCs (left) vs. Adv-GFP infected SMCs (right).

DISCUSSION & CONCLUSIONS: FRNK displaces FAK from focal adhesion complexes in vascular smooth muscle cells, and it is readily expressed in SMCs by adenovirus delivery that are grown on/in both 2-D and 3-D culture environments. Substrate specific gene delivery of adv-FRNK may allow down-regulation of FAK-mediated SMC migration, proliferation, and matrix deposition. Cellular activity in 3-D systems is poorly understood, yet these conditions are more similar to the in vivo cellular milieu. This study provides mechanistic and pragmatic proof of concept for the selective manipulation of SMC activity in 3-D matrices.

REFERENCES: ¹ MC Heidkamp, AL Bayer, JA Kalina, DM Eble, AM Samarel (2002) *Circ Res* 90:1282-89. ² L Xue, HP Greisler (2002) *Surgery* 132:259-67.

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