Anastellin irreversibly alters the mechanical properties of extracellular matrix fibronectin fibers

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Statement of Purpose: Anastellin (An), a fragment derived from the extracellular matrix (ECM) of the tumor microenvironment, has been reported to regulate tumor growth in mouse models of human cancers. However it is not yet known whether An directly affects the cell's microenvironment and how it could do so. Here we examined the activity of An both *in vitro* on fibroblast-derived extracellular matrix fibronectin (Fn) fibers and on *manually pulled* Fn fibers used as model systems. More specifically, we investigate whether (and how it could do so) An binding to Fn fibers in turn alters the mechanical response of the Fn fibers.

Methods: We used a fluorescence resonance energy transfer (FRET)-based technique as an indirect indication of Fn conformation [1] to address whether newly incorporated An molecules were affected by the local conformation of the pre-existing Fn matrix. In a first series of cell-based assays, cells were grown for 24 hours in the presence of FRET-labeled Fn (double labeled with Alexa Fluor® 488 and Alexa Fluor® 546), and subsequently the media was exchanged to contain An labeled with a third color (Alexa Fluor® 633) to map the location of newly incorporated molecules within the FRET-labeled pre-existing matrix fibers. In a second series of experiments, manually pulled (pre-strained) Fn fibers were used as model systems to measure individual fibers mechanical behavior upon deformation (using a MEMS pulling/sensing device [2]) and to correlate it with exposure of cryptic sites for binding An molecules.

Results: In this report we investigated how An bound to cell-made Fn fibers, more specifically we explored whether such binding was specific, and how it was affected by the conformation of the underlying fibrillar network. Figure 1 shows the quantitative colocalization analysis we developed to effectively measure the amount of An binding as a function of the conformation of underlying fibrillar Fn represented by the fluorescence resonance energy transfer (FRET) ratio. These results indicate that fibrillar Fn in the unfolded conformations (blue FRET pixels) are the most frequent sites for binding of An. In addition we show that the binding of An to those unfolded (strain-induced) Fn regions is irreversible, which in turn results in the permanent alteration both of the fibers elasticity (increased stiffness at high strains) and of their refolding capability, leaving a long-lived residual strain in the An-treated Fn fibers.

Colocalization analysis of anastellin binding to Fn matrix

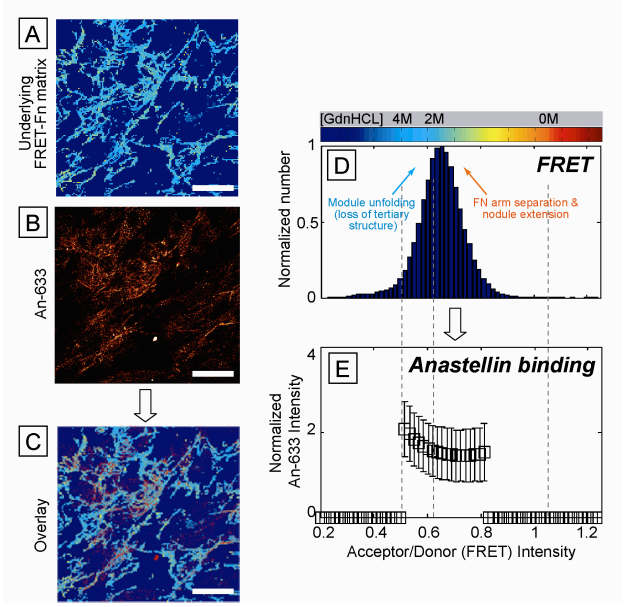

Figure 1. Colocalization analysis of anastellin binding to a pre-existing fibronectin matrix. (A) Fibronectin matrix after 24 hours culture of HFFs: fibronectin fibers have incorporated 10% FRET-labeled Fn (B) Anastellin tagged with Alexa Fluor® 633 (An-633) was allowed to adsorb to the underlying fibronectin matrix for 2 hours. (C) Overlay of (B) with (A). The FRET intensity for each pixel is color-coded and mapped to the image shown in (A), and (D) displays the corresponding histogram of FRET values for that field of view. (E) Normalized An-633 intensity measured from (B) vs. FRET intensity measured from (A). Scale bar: 50 μm.

Conclusions: Both the altered stiffness and the residual strain measured in mature Fn fibers after An treatment play critical roles in mechano-transduction processes (since reversibility is a key feature of mechano-chemical protein switches such as Fn) by disrupting the functionality of Fn fibers when stretched by cells into non-equilibrium states. These results further suggest that anastellin acts primarily as a mechano-regulator of the ECM network stiffness, which in turn may be a key mechanism for regulating malignant behavior of stiffness-sensitive cancer cells phenotype.

References:

[1] Smith ML, Gourdon D et al. PloS Biol. 2007;5:e268.
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