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## **Exogenous FNIII 12-14 Regulates TGF- $\beta$ 1-Induced Markers**

A thesis submitted in partial fulfillment of the requirement for the degree of Master's in  
Biomedical Engineering at Virginia Commonwealth University.

by

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## List of Abbreviations

Anti-EMT GF:	Growth factors that are known to block or reverse EMT processes
BSA:	Bovine serum albumin
ECM:	Extracellular matrix
EGF:	Epidermal growth factor
EMT:	Epithelial-Mesenchymal Transition
FGF-2:	Fibroblast growth factor
FITC:	Fluorescein isothiocyanate molecules
FN:	The extra cellular protein, Fibronectin, as a whole, consisting of Types I, II, and III.
FNIII 12-14:	The repeats 12, 13 and 14 of Fibronectin type III.
FNIII 2-4:	The repeats 2, 3 and 4 of Fibronectin type III
FUD:	The 49-residue functional upstream domain (FUD) of Streptococcus pyogenes F1 adhesion
GF:	Growth factor
LTBP1:	Latent TGF- $\beta$ 1 binding protein
PBS:	Phosphate-buffered saline
PDGF:	Platelet-derived growth factor
Pro-EMT GF:	Growth factors that are known to promote EMT processes
TGF- $\beta$ 1:	Transforming Growth Factor Beta 1
ZEB1:	Zinc finger E-box-binding homeobox 1 transcription factor



## Abstract

The extra-cellular matrix protein Fibronectin (FN) plays an important role in cell contractility, differentiation, growth, adhesion, and migration. The 12th -14th Type III repeats of FN (FNIII 12-14), also referred to as the Heparin II domain, comprise a highly promiscuous growth factor (GF) binding region. This binding domain aids in cellular signaling initiated from the ECM. Additionally, FN has the ability to assemble into fibrils under certain conditions, mostly observed during cell contractile processes such as those that initiate due to upregulation of Transforming Growth Factor Beta 1 (TGF- $\beta$ 1) [1], [2]. Previous work from our lab has shown that self-assembly of FN into insoluble fibrils is crucial for Epithelial-Mesenchymal Transition (EMT) [3]. The transition from epithelial to mesenchymal cell type has been implicated as an early event in tumor formation and breast cancer. We were previously able to find that upregulation of FN fibrils drive EMT through contractility due to the increase of the GF latent TGF- $\beta$  complex concentration at the cell membrane [3].

The challenge in the current work is to exploit the role of Heparin II binding domain and to concentrate growth factors of interest, such as those that are pro-EMT or anti-EMT at the signaling sites of the cell membrane. Initially, we investigated the localization of the fragments FNIII 12-14 delivered to cell membrane using FITC conjugated protein. We then investigated the effects of exogenous FNIII 12-14 on EMT using breast epithelial cells (MCF10A) in the presence or absence of TGF- $\beta$ 1 to determine whether FNIII 12-14 alters EMT signaling. Quantification of mRNA expression, for EMT markers such as Slug, Snail, Twist and ZEB1 were analyzed. Results showed that dosage increase of FNIII 12-14 appears to inhibit EMT

transcription factors. This study will develop a new understanding of disease and gene control using ECM proteins. The exploitation of ECM natural protein interactions could become a new method in turning on/off genes of interest. While we are currently investigating this as a mechanism of blocking EMT, it could also have implications in wound healing, fibrosis, and tissue engineering, where EMT is an important aspect of the physiologic progression.

## Chapter 1: Introduction

Estimates from the American Cancer Society (ACS) for new diagnosed cases of and deaths from invasive breast cancer have been on the rise; hence, placing breast cancer as the second leading cause of death in women after lung cancer [4]. Both, the malignancy of the disease and lack of an absolute cure, in late stages, have encouraged our research team in investigating and helping to enrich the field with better assessments, diagnosis, and potentially effective treatments. Combined with our knowledge and passion of extracellular matrix (ECM) biology, we are able to further investigate cancer-causing processes, such as Epithelial-Mesenchymal Transition (EMT), and the ability of ECM proteins, such as Fibronectin (FN), in disease control.

ECM of an animal cell is commonly used as a guide to comprehend activities related to early invasion and tumor metastasis. One of the most commonly unregulated ECM proteins in cancer is FN. It has been widely studied for its significance in supporting embryogenesis [3], [4], differentiation [5], [6], and cell adhesion [9], [10]. It also plays an important role in cell stiffness and migration[11], and wound healing [12], [13]. It has been implicated that abnormally high expression of FN is an indication of early EMT; Followed by high rate of FN cleavage leads to metastasized cancer cells [14], [15]. Cancerous and fibrotic related health conditions usually initiate due to EMT processes known to uncontrollably up-regulate cell signaling and cause redundant pre-inflammatory response. These processes have the ability to alter cell fate through back-differentiation from a unipotent-epithelial to multipotent-mesenchymal cell type. In adult tissue, these transitions lead cells to gain stem-cell like properties, such as migration and contractility, and become at risk of tumorigenesis and metastasize.

Consequently, FN is considered a significant factor in the initiation of EMT processes. Due to its highly promiscuous growth factor (GF) binding domain, FNIII 12-14, it is thought to enhance cellular signaling initiated from the ECM [16]–[18]. FNIII 12-14 is also referred to Heparin II domain of FN, where it binds to cellular heparin. FN assembly into FN fibrils increases the number of localized FN, therefore FNIII 12-14, at the cell membrane. Previous work from our lab suggests that insoluble FN fibrils play a role in Transforming Growth Factor Beta 1 (TGF- $\beta$ 1)-induced EMT, due to their capacity in enhancing cell signaling through increased localization of the growth factor. This increase of co-localized growth factors cause an abnormal increase in cell signaling [3]. In other words, FN fibrils drive EMT by concentrating GFs, such as latent TGF- $\beta$  complex, at the cell surface through FN repeats with notable affinity to growth factors.

Because this increase in cell signaling is due to the distinguished affinity to GFs, Heparin II domain is used in the study as exogenous fragments to potentially create a binding competition to GFs and alter the end outcomes of EMT. The challenge of our work is to prospectively reduce or block abnormal EMT processes through utilizing the role of FNIII 12-14 to seize growth factors from the ECM. We investigated the effects of exogenous FNIII 12-14 on EMT in the presence of the pro-EMT, TGF- $\beta$  1, growth factor using the human breast epithelial MCF10A cell line. Through immunofluorescence and FITC-conjugated FN fragments, we were able to verify the activity of the exogenous FNIII 12-14. Furthermore, we investigated other potential interactions between the ECM and FNIII 12-14. Gene expression studies were then developed to investigate the effects of the exogenous FNIII 12-14 on transcription factors known to elevate during EMT. We observed great changes in levels of Slug, Snail, Twist, and ZEB1.

## Chapter 2: Background

The 2018 estimates from the American Cancer Society (ACS) show about 268,670 new cases of breast cancer to be diagnosed, and 41,400 new deaths, of which most are females. The numbers estimated are approximately six percent higher than last year, keeping breast cancer in second place of leading cause of deaths in women after lung cancer [4], [19]. The increasing death toll every year is due to lack in absolute cure, which is a great motivation to our research team and other labs around the globe to investigate and help to enrich the field with better assessments, diagnosis, and potentially effective treatments. Combining our knowledge of molecular biology and extracellular matrix (ECM) biology, we are able to investigate the ability of fragments of extracellular proteins, such as Fibronectin (FN), and its interactions in disease control.

ECM is commonly used as a guide to comprehend activities related to wound healing, fibrosis, early invasion and tumor metastasis. Yet, cells use common signaling pathways for both wound healing and Epithelial-Mesenchymal Transition (EMT) [20]. These signaling pathways include biochemical, bioelectrical and/or biomechanical interactions between cellular components. The balance between all three is highly regulated in healthy conditions. On the other hand, and in some cases, small disruptions in key signal-regulators can propagate and cause nonreversible damage to cells. FN, for instance, has been widely studied for its significance in supporting embryogenesis [3], [4], differentiation [5], [6], cell adhesion [9], [10], cell stiffness and migration [11], and wound healing [12], [13]. It has been implicated that abnormally high expression of FN is an indication of early EMT; Followed by FN degradation, leading to metastasized cancer cells breaking through the matrix [14], [15].

Epithelial to mesenchymal transition or EMT processes naturally occur in three forms: embryogenic and growth; fibrotic; and cancerous [21]–[24]. EMT processes, depending on the micro-environment, can lead to one of the three cell types; secondary epithelial cells, fibroblast cells, or secondary cancer cells [25]. EMT is critical and highly regulated during embryogenesis and wound healing, which leads to organ development and tissue remodeling [22], [24], [26]. These processes have the ability to alter cell fate through back-differentiation from a unipotent-epithelial to multipotent-mesenchymal cell type for growth and healing. On the other hand, **misregulation** of EMT can reprogram adult epithelial cells to become fibrotic or cancerous. Cancer and fibrotic-related health conditions have been suggested to initiate due to EMT processes known to up-regulate chemical and mechano-chemical cell signaling, causing redundant pre-inflammatory responses [27]–[29]. Nevertheless, in adult tissue, this transitioning leads cells to gain stem-cell like phenotypic properties, such as migration and increased rate in proliferation, becoming at risk for tumorigenesis and metastasis [30]. Metastasized cells have the ability to dissociate from original tissue, travel through the blood stream, and localize somewhere else in the body to form secondary tumors.

ECM composition differs from tissue to tissue, and can dictate a cell's morphological properties. The ECM provides epithelial cells with strong E-cadherin-based cell-cell junctions, an apicobasal polarity, and cortical actin cytoskeletal components. In mesenchymal-like cells, upregulated EMT individualizes cells, creates front-back polarity, causes spindle-like morphology of actin stress fibers, expresses N-cadherin, FN and vimentin in addition to increasing motility and invasion [21]. EMT is also known to play a role in angiogenesis important for tumor survival [31]. Although

Transforming Growth Factor Beta 1 (TGF- $\beta$ 1) plays an important role in proliferation control of epithelial cells, it has been found to induce EMT [23], [32], [33], and promote tumor metastasis [34], [35]. Consequently, FN is considered a significant factor in the aiding of upregulation of EMT processes in epithelial cells [3], [36].

FN is a long extracellular protein which acquires a compact bulky shape in the soluble form. When bound to integrins on the cell surface, it becomes stretched along its two ends to form fibril-like orientated fibrils [37], [38]. FN becomes more abundant during tumor initiation processes, as well as embryogenesis and wound healing through EMT [39], [40]. When FN is bound to cells and stretched, domains of the protein, which were initially blocked from interactions due to the soluble form, become exposed and able to participate in signaling processes. The soluble bulky conformation of FN is usually due to amino acid interactions between itself. Such electrostatic interactions are usually seen between FNIII domains 2-4 and 12-14 [41].

Both soluble growth factors (GFs) and FNIII 2-4 compete to bind with the heparin domain III 12-14. Studies suggest that assembled FN fibrils play a role in GF delivery when TGF-  $\beta$ 1 is bound in the activated form (LTBP-1 complex) to the heparin domain [42], [43]. FN type III 12, 13, and 14 repeats (FNIII 12-14) domains are a highly promiscuous GF binding region. This fragment is also a heparin binding domain (also called heparin domain II) [38]. FNIII 12-14 binds to many growth factors including TGF- $\beta$ 1, and TGF- $\beta$ 1 appears to have a medium binding affinity to FNIII 12-14, in comparison to PDGF (low), FGF-2 and EGF (high) [44].

EMT signaling, which may play a role in the initiation of carcinoma cells, remains an active area of scientific research. It is suggested that initial EMT-inducing signals are

released by primary cancer cells, which are capable of altering neighboring cells genetically and epigenetically [25]. EMT signals of cancer cells are able to inhibit senescence causing the aggression in tumorigenesis [45]–[47]. Pro-EMT growth factors, such as EGF, PDGF, and TGF- $\beta$ 1, are linked to the activation of Snail, Slug, ZEB1, and Twist transcription factors [48]–[52]. The upregulated expression of any, or more than one, of those transcription factors is an indication of an oncogenesis activity.

Snail, Slug, ZEB1, and Twist are epithelial marker repressors and mesenchymal phenotypic promoters. For instance, when activated by downstream signaling pathways of TGF- $\beta$ 1, the transcription factors down-regulate cell-cell junctions by repressing E-cadherin, as well as digest and remodel the ECM through over expression of FN and MMPs, giving the cell the ability to metastasize [53], [54]. Snail has also been found to promote cell invasiveness in mice [55], as well as oral and breast cancer in humans [56], [57]. Through gene expression analysis, researchers were able to identify Twist transcription factor as an EMT and metastasis inducer [58]. Therefore, using immunofluorescence staining techniques, markers such as E-cadherin, and peripheral actin filaments are here used to identify epithelial phenotypes, while FN, vimentin, and N-cadherin are used to identify cells with mesenchymal-like phenotype.

Since this increase in cellular signaling is due to the affinity and clustering of the pro-EMT growth factor on the cell membrane by FN Heparin II domain, introducing FNIII 12-14 fragments to the cell media could potentially create a binding competition and alter the end outcomes of the pro-EMT GF TGF- $\beta$ 1. The hypothesis of our work is that EMT can be inhibited through the utilization of FNIII 12-14's binding activity to competitively bind soluble growth factors around the cells. We investigated the effects of



exogenous FNIII 12-14 on EMT in the presence of TGF- $\beta$ 1 growth factor using the breast epithelial cell line MCF10A. Through immunofluorescence techniques along with conjugated protein, we were able to verify the localization of the exogenous protein, and investigate the mechanism of possible interactions with the ECM. Gene expression studies were then used to investigate the effects of the exogenous FNIII12-14 on mRNA expression of EMT transcription factors Slug, Snail, Twist and ZEB1. This study will develop a new understanding of how FN domains can be used to regulate GF pathways that bind to FN. While we are currently investigating this as a mechanism of blocking EMT, it could also have implications in wound healing, fibrosis, and tissue engineering, where FN fibril assembly is an important component of the physiological progression.

## Chapter 3: Methods and Materials

### Cell culture and reagents

Human mammary epithelial cells (MCF10A) were purchased from the Oncology Bioresource Core Facility of the National Cancer Institute Physical Sciences, in conjunction with American Type Culture Collection (Manassas, VA). Cells were cultured in a humidified incubator at 37 °C and 5% CO<sub>2</sub>. Culture conditions for the cells were maintained using DMEM/F-12 HEPES obtained from Life Technologies, Carlsbad, CA, 5% horse serum, 0.05% hydrocortisone, 0.01% cholera toxin, 0.1% insulin, 0.02% EGF and 1% antibiotics.

MCF10A cells were split and/or used for experiments just before cell monolayers reached full confluence (approximately 85-95%). This was done by incubating cells in 0.05% Trypsin solution for 20 minutes and re-suspending cells with culture medium. Following a centrifugation step at 500 RPM for five minute at room temperature, supernatant solution was vacuumed, and fresh media was added to resuspend the pellet of cells. A ratio of 1:5 was used in the process. Active Transforming Growth Factor Beta 1 (TGF- $\beta$ 1) recombinant protein was obtained from Sigma Aldrich, St. Louis, MO. Antibody staining for immunofluorescence imaging was performed using the following primary antibodies; Mouse anti-Human E-cadherin (Abcam, Cambridge, United Kingdom), Rabbit anti-Human FN (Sigma Aldrich, St. Louis, MO). For F-actin imaging, cells were labeled using AlexaFluor555 Phalloidin (Life Technologies, Carlsbad, CA).

## Expression and purification of FNIII 12-14

Fibronectin (FN) III 12-14 cDNA was received as a gift from Dr. Harold Erickson at Duke University. The DNA was previously inserted into ampicillin resistant *E. coli* bacteria vector (pET15b) containing a poly-histidine C-terminal tag and maltose binding protein (MBP), which aided in protein purification. Isopropyl  $\beta$ -D-1-thiogalactopyranoside (IPTG) was used as a transcription promoter. For bacteria cell-lysing and protein-degradation inhibition, reagents phenylmethane sulfonyl fluoride (PMSF) in isopropanol, ethylenediaminetetraacetic acid (EDTA), lysozyme, and 10% Triton-X 100 were used, in addition to -20°C overnight freeze. PMSF, MgCl<sub>2</sub>, and DNase were then added to the frozen culture and protein purification process was done using HisPur™ Cobalt Resin from Thermo Scientific.

During protein purification, Phosphate-buffered saline (PBS) was used for the washing steps, and 0.2  $\mu$ M of imidazole in PBS was used for protein elution. For proper storage of the fragments, FNIII 12-14 protein, Sephadex™ G-25 M kit from GE healthcare, Life Sciences, was used to perform buffer exchange using PBS as equilibrium, elution and storage buffer. FNIII 12-14 determination of A280 recombinant protein concentration was measured using NanoDrop 2000c spectrophotometer and software, Thermo Scientific.

Protein conjugation was performed using Fluorescein isothiocyanate isomer I (FITC) which was obtained from Sigma Life Sciences. FITC-FNIII 12-14 labeling was carried out using the following steps. FNIII 12-14 protein solution, of known concentration, was combined with freshly-made 1 M sodium bicarbonate buffer in 1:10

ratios, followed by mixing with newly dissolved 1mg/ml amine-reactive compound powder in DMSO. A total of 50 uL of the reactive dye was added per 1 mL of protein solution, in aliquots of 5 uL, with gentle vortexing in a dark room. The solution then was incubated at 4°C in the dark. Eight hours later, NH<sub>4</sub>Cl of 50 mM was added followed by a two hour incubation at 4°C.

Purification of the protein solution from unbound dye was done using 5% of Glycerol and 1% of BME followed by the use of Sephadex™ G-25 M kit from GE healthcare. Concentration of conjugated protein, FITC-FNIII 12-14, was measured using NanoDrop 2000c spectrophotometer and software, Thermo Scientific, through UV-vis option set at 280 and 495 nm wavelengths. Excel software, was used for calculations to verify the rate of conjugation and protein concentration in moles, refer to Figure 1 for mathematical equations used in this step.

$$\text{Ratio of } \frac{\text{FITC}}{\text{Protein}} = \left( \frac{\epsilon}{389} \right) \times \left[ \frac{\frac{A_{495}}{195}}{A_{280} - (0.35 * A_{495})} \right]$$

$$\text{Molarity} = [A_{280} - (A_{495} \times 0.35)] \times \left( \frac{1000000}{\epsilon} \right)$$

- 
- ε: Molar extinction coefficient of FNIII 12-14
  - 389: The molecular weight of FITC
  - 195: The absorption of bound FITC at 490 nm at pH 13.0
  - 0.35: Correction factor due to the absorbance of FITC at 280 nm
  - A<sub>280</sub>: Absorbance of FNIII 12-14 at 280
  - A<sub>495</sub>: Absorbance of FNIII 12-14 at 495

Figure 1: Equations Used for Protein Concentration Calculations

## Immunofluorescence imaging

Immunofluorescence experiments were conducted using glass coverslips previously washed with ethanol and coated with 50 µg/ mL laminin (Sigma Aldrich, St. Louis MO) for two hours at 37 °C. MCF10A cells were seeded onto laminin-coated 12 or 25-mm glass coverslips, in which cell count resulted in near confluent monolayers after overnight incubating (approximately 400,000 seeded cells per well). EGF and serum free medium was then used to growth factor-starve cells for two hours. Following the GF-free medium incubation, cells were treated with different dosages of FITC-FNIII 12-14 (0, 1nM, 10nM, 100nM, 1µM, 5µM and 10µM) followed by treatment with/without TGF-β1 (10 ng/mL) and incubated for an additional 96 hours (See Table 1 for experiment setup). Cells' plasma membrane was made permeable using 0.5% Triton in 4% paraformaldehyde for two minutes, and incubated in 4% paraformaldehyde for 20 minutes. Two PBS rinses were done, followed by 1% BSA incubation for five minutes and primary antibody incubation for 30 minutes at 37°C. Cells were then rinsed once with PBS for two minutes, and nonspecific-binding was eliminated by a five-minute incubation in 1% BSA followed by the appropriate secondary antibody application for 30 min at 37C°. Nonspecific-binding blocking technique was performed for the last time following the use of fluoromount to attach coverslips to glass slides, which were then placed in the dark at room temperature for one hour to stabilize. Images were captured using Zeiss AxioObserver Z1 fluorescence microscope and ZEN2011 software.

	FNIII 12-14 ( $\mu\text{M}$ )						
	0	0.001	0.01	0.1	1	5	10
(-)TGF- $\beta$ 1							
(+)TGF- $\beta$ 1							

Table 1: Immunofluorescence and Gene Expression Experiment Setup

## Real time quantitative PCR analysis

Experiments were also conducted using glass coverslips previously washed with ethanol and coated with 50 µg/ mL laminin (Sigma Aldrich, St. Louis MO) for two hours at 37 °C. MCF10A cells were seeded on the laminin-coated 25-mm glass coverslips, in which cell counts (approximately 400,000) result in near confluent monolayers after overnight incubation period. EGF and serum free medium was then used to growth factor-starve cells for two hours. Following the GF-free incubation, cells were treated with different dosages of FNIII 12-14 (0, 1nM, 10nM, 100nM, 1µM, 5µM and 10µM) followed by treatment with/without TGF-β1 (10 ng/mL) and incubated for an additional 96 hours, refer to table 1 above for experiment setup.

Extraction of total RNA was performed using RNeasy kit (Qiagen). Concentration of mRNA for each condition was then verified at or above the minimum concentration (100 ng/µL) via Nucleic acid built-in option on NanoDrop 2000c spectrophotometer and software, Thermo Scientific. The cDNA for mRNA sequences were then acquired using reverse transcriptase obtained from an iScript cDNA Synthesis Kit (Bio-Rad), and one PCR cycle using eppendorf Mastercycler pro equipment device.

The selective amplification of the cDNA was conducted by predesigned forward and reverse primers to genes of interest. The data for mRNA expressions were analyzed in response to primers for Snail, Slug, Twist, and ZEB1 (IDT). In addition, a housekeeper gene, 18s, specific primer set (IDT) was used for the normalization of data, refer to table 2 below for the complete primer sequence list. A 2X SYBR Green qPCR Master Mix, obtained from QUARTZY Bimake, was lastly added to qRT-PCR plates for the mRNA quantification assay which was performed using CFX Connect



Real-time System (Bio-Rad). Experiment data were collected and compared using gene study analysis using Bio-Rad CFX Manager and Microsoft Excel.

<b>Marker</b>	<b>Direction</b>	<b>Nucleotide Sequence</b>
<b>18s</b>	<b>Forward</b>	5'-GCA ATT ATT CCC CAT GAA CG-3'
	<b>Reverse</b>	5'-GGG ACT TAA TCA ACG CAA GC-3'
<b>Slug</b>	<b>Forward</b>	5'-CTC ACC TCG GGA GCA TAC AG-3'
	<b>Reverse</b>	5'-GAC TTA CAC GCC CCA AGG ATG-3'
<b>Snail</b>	<b>Forward</b>	5'-AAG ATG CAC ATC CGA AGC CA-3'
	<b>Reverse</b>	5'-CTC TTG GTG CTT GTG GAG CA-3'
<b>Twist</b>	<b>Forward</b>	5'-CTG CCC TCG GAC AAG CTG AG-3'
	<b>Reverse</b>	5'-CTA GTG GGA CGC GGA CAT GG-3'
<b>ZEB1</b>	<b>Forward</b>	5'-GTT CTG CCA ACA GTT GGT TT-3'
	<b>Reverse</b>	5'-GCT CAA GAC TGT AGT TGA AG-3'

Table 2: Nucleotide Sequences for Forward and Reverse Primers Used in mRNA Quantification Analysis (Real-Time qPCR)

## Chapter 4: Results

Immunofluorescence staining for each condition of MCF10A cells was completed using antibodies for E-cadherin (blue), F-actin (red), and FN (white). FNIII12-14 treatments were added as FITC-tagged protein and therefore are presented in green. Based on multiple trials, presented below are representative images for the Epithelial-Mesenchymal Transition (EMT) morphological markers (E-cadherin containing adherens junctions, actin organization, and FN assembly) for the negative TGF- $\beta$ 1 conditions displayed below in Figure 2. The increase and the presence of FNIII 12-14 alone appear to cause minimal morphological changes to E-cadherin junctions and actin (Figure 2). Furthermore, FN expression in the FN channel is seen in white inside cells and around the nuclei while assembly of FN can be seen as fibril-like aggregates on top or around cells. It appears that in response to the dose increase assembly and expression of FN stay consistently at low levels.

On the other hand, FNIII 12-14 effects on TGF-  $\beta$ 1-induced EMT morphological markers in MCF10A cells, shown in Figure 3, appear to inhibit EMT, as cells appear similar to morphologies observed in the control conditions in Figure 2. Cells were also cultured for 96 hours, yet this time with 10 ng of TGF- $\beta$ 1 in the presence of FNIII 12-14 treatment (ranging from 1nM to 10 $\mu$ M). In comparison with the positive control, molar concentration increase in FNIII 12-14, in the presence of 10ng/mL TGF- $\beta$ 1, appears to reduce morphological changes seen in the organization of E-cadherin junctions, yet morphological changes in actin appear to take place regardless of the amount present of the FN fragment. Interestingly, FN show visible reduction in protein expression and assembly in response to the dosage treatment of FNIII 12-14 (Figure 3).

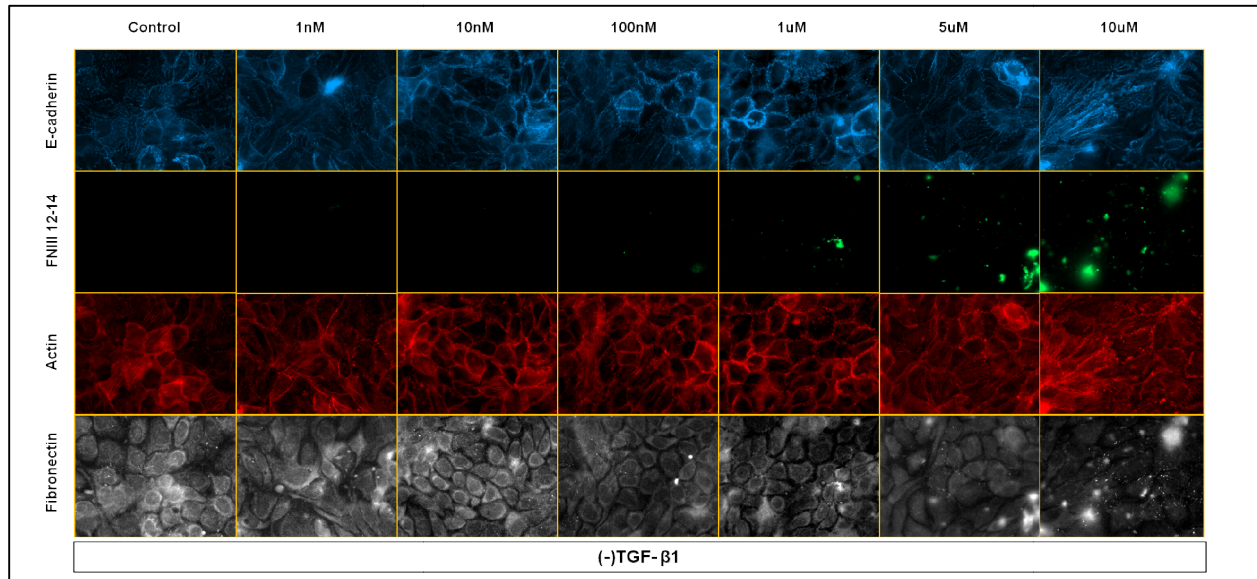


Figure 2: Representative images of FNIII 12-14-induced morphology in MCF10A cells. MCF10A cells were cultured for 96 hours without any exogenous TGF-β1, yet with the presence of FITC-FNIII 12-14 treatment ranging from 1nM to 10μM. Immunofluorescence staining was completed using antibodies for E-cadherin (blue), F-actin (red), and FN (white). FNIII12-14 treatments were added as FITC-tagged protein (green). The exact light intensity and exposure was used throughout the experiment. In the FN channel, white immunofluorescence inside MCF10A cells and/or around the nucleus represents expressed FN protein by the cell while fibril-like white elements on or around the cell represent assembled FN fibrils.

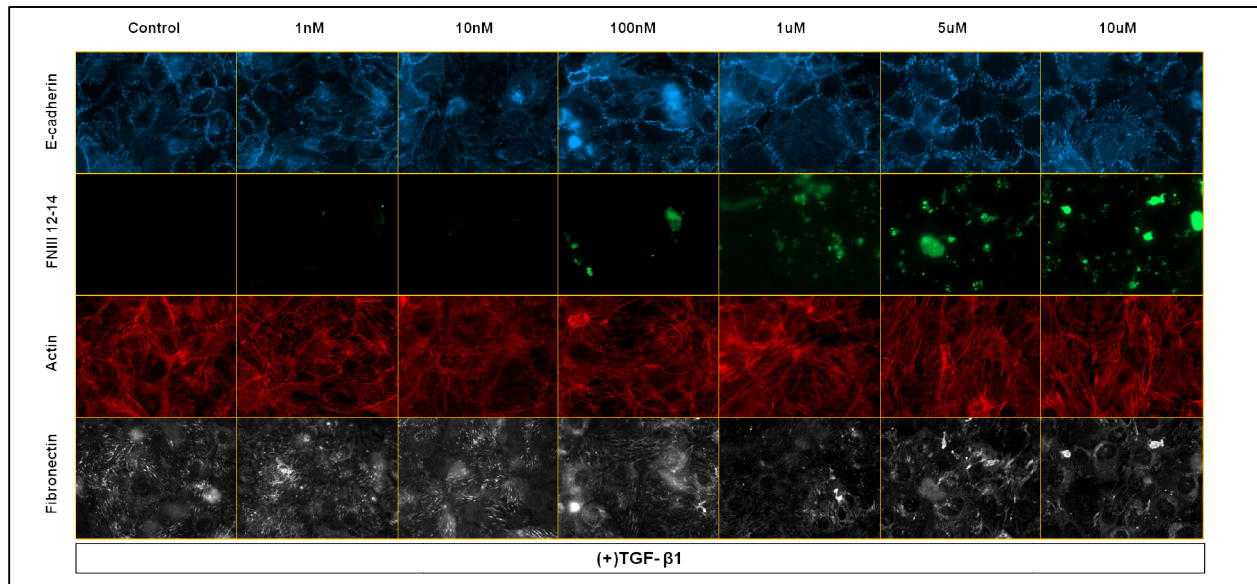


Figure 3: Representative images of FNIII 12-14 effects on TGF-  $\beta$ 1-educed EMT morphological markers in MCF10A cells. MCF10A cells were cultured for 96 hours with 10 ng of TGF- $\beta$ 1 in the presence of FNIII 12-14 treatment (ranging from 1nM to 10 $\mu$ M). Immunofluorescence staining was performed using antibodies for E-cadherin (blue), F-actin (red), and FN (white). FNIII12-14 treatment was added in the form of FITC-tagged protein (green). The exact light intensity and exposure was used throughout the experiment. In the FN channel, white immunofluorescence inside MCF10A cells and/or around the nucleus represents expressed FN protein by the cell while fibril-like white elements on or around the cell represent assembled FN fibrils.

To further investigate the effects of FNIII 12-14 on TGF- $\beta$ -induced EMT, transcription factor levels of Slug, Snail, Twist and ZEB1 from mRNA extraction following a 96 hour incubation were analyzed. The EMT related gene expression levels were evaluate from 14 conditions of two groups, (-) TGF- $\beta$ 1 and (+) TGF- $\beta$ 1. Each sample was run in triplicate during three trials.

Exogenous FNIII 12-14 with varying molar concentrations does not appear to influence TGF- $\beta$ 1-induced upregulation of Slug transcription factor in MCF10A cells (See Figure 4). Even though expression levels appear to fluctuate and reduce in response to treatments of 10nM, 100nM or 10 $\mu$ M of FNIII 12-14, statistical analysis show high percentage of random observations in the experiment (approximately 17%) in comparison to 0.01% of randomness in observed TGF- $\beta$ 1 effects. This resulted in insignificant affect interaction of the FN fragment to (+) TGF- $\beta$ 1 (See Figure 5). In the absence of exogenous TGF- $\beta$ 1, levels of Slug expression remain relatively low and constant as expected, regardless of the amount of FNIII 12-14 present. The inconsistent response of Slug levels seen in the analysis can be further seen in the ratio of change graph presented in Figure 4.

In contrast, FNIII12-14 treatments resulted in a consistent drop changes to Snail transcription factor levels in the presence of TGF- $\beta$ 1. This reduction in the transcription factor Snail was observed in the presence of all molar concentrations of the FN fragment along with (+) TGF- $\beta$ 1, except in the positive control where no FNIII 12-14 was added (See Figure 6). More specifically, at 10 $\mu$ M of FNIII 12-14, Snail levels appear to exhibit the maximum response of the inhibition interaction between the two factors. This can also be observed in the response change graph in Figure 6. Statistically, Snail

levels of expression changes due to the FN fragment in (+) TGF-  $\beta$ 1 conditions appear to be rather nonrandom with a success chance of at least 96.9% (See Figure 7). Snail levels are confirmed unchanged in response to the presence of FNIII 12-14 alone.

Normalized mRNA expression levels for Twist in the presence of FNIII 12-14 and TGF- $\beta$ 1 show some degree of fluctuation. Yet, all conditions treated with both factors exhibit significant reductions in Twist expressed levels (See Figure 8). The concentration increase of FNIII 12-14, in the presence of TGF- $\beta$ 1 appears to maintain Twist expression at relatively close levels to those of (-) TGF-  $\beta$ 1 (See Figure 8). The presence of FNIII 12-14 alone does not appear to promote Twist expression, especially. The change in Twist expression levels is highly significant with at least 97% success. FNIII 12-14 appear to be somehow interacting with TGF- $\beta$ 1 preventing changes to Twist expression levels, significantly seen in Figure 9.

Averaged and normalized transcription factor ZEB1 expression levels were obtained in relationship to the presence of 1nM to 10 $\mu$ M of FNIII 12-14, with and without TGF-  $\beta$ 1. As seen in Figure 10, the concentration increase of FNIII 12-14 appears to cause a reduction in (+) TGF- $\beta$ 1-induced ZEB1 expression levels. No increase in ZEB1 expression levels in response to presence of exogenous FNIII12-14 alone is observed. Levels of ZEB1 expression appear to stay relatively low in response to the presence of both factors, which was statistically verified to be at 99.9% significance (Figure 11).

Observations from overlapped line charts in Figure 12 for the transcription factors suggest that ratio of change takes almost the same pattern with dose increase of FNIII 12-14. It is also statistically evident that significant transcription factor reduction changes take place in Snail, Twist and ZEB1 in the presence of TGF- $\beta$ 1. Although each

molar concentration higher than 0nM of FNIII 12-14 result in significant inhibition of EMT-induced Snail, Twist and ZEB1, most significant drop change in all three transcription factors is observed at treatments of 10 $\mu$ M (See Figure 12). In other words, exogenous treatments of FNIII 12-14 between 1nM and 10 $\mu$ M display signaling regulation opposed to what is normally seen due to the solely presence of recombinant TGF- $\beta$ 1 in Slug, Twist and ZEB1, yet does not have observed significant effects on Slug nor exhibits prime influence on any of the transcription factors in the absence of TGF $\beta$ 1 (See Figure 12).



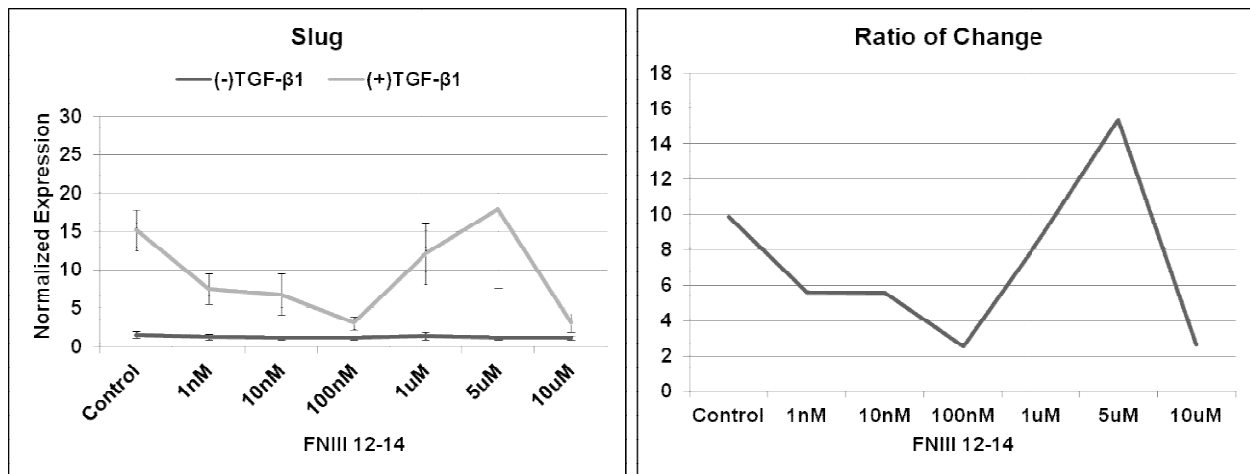


Figure 4: Effects of FNIII 12-14 on EMT marker Slug mRNA expression. Cells were incubated for 96 hours before mRNA was extracted for gene expression analysis. All conditions were normalized to the (-) control. Ratio of change analysis was completed using the ratio of (+)/ (-) TGF-β1 for Slug expressed levels for each molar concentration of FN fragment used. Levels of mRNA were determined by means of RT-qPCR. N = 9 for each condition of the three trials.

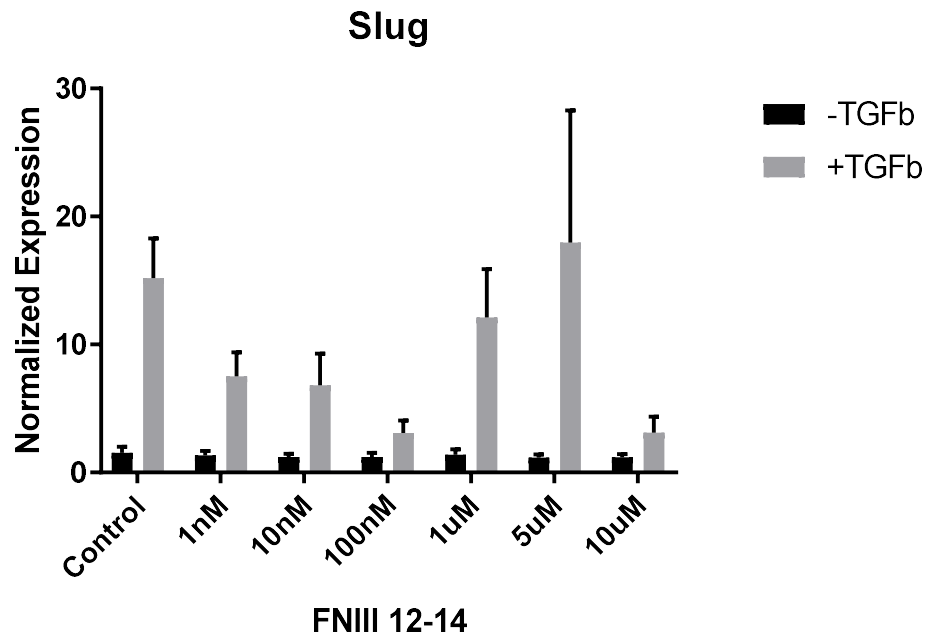


Figure 5: Statistical analysis for FNIII 12-14 effects on the EMT marker Slug expression levels. Cells were incubated for 96 hours before mRNA was extracted for gene expression analysis. Changes of Slug expression in response to 1nM to 10 $\mu$ M of exogenous FNIII 12-14 were averaged between three experiment trials and normalized to the (-) control. Levels of mRNA expression were determined by means of RT-qPCR. N = 9 for each condition of the three trials. Grouped statistical analysis using two-way ANOVA and Bonferroni's multiple comparisons tests were performed using GraphPab Prism 7.04 software. P-value>0.09.

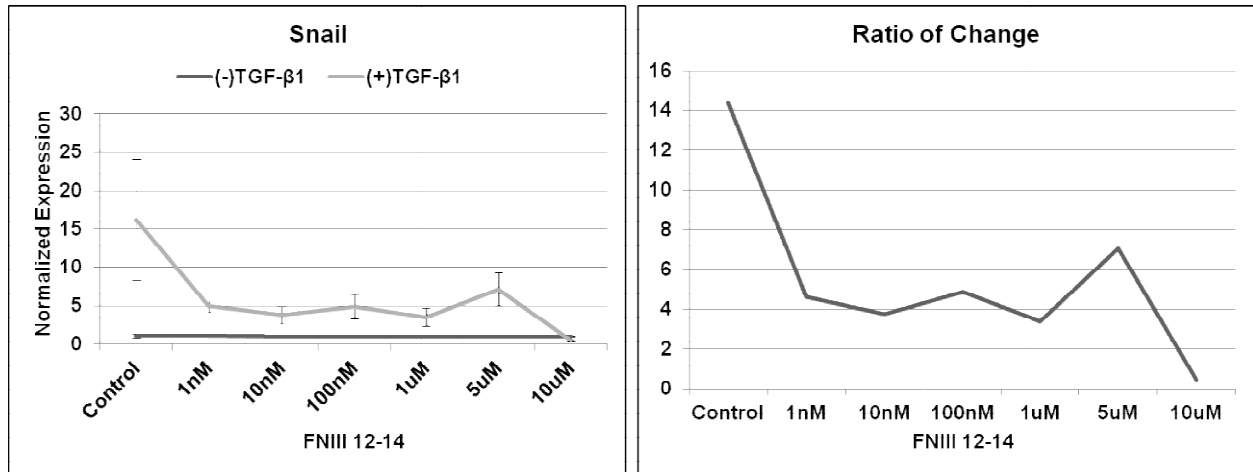


Figure 6: Effects of FNIII 12-14 on the EMT marker Snail expression levels. Cells were incubated for 96 hours before mRNA was extracted for gene expression analysis. All conditions were normalized to the (-) control. Ratio of change analysis was completed using the ratio of (+)/ (-) TGF-β1 for Snail expressed levels for each molar concentration of FN fragment used. Levels of mRNA were determined by means of RT-qPCR. N = 9 for each condition of the three trials.

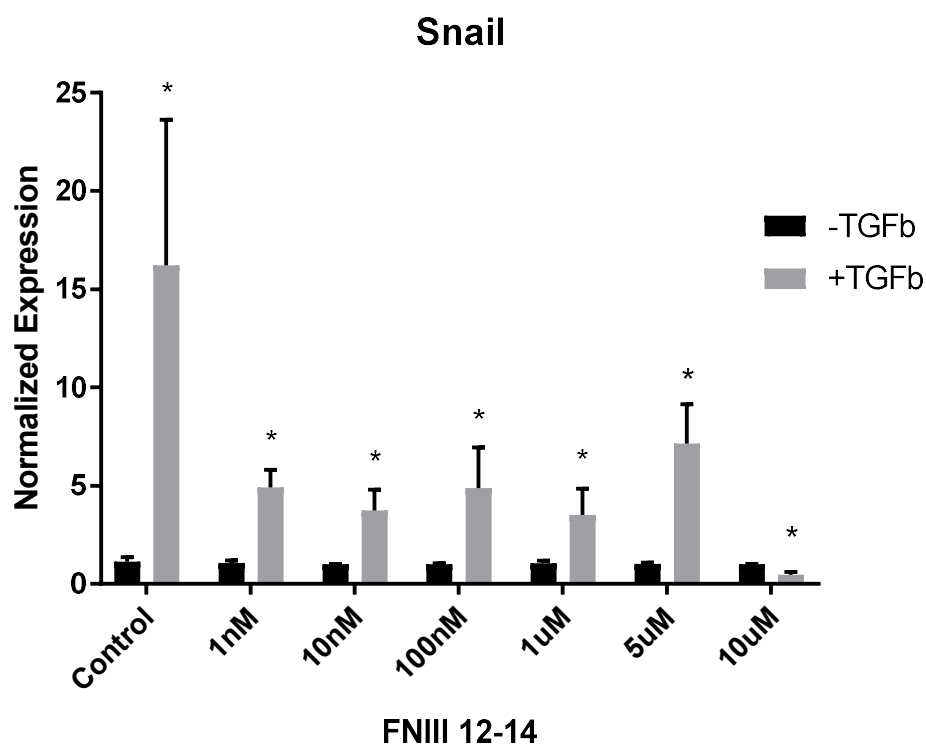


Figure 7: Statistical analysis for FNIII 12-14 effects on the EMT marker Snail expression levels. Cells were incubated for 96 hours before mRNA was extracted for gene expression analysis. Changes of Snail expression in response to 1nM to 10 $\mu$ M of exogenous FNIII 12-14 were averaged between three experiment trials and normalized to the (-) control. Levels of mRNA were determined by means of RT-qPCR. N = 9 for each condition of the three trials. Grouped statistical analysis using two-way ANOVA and Bonferroni's multiple comparisons tests were performed using GraphPab Prism 7.04 software. (\*) P-value<0.03.

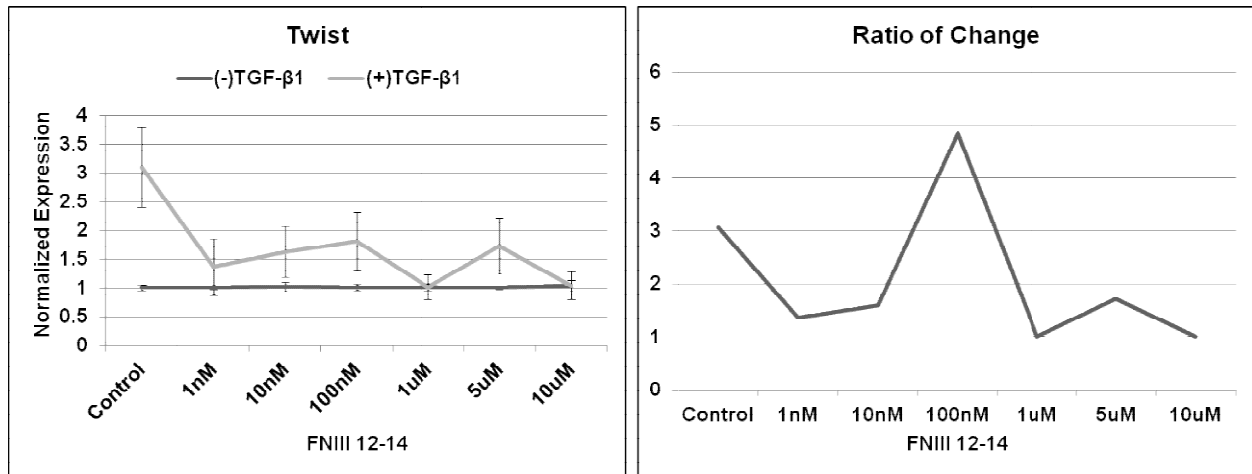


Figure 8: Effects of FNIII 12-14 on the EMT marker Twist expression levels. Cells were incubated for 96 hours before mRNA was extracted for gene expression analysis. All conditions were normalized to the (-) control. Ratio of change analysis was completed using the ratio of (+)/ (-) TGF-β1 for Twist expressed levels for each molar concentration of FN fragment used. Levels of mRNA were determined by means of RT-qPCR. N = 9 for each condition of the three trials.

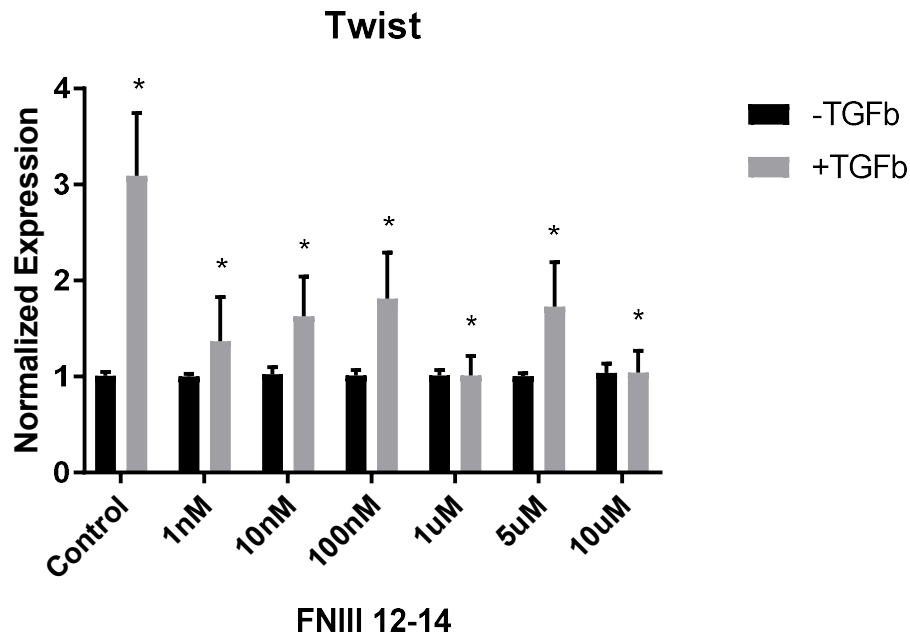


Figure 9: Statistical analysis for the effects of FNIII 12-14 on EMT marker Twist mRNA expression. Cells were incubated for 96 hours before mRNA was extracted for gene expression analysis. Changes in Twist levels in response to 1nM to 10 $\mu$ M of exogenous FNIII 12-14 were averaged between three experiment trials and normalized to the (-) control. Levels of mRNA were determined by means of RT-qPCR. N = 9 for each condition of the three trials. Grouped statistical analysis using two-way ANOVA and Bonferroni's multiple comparisons tests were performed using GraphPab Prism 7.04 software. (\*) P-value<0.02.

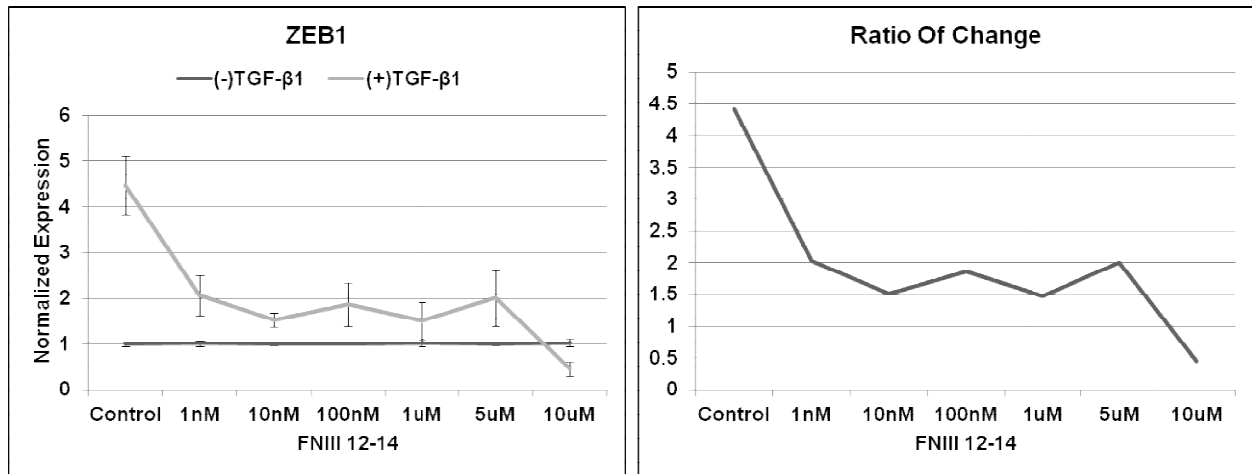


Figure 10: Effects of FNIII 12-14 on the EMT marker ZEB1 expression levels. Cells were incubated for 96 hours before mRNA was extracted for gene expression analysis. All conditions were normalized to the (-) control. Ratio of change analysis was completed using the ratio of (+)/ (-) TGF-β1 for ZEB1 expressed levels for each molar concentration of FN fragment used. Levels of mRNA were determined by means of RT-qPCR. N = 9 for each condition of the three trials.

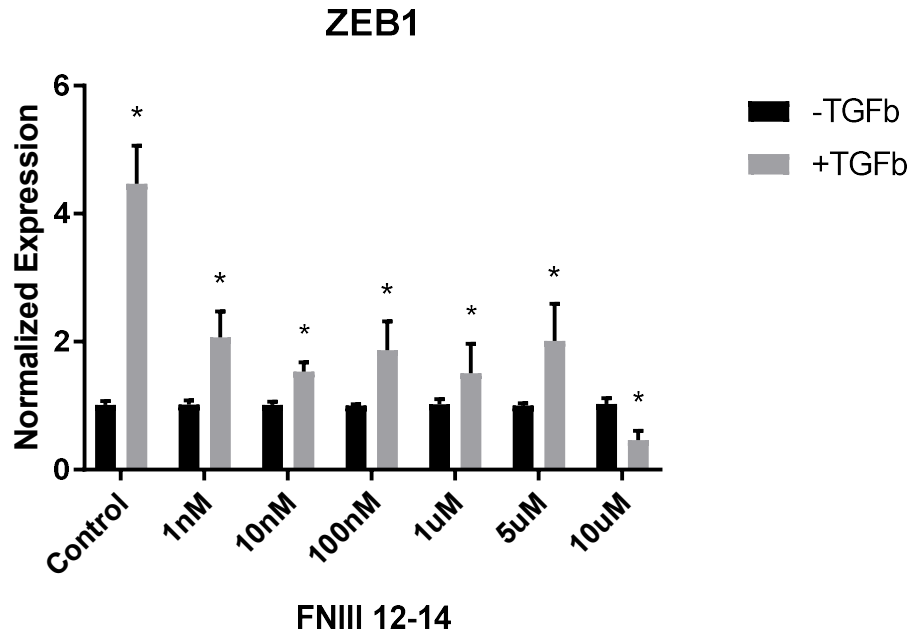


Figure 11: Statistical analysis for the effects of FNIII 12-14 on EMT marker ZEB1 mRNA expression. Cells were incubated for 96 hours before mRNA was extracted for gene expression analysis. Changes in ZEB1 levels in response to 1nM to 10 $\mu$ M of exogenous FNIII 12-14 were averaged between three experiment trials and normalized to the (-) control. Levels of mRNA were determined by means of RT-qPCR. N = 9 for each condition of the three trials. Grouped statistical analysis using two-way ANOVA and Bonferroni's multiple comparisons tests were performed using GraphPab Prism 7.04 software. (\*) P-value<0.0001.



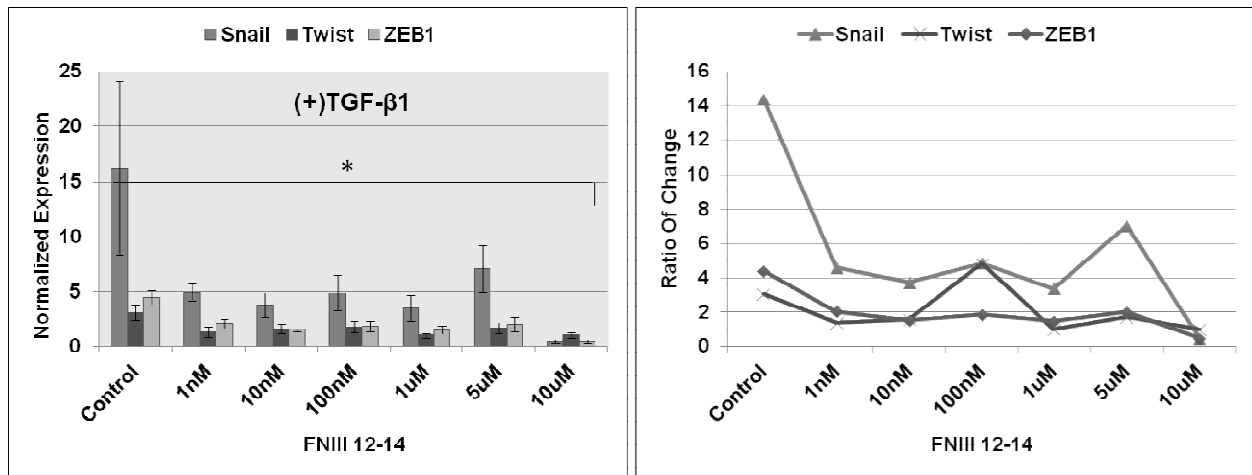


Figure 12: Exogenous FNIII 12-14, at different concentrations, alters TGF-β1–induced upregulation of Snail, Twist, and ZEB1 in MCF10A cells. (+)/ (-) TGF-β1 for Snail, Twist, and ZEB1 expression level changes were analyzed after 96 hours in response to the presence of 1nM to 10µM of FNIII 12-14. Levels of mRNA were determined by means of RT-qPCR. N = 9 for each condition of the three trials. Grouped statistical analysis using two-way ANOVA and Bonferroni's multiple comparisons tests were performed using GraphPad Prism 7.04 software. (\*) P-value<0.05.

## Chapter 5: Discussion

FNIII 12-14 is recognized as a promiscuous growth factor (GF) binding protein, aids in Transforming Growth Factor Beta 1 (TGF- $\beta$ 1) localization which promotes TGF- $\beta$ 1-induced upregulation of Epithelial-Mesenchymal Transition (EMT) during Fibronectin (FN) polymerization. Previous work from our lab has shown that self-assembly of FN into insoluble fibrils is crucial for EMT [3]. Additionally, our lab previously suggested that upregulation of FN fibril formation further drive EMT through contractility due to the increase amount of the GF latent TGF- $\beta$  complex at the cell membrane [3].

Immunofluorescence images for TGF- $\beta$ 1-induced cell markers suggest that exogenous FNIII 12-14 is able to reduce EMT morphological transformation in MCF10A cells. Transdifferentiation markers for EMT remain mostly epithelial-like, even in the presence of exogenous 10ng/mL of TGF- $\beta$ 1. Following 96 hours incubation of MCF10A cells with FNIII12-14 and TGF- $\beta$ 1, E-cadherin cell junctions appear to somehow maintain its morphological organization opposing the effects of EMT induction. Actin spindle-like morphology did not show significant changes in response to the FN fragment-TGF- $\beta$ 1 interactions. FN expression and assembly appeared to be both reduced, yet immunoprecipitation and protein quantification techniques could confirm these observations in future studies. This supports results from other studies suggesting that FN assembly can be inhibited by exogenous FNIII 12-14 or other fragments containing heparin domain [59]. Moreover, FN fragments appear to have relatively higher cell binding affinity when recombinant TGF- $\beta$ 1 is added. This is likely due to initial TGF- $\beta$ 1-induced cell contractility [60]; which facilitates essential ligand

binding protein interactions at the plasma membrane before leaving the receptor to be captured by the soluble FN fragment [1], [61].

Upregulation in expression levels of transcription factor Slug, Snail, Twist and ZEB1 are recognized as EMT-induced cellular signaling [62]–[68]. Upon multiple trials of mRNA quantification, we demonstrated that exogenous FNIII 12-14 at concentrations between 1nM and 10 $\mu$ M produces reduction responses in Snail, ZEB1, and Twist mainly in the presence of TGF- $\beta$ 1. Yet Slug expression levels fail to provide statistical evidence for which response to FNIII 12-14 could be happening. Additional tested considered to be performed to reveal more evidence for whether Slug signaling participates due to the interaction activity of exogenous FNIII 12-14 and TGF- $\beta$ 1.

Although it is insignificant in Slug, transcription factors Snail, Twist, and ZEB1 expression levels appear to have maximum decline roughly at 10 $\mu$ M of the molar concentration of FN fragment. Snail, Twist and ZEB1 levels are somewhat reduced and stay constant between 1nM and 5 $\mu$ M. It is likely that molecular diffusion and binding affinity play a role in the fate of TGF- $\beta$ 1-FNIII 12-14 complex across the multiple conditions. Therefore, our theory is that exogenously added low amounts of FN fragment bind most soluble TFG- $\beta$ 1 factors through heparin and electrostatic interactions [18]; reducing GFs available for cell signaling, and therefore EMT effects. At higher concentrations of FNIII 12-14 (approximately 10 $\mu$ M), FN assembly is completely inhibited due to the presence of high amounts of heparin domain fragments [59]; therefore further inhibiting stable binding of TGF- $\beta$ 1 to cell membrane. Interestingly, studies have shown a degree of success the use of FN fragments for protein delivery [16], [44].

FNIII 12-14-TGF- $\beta$ 1 complex could also be inhibiting back differentiation of epithelial to mesenchymal cell type activity, yet the FN fragment might be partially ineffective towards a number of genes activated by TGF- $\beta$ 1, such as those that are transcribed by Slug and/or transforming actin organization. This could explain why changes in Snail and actin are rather non-responsive to the presence of FNIII 12-14. It is also essential to take into consideration that FNIII 12-14-TGF- $\beta$ 1 binding affinity is intermediate in contrast to other GFs; and that FNIII 12-14 is capable of maintaining the GF bounds for multiple days [44].

## Chapter 6: Conclusion and Future Directions

In the study, we hypothesized that TGF- $\beta$ 1-induced misregulation of cellular morphology and transcription factors (Snail, Twist and ZEB1) can be inhibited by exogenous FNIII 12-14 in amounts of 1nM to 1 $\mu$ M. This will significantly hinder EMT markers: E-cadherin degradation and FN assembly as seen in Figure 2 and 3. Nevertheless, Normalized changes in Slug, Twist and ZEB1 transcription factor expression levels between all conditions appear to further support our conclusion in that the overall upregulation effect of TGF- $\beta$ 1 on EMT transcription factors can be significantly reduced as much as 60% using 1nM of exogenous FNIII 12-14. Therefore, we characterize FNIII 12-14 as an inhibitor for Snail, ZEB1 and Twist signaling due to its competitive binding that leads to minimizing the reach of growth factors (GFs) to the cell membrane. This characterization of the FNIII 12-14 is verified in our study by blocking TGF- $\beta$ 1 upregulatory activity when 1nM - 10 $\mu$ M exogenous FN fragments is used. The study does not provide significant evidence for TGF-  $\beta$ 1 delivery by FNIII 12-14. Additionally, our study confirms that exogenous FNIII 12-14 alone does not induce transcriptional or morphological EMT markers in human epithelial MCF10A cells.

Due to response behavior seen in Snail, Twist and ZEB1 in the presence of FNII 12-14 and TGF- $\beta$ 1, we plan to perform additional tests to reveal whether other signaling pathways might be altered by the FN fragment FNIII 12-14. Using quantitative analysis, we will further verify the effects of the FN fragments on FN expression levels. Investigations of anti-EMT GF signaling activities hold potential discoveries in mesenchymal-epithelial transition (MET) and the future of cancer medicine. Therefore, we have considered studying the effects of treating pre-EMT transformed human

epithelial cells with exogenous FNIII 12-14 and anti-EMT GFs such as CTGF [69]; or BMP-2 [70] by means to enhance the processes of MET. We encourage the field to further investigate the molecular interactions between FNIII 12-14, GFs, and cell membrane proteins, competitive affinity binding and release, other possible cellular signaling cascades altered by the FN fragment, and variety of proteins for the ECM delivery. Studying binding affinities between FNIII 12-14 and membrane proteins, such as integrins, could provide us with more insights on FNIII 12-14 fragments favorable interactions in vitro.

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## Curriculum Vita (CV)

**HILMI MAJED HUMEID**  
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7136 Aquarius Drive  
Mechanicsville, VA 23111

### OBJECTIVE

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Driven biomedical-engineer professional able to maintain a strong work ethic with a positive attitude and productive work environment. Experienced in the field of clinical & laboratory research, biomedical, cellular, tissue, and genetic engineering.

### EDUCATION

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<b>VIRGINIA COMMONWEALTH UNIVERSITY, SCHOOL OF ENGINEERING</b> <i>Master of Science</i> in Biomedical Engineering	<b>Richmond, VA</b> May 2018
<b>VIRGINIA COMMONWEALTH UNIVERSITY, COLLEGE OF HUMANITIES AND SCIENCES</b> <i>Bachelor of Science</i> in Biology	<b>Richmond, VA</b> May 2015
<b>NORTHERN VIRGINIA COMMUNITY COLLEGE, BIOLOGY AND NATURAL SCIENCES</b> <i>Associate of Applied Sciences</i> in Biology	<b>Annandale, VA</b> May 2011

### GRANTS AND AWARDS

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<b>VIRGINIA COMMONWEALTH UNIVERSITY</b> <ul style="list-style-type: none"><li>• <b>Second Professional Judgment Grant</b></li><li>• <b>First Professional Judgment Grant</b></li></ul>	<b>Richmond, VA</b> Spring 2015 Fall 2014
<b>KING FAHD HOSPITAL</b> <ul style="list-style-type: none"><li>• <b>Medical Trainee Certificate</b></li></ul>	<b>Jeddah, Saudi Arabia</b> Spring 2012
<b>INOVA FAIRFAX HOSPITAL</b> <ul style="list-style-type: none"><li>• <b>Certificate Of Volunteering</b></li></ul>	<b>Fairfax, VA</b> Fall 2011

### PUBLICATIONS

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- **Humeid, H. Deal, H. Lemmon, C.** “Exogenous FNIII 12-14 Regulates TGF- $\beta$ -Induced EMT Markers”  
(In preparation)

### RESEARCH EXPERIENCE

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<b>VIRGINIA COMMONWEALTH UNIVERSITY</b> <b>Graduate Research Assistant</b> , Department of Biomedical Engineering <b>M.S. Candidate:</b> Completed all course work and dissertation prospectus	<b>RICHMOND, VA</b> Summer 2015-Present
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Research Topic: Fibronectin interactions in Epithelial to Mesenchymal Transition (EMT)

*Advisor:* Dr. Christopher Lemmon

- Investigated mechanico-signal generation in ECM
- Analyzed morphological cell markers, protein expressions, protein assembly, protein aggregations, and growth factor signaling in EMT
- Managed scheduled sterilization processes for lab tools and equipment



# HILMI MAJED HUMEID

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## RESEARCH SKILLS

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**LABORATORY SKILLS:** Cell Culture, Gel Electrophoresis, Immunofluorescence Labeling & Microscopy, Microfabrication, Optical & SEM Imaging, PCR & qRT-PCR, Animal & Bacterial Cell Transfection, DNA & RNA Extraction, Site-Directed Mutagenesis, Primer & Oligos Designing, Protein Expression & Purification, Protein-Fluorescence conjugation (GFP & FITC), Western Blotting, ELISA, Gene Barcoding, Scientific Writing, Data Reporting, and Experiment Designing.

**COMPUTER SKILLS:** CleWin, HTML, Java, Image J, MATLAB, Microsoft Office, Photoshop, SOLIDWORKS, ZEN2011, Nanodrop2000, Bio-Rad CFX Manager, GraphPad Prism & Adobe Acrobat PDF.

## CLINICAL EXPERIENCE

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### STATEN ISLAND HOSPITAL

Staten Island, NY  
Summer 2012

*Mentor:* Dr. Mohammad Ayoub, Internal Medicine

- Completed 153 hours of shadowing in the intensive care unit.
- Observed recovery of patients, in addition to attending some surgical procedures.
- Recorded patient's vitals and managed medical charts

### KING FAHD HOSPITAL

Jeddah, Saudi Arabia  
December-January 2012

**Medical Trainee**, Department of Nursing Admissions

**Certificate:** Completed 110 hours (an approximate of eight hrs/day) in clinical observations and patient relations

*Mentor:* Amin Almadani, Assistant Nursing Director

- Assigned to three different nursing units: operation room, ward, and the emergency room.
- Engaged in learning discussions with physicians while presently observing medical procedures
- Assisted in providing care toward the recovery of diabetic foot and patients in the internal medicine wards
- Completed 32 hours of assisting Dr. Emad Aljurf (general medicine) in the ER.

### INOVA FAIRFAX HOSPITAL

Fairfax, VA  
Feb-Dec 2011

**Patient-Support Volunteer**, Heart and Cardiovascular Institution

**Certificate:** Completed 127 hours of volunteering services

*Volunteering Chair:* Mrs. Lyn McDonald

- Helped the healthcare community of Fairfax, Virginia, by easing patients stay and providing the medical staff extra time to help other patients, through:
  - Patient transportation between the medical facilities
  - Delivering flowers, balloons and greeting cards
  - Maintaining a sterile environment using clinical techniques
- Developed communication skills sensitive for patients and relatives
- Implemented hospital emergency codes and protocols
- Maintained excellent time management and dress code

## HILMI MAJED HUMEID

Page Three

### CONFERENCE PRESENTATIONS

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#### ORAL PRESENTATIONS

- **The Biomedical Engineering Society (BMES)** **Phoenix, AR**  
2017  
**Humeid, H.** Deal, H. Lemmon, C. "Exogenous effect of FNIII 12-14 on EMT"

#### POSTER PRESENTATIONS

- **The America Society of Matrix Biology (ASMB)** **Tampa, FL**  
2016  
**Humeid, H.** Lemmon, C. "Localization of Exogenous FNIII 12-14"

### TEACHING EXPERIENCE

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#### VIRGINIA COMMONWEALTH UNIVERSITY

**Instructor**, Matrix Mechanobiology Laboratory For New-hired Students **Richmond, VA**  
Summer 2017

**Course #1:** Epithelial To Mesenchymal Transition (EMT) Review

**Course #2:** The Effects of Exogenous FNIII 12-14 on EMT Markers

**Course #3:** Pipetting, Measuring and Weighing Techniques

**Course #4:** Hands-On Training For Laboratory Equipment Use, Calculations For Solution Concentrations, And Methods of Dilutions.

- Designed, planned and instructed four-day, two-hour course for four newly-hired undergraduate students, majoring in Biomedical Engineering
- Provided students with detailed background, associated findings, key resources, and the laboratory's related research topics and future directions in the field
- Held an extensive wet-lab training session for efficient and accurate use of lab equipment
- Provided practice session on solving complex mathematical equations used in daily lab routine

**Mentor**, Undergraduate Student Halston Deal, Matrix Mechanobiology Laboratory **Richmond, VA**  
Summer 2017

- Provided Mr. Deal with hands-on training for:
  - Cell culture
  - Protein expression and purification
  - DNA amplification /cloning
  - DNA extraction and purification
  - Immunofluorescent-cell labeling and Imaging
- Helped improve critical thinking, and develop experiment designing skills
- Advised with key solutions during experiment and trouble shootings

#### DAR AL-HIJRAH ACADEMIC CENTER

**Teaching**, Dar Al-Hijrah Summer School **Falls Church, VA**  
Summer 2011

**Course:** Arabic Language

- Designed, planned and instructed six-week, four-hour course for 14 students between ages of 11 and 15 years old.
- Built a strong teacher-student relationship by establishing a friendly environment and intellectual games.
- Supervised physical activities, helped with creative self-expression and social interactions, and involved students in meaningful participations.

# HILMI MAJED HUMEID

Page Four

## ADMENISTRATIVE EXPERIENCE

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### WAL-MART

#### Sales Associate, Department of Electronics

- Provided costumer services
- Managed secretary tasks and inventory
- Marketed and sold electronics
- Activated new cell-phone lines and plans

Fair Oaks, VA

2011- 2012

### MAFAZA TRAVEL AGENCY

#### Travel Agent Representative

- Provided costumer services for air travelers
- Managed secretary tasks for supervisors
- Engaged in marketing for the agency

Falls Church, VA

Summer-Fall 2008 & 2011

## LANGUAGES

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**English** – fluent (speaking, reading, writing)

**Arabic** – native language

## PROFESSIONAL AFFILIATIONS

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### GENERAL MEMBERSHIP

- |   |                |
|---|----------------|
| ▪ The Biomedical Engineering Society          | 2017 – Present |
| ▪ American Society for Matrix Biology         | 2016 – Present |
| ▪ The national society of collegiate scholars | 2010 – Present |
| ▪ Muslim American Society                     | 2009 – Present |