Analysis of the effects of TGF-β mediated reduction of caveolin-1 expression following cellular interaction with a biological extracellular matrix

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Background
Breast cancer is the most commonly diagnosed cancer in women and the second most common overall. Extensive work has been done on the underlying genetic mechanisms concerning the development of malignancy(Van’t Veer et al 2002), but the focus is shifting to reflect evidence that the tumor microenvironment is critical to understanding the disease(Liao et al 2009). TGF-β has been implicated in the induction of cancer associated fibroblasts and subsequent development of cancer in breast epithelial cells(Kupershaw et al. 2004), and it is known to be stored in the fibroconnect of the ECM via the LTBP-1 protein(Zilberberg 2012). With increased deposition of fibroconnect in a cancerous state(Chiarugi et al. 2011), and knowing that TGF-β is stored in the fibroconnect matrix of the ECM, we propose that TGF-β in cooperation with the biological matrix downregulates caveolin-1 in human mammary fibroblasts.

Hypothesis
We believe that fibroconnect matrix alone does not downregulate caveolin-1. Additionally, we propose that TGF-β is stored in the biological matrix in the form of LTBP-1, and, in cooperation with the matrix, downregulates cav-1 in human mammary fibroblasts.

Experimental Methods
HMFs were plated on 6-well plates that were coated with varying densities of ECM protein and observed for downregulation of caveolin-1 via Western Blot. Additionally, LTBP-1 was visualized using immunofluorescence with DAPI to visualize the nucleus, GFP to visualize LTBP-1, and RFP to visualize the fibroconnect. Additionally, to observe the effects of TGF-β on caveolin-1 expression levels, HMFs were plated on a control plate or biological ECM and observed for 24, 48, and 72 hours then collected and lysed to quantify protein expression with Western Blot.

Conclusions
- Cav-1 is not downregulated through fibroblast interaction with the fibroconnect matrix alone
- LTBP-1 is present in HMFs and more specifically in the biological ECM as evidenced via western blot and immunofluorescence
- TGF-β decreases cav-1 expression in HMFs grown on a biological matrix and not control plates, indicating a mechanism of cav-1 downregulation via TGF-β interaction with the matrix

References
Ciri P; Chiragji R; Cancer associated fibroblasts: the dark side of the coin. Am J Cancer Res 64(4): 482-497 (2011)

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