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The Induction of EMT and Activation of Adipose Stem Cells in Correlation with the Secretion of LTBP-1 in Mammary Cells

By Roshni Malik, Lauren Griggs,
Dr. Christopher Lemmon, PhD.

Abstract

This work is part of an ongoing study that investigates the upregulation of LTBP-1 in mammary epithelial cells as well as the differentiation of breast adipose stem cells (BASCs) in the presence of TGF- β 1. Through immunofluorescence imaging, LTBP-1 is shown to co-localize with fibronectin fibrils in adipose stem cells. Previous work from our lab has shown that blocking fibronectin fibril formation can inhibit Epithelial-Mesenchymal Transition. Thus, targeting of fibronectin assembly could be a potent new therapeutic in cancer treatment. In the current work, we focus on the pharmacodynamics of a FN assembly inhibitor derived from the protein Adhesin F1 (referred to as FUD). FN Fibril area was quantified in samples with different FUD dosages to determine the optimal concentration. The optimal dosage for this inhibitor was obtained for both mammary epithelial cells and breast adipose stem cells through image processing. Additionally, toxicity studies were performed using MTT assays. Results suggest that in both the mammary epithelial cells and the breast adipose stem cells, there is a range of dosing for which FN fibril formation is blocked but toxicity is low.

Introduction

This study investigates the correlation between the secretion of Latent Transforming Growth Factor Beta Binding Protein-1 (LTBP-1) and the occurrence of Epithelial to Mesenchymal Transition (EMT) in mammary epithelial cells (MCF10As). EMT is a process by which epithelial cells, which are the cells that line all of the organs within our body, change their phenotype and function to become mesenchymal cells, which are cells responsible for tissue growth and assembly throughout the body. This process has been implicated as an early step in cancer progression (1). Upon addition of certain growth factors, such as TGF- β 1, epithelial cells have been shown to transdifferentiate into mesenchymal cells.

TGF-1 also causes the activation of breast adipose stem cells (BASCs). Experimental data indicate that BASCs can differentiate into cancer-associated fibroblast (CAF)-like cells under the influence of breast cancer-derived soluble factors (2). BASCs stimulate desmoplasia as well as breast cancer cell growth and invasion in vivo (3), making this resident stromal cell of particular interest.

Previous research has demonstrated that adding TGF- β 1 to mammary cells increases fibronectin secretion, which can serve as a growth factor delivery system by concentrating growth factors at domains III12-14. This research seeks to determine whether the addition of TGF- β 1 causes an increase in latent TGF- β 1 which then concentrates at the cell surface. We believe that upon the activation of LTBP-1, signal transduction and gene transcription associated with EMT is initiated, resulting in a positive feedback cycle. We hypothesize that the mechanism of EMT in epithelial cells involves a positive feedback loop by which increased TGF- β 1 increases the expression/assembly of fibronectin, which then increases the secretion of LTBP-1, the inactive form of TGF- β 1, initiating the process of EMT. In BASCs, we expect differentiation into a myofibroblast phenotype in the presence of this growth factor. Thus we targeted FN assembly inhibition in mammary cells through the functional upstream domain

of the bacterial cell wall protein adhesin F1, better known as FUD.

Methods

To analyze the relationship between EMT growth factor signaling and fibronectin assembly, approximately 0.2×10^5 BASCs or 0.1×10^6 MCF10As were seeded on coverslips in the presence or absence of 2 ng/ml of TGF- β 1 to determine whether the addition of TGF- β 1 increased the formation of fibronectin fibrils and the co-localization of LTBP-1. Additionally, the correlation between FUD dosage and fibril formation was also observed quantitatively and qualitatively in MCF10As and BASCs through immunofluorescence. MTT assays were conducted to analyze the effect of FUD concentration on cell death in both BASCs and MCF10As.

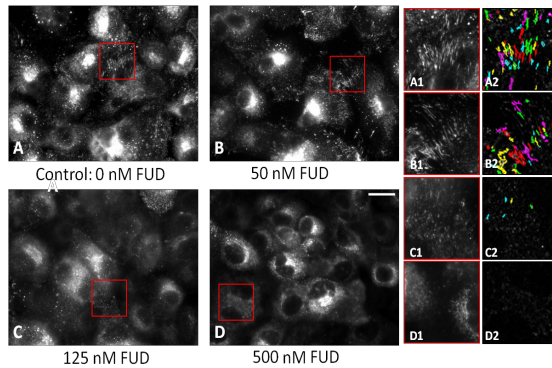


Figure 1: The Effect of FUD Concentration on Fibril Formation. Immunofluorescence images of MCF10As. A) Control 0 nM FUD: Abundance of short fibrils; B) 50 nM FUD: Less fibril formation as compared to control; C) 125 nM FUD: FN assembly sufficiently inhibited; D) 500 nM FUD: No fibril formation; A1-D1) Representative sections of A-D; A2-D2) Representative images of total fibronectin fibril area.

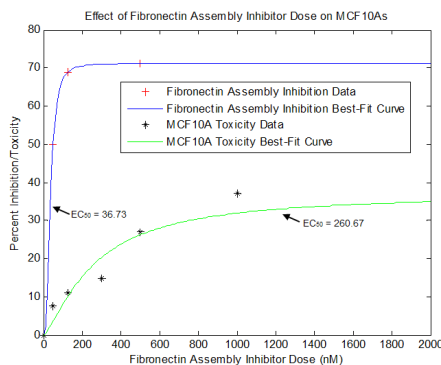


Figure 3: Dose Response and Toxicity of Fibronectin Inhibitor on MCF10As. Percent fibronectin fibril inhibition and percent toxicity as a function of inhibitor dose.

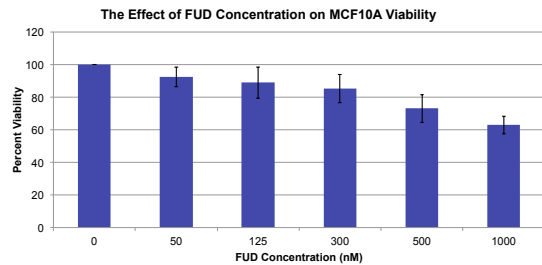


Figure 2: The Effect of Fibronectin Inhibitor FUD on Toxicity of MCF10As.

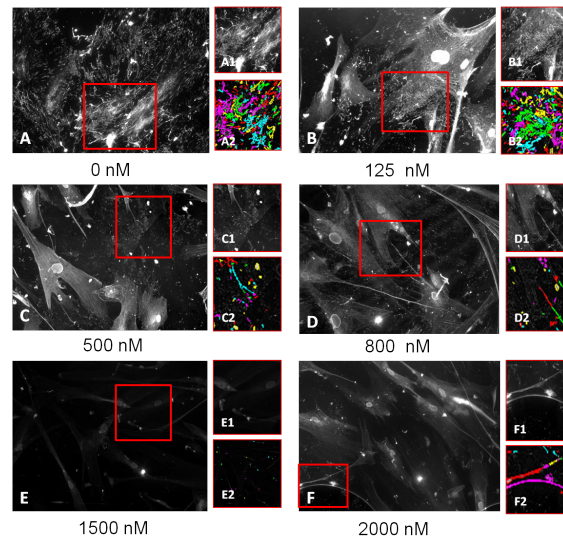


Figure 4: The Effect of FUD Concentration on Fibril Formation. Immunofluorescence images of BASCs. A) Control 0 nM FUD: Abundance of short fibrils; B) 125 nM FUD: Less fibril formation as compared to control; C) 500 nM FUD: Substantially decreased FN assembly D) 800 nM FUD: FN assembly sufficiently inhibited; E) 1500 nM FUD: No fibril formation; F) No fibril assembly; A1-F1) Representative sections of A-D; A2-F2) Representative images of total fibronectin fibril area.

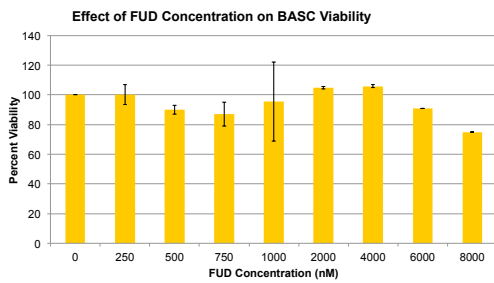


Figure 5: *The Effect of Fibronectin Inhibitor FUD on Toxicity of bASCs.*

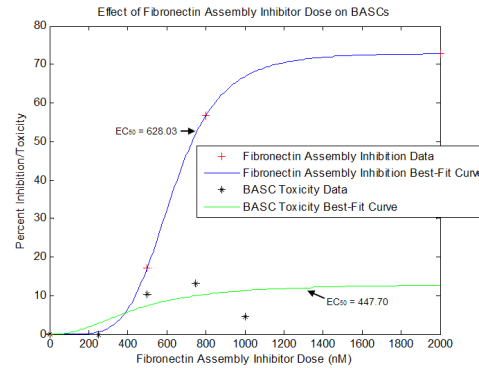


Figure 6: *Dose Response and Toxicity of Fibronectin Inhibitor on bASCs. Percent fibronectin fibril inhibition and percent toxicity as a function of inhibitor dose.*

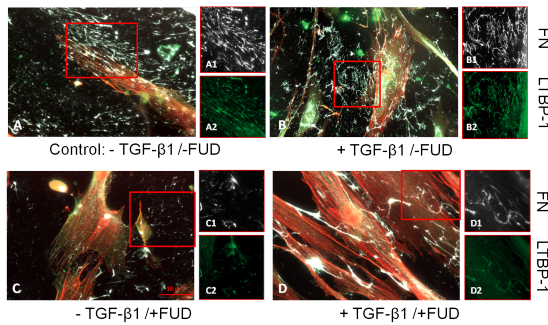


Figure 7: *The Effect of FUD on Fibril Formation and localization of LTBP-1. Composite immunofluorescence images of MCF10As. Red channel: actin, green channel: LTBP-1 and white channel: fibronectin. Addition of TGF-β1 causes an increase in fibronectin fibrillogenesis and corresponding LTBP-1 secretion, which then becomes localized on fibronectin fibrils. This increased secretion and colocalization of LTPB-1 is blocked when fibril formation is inhibited.*

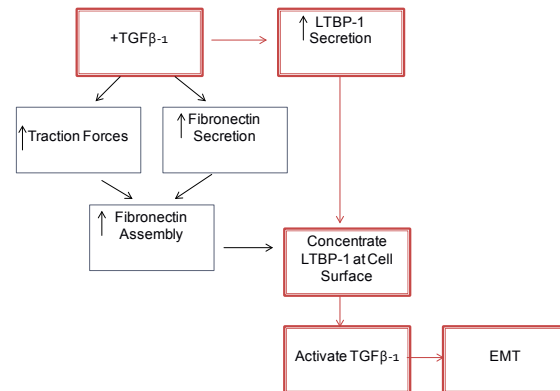


Figure 8: *Schematic Representation of Hypothesized Mechanism. Illustration of the specific mechanism of EMT being studied. The red boxes signify the hypothesized positive feedback loop that is inducing EMT.*

Discussion

We have established a correlation between fibronectin assembly and LTBP-1 co-localization. We believe that a mechanism behind TGF-β1-induced EMT involves a positive feedback loop of which addition of TGF-β1 increases the secretion of LTBP-1, its co-localization, and its activation, causing the induction of EMT (Figure 1). Furthermore, fibronectin assembly on BASCs decreased with increasing FUD dosage. MTT results showed that there was no significant effect of FUD dosage on BASCs, however MCF10as are less tolerable to FUD as cell death started to occur at 500nM. These results indicate that LTBP-1 secretion is linked to TGF-β1 activation. In order to stop the cycle of EMT the optimal FUD dosage was obtained for BASCs as 600 nM and MCF10As as 125 nM.

References

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