THE PENNSYLVANIA STATE UNIVERSITY
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DEPARTMENT OF BIOCHEMISTRY AND MOLECULAR BIOLOGY

THE ROLE OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR-β/δ (PPARβ/δ) IN TRANSFORMING GROWTH FACTOR β-INDUCED EPITHELIAL-MESENCHYMAL TRANSITION (EMT) OF HUMAN LUNG ADENOCARCINOMA CELL LINE

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ABSTRACT

Epithelial-mesenchymal transition (EMT) is necessary during certain stages of development; however, it can be detrimental when acquired by tumor cells. Epithelial mesenchymal transition can allow tumor cells to invade to surrounding tissues resulting in metastasis, and subsequent tumors can resist apoptic agents making the cancer very aggressive. Transforming Growth Factor-β (TGFβ), which induces epithelial mesenchymal transition, facilitates this transition. Peroxisome proliferator-activated receptor-β/δ (PPARβ/δ) is a ligand-activated transcription factor that regulates proliferation and differentiation among other processes in the cell. The role of PPARβ/δ is controversial but there is some evidence that PPARβ/δ promotes terminal differentiation of epithelial tissues. This study examined the effect of ligand activation of PPARβ/δ ligand GW0742 on TGFβ-induced epithelial mesenchymal transition in human lung adenocarcinoma cell line A549. Epithelial mesenchymal transition was assessed by observing changes in the morphology of the cells and measuring the expression of proteins involved in epithelial mesenchymal transition, such as E-CADHERIN and VIMENTIN by western blot analysis. The analysis of morphology did not show the suppression of TGFβ-induced epithelial mesenchymal transition in A549 cells post- or co-treated with PPARβ/δ agonist. The results from western blot analysis were inconclusive due to inadequate amounts of protein. Therefore, the experiments conducted in this study must be repeated and additional studies with PPARβ/δ overexpressed cells treated with specific ligands should be completed.

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INTRODUCTION

The role of peroxisome proliferator-activated receptors (PPARs) in carcinogenesis

PPARs are nuclear receptors that form heterodimers with retinoic X receptors (RXR) and upon ligand activation, regulate gene expression. Ligands signal triggering molecules by binding to a particular site on designated proteins. Additionally, sequence comparisons have shown that DNA-binding domains are conserved, while the ligand-binding domains have a slightly lower level of conservation across the subtypes[1]. Specifically, the N-terminal domain in PPAR genes is responsible for differences in the biological function of different subtypes[2].

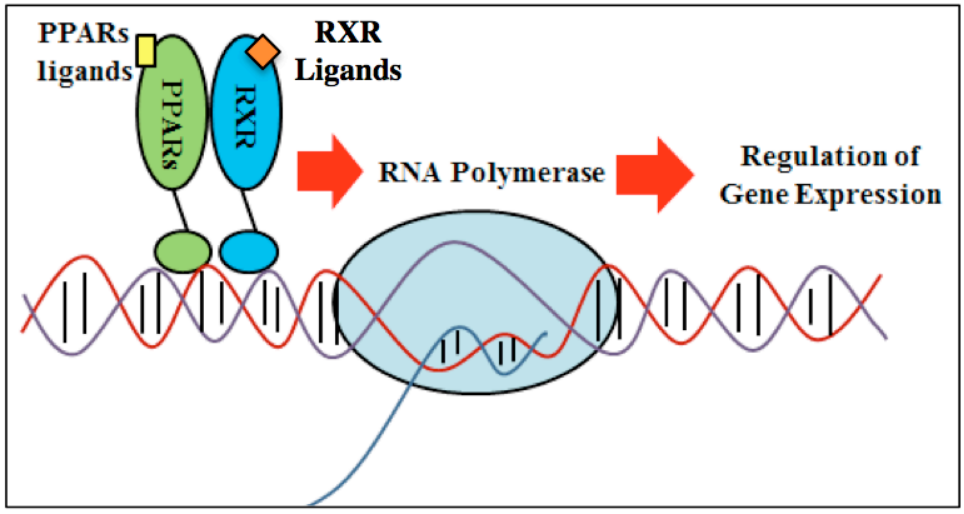

Figure 1. PPARs' mode of action: PPARs form heterodimers with RXRs and bind to DNA-binding domains, which results in the modulation of transcription eventually leading to the regulation of gene expression. These ligand-activated transcription factors aid in the regulation of glucose and lipid homeostasis, inflammation, proliferation and differentiation[3]. The DNA-binding domains of PPARs are highly conserves while ligand-binding domains are slightly less conserved and may be responsible for differences in the biological function of the subtypes[1,2].

Upon ligand activation, PPARs dissociate from co-repressors, and recruit co-activators, chromatin remodeling factors and the machinery of gene transcription, which along with the fatty acid binding proteins, can translocate from the cytoplasm to the nucleus[2]. PPARs have key roles not only in embryonic development and lipid metabolism in peripheral tissues, but also in important cell functions such as adhesion, proliferation, differentiation and survival. Since the receptors have a pivotal role in the control of these functions, they can be implicated in regulating carcinogenesis and metastatic progression of tumors[4].

As a result, they carry out cell-type specific regulation of proliferation, differentiation and cell survival. There are three different PPAR isoforms: PPARα, PPARβ/δ and PPARγ. PPARα can improve insulin resistance in high fat and genetic models of diabetes by preventing weight gain and adiposity through gene expression changes. It has also been shown to cause liver cancer in rodents[3]. PPARγ has been shown to prevent cancer in the colon, breast, prostate, lung and several others. PPARα and PPARγ ligands are currently being used clinically to treat hyperlipidemia and type 2 Diabetes respectively.

More recently, PPARβ/δ has come into focus. It has been found that this isoform is highly expressed in skin, skeletal muscle, adipose tissue, inflammatory cells, heart and various types of cancer and has a variety of different functions. Its diverse functionality can be attributed to the different binding of ligands, along with the recruitment of co-activators and co-repressors[1]. PPARβ/δ has been shown to promote terminal differentiation in keratinocytes, intestinal epithelium, oligodendrocytes, and osteoblasts and urothelial breast, colon and nueroblastoma cancer cell lines[8]. Although several roles

of PPARβ/δ have been established, its relation to carcinogenesis remains controversial due to differing results in various cell culture and animal models. It has been suggested, however, that PPARβ/δ has an antitumorigenic effect in endometrial cancer upon activation[9]. These findings suggest that there is a possibility of cell type and organ-specific effects mediated by PPARβ/δ.

Endogenous ligands for PPARβ/δ include fatty acids, and prostacyclin[10,11,12]. Cow milk, ice cream, butter and yogurt were also described as activators but the specific compound or molecule was not identified[13]. Synthetic ligands with a high affinity for the human receptor include L165041, GW501516 and GW0742. These high-affinity ligands help determine the role of PPARβ/δ but must be controlled for as their specificity can be limited and off-target effects are possible[8]. The Peters Lab has used ligand activation as a tool to test different theories of PPARβ/δ's activity in toxicology and carcinogenesis[14]. Therefore, in the present study, a special system using retroviral transduction to over-express PPARβ/δ coupled with ligand activation can provide information regarding the role of PPARβ/δ on epithelial mesenchymal transition.

Epithelial mesenchymal transition and carcinogenesis

Epithelial mesenchymal transition is a biological process that allows a polarized epithelial cell to undergo multiple biochemical changes that enable it to assume a mesenchymal cell phenotype, which includes enhanced migratory capacity of invasiveness and elevated resistance to apoptosis[15]. The term transition is used due to possible reversibility of epithelial mesenchymal transition[16].

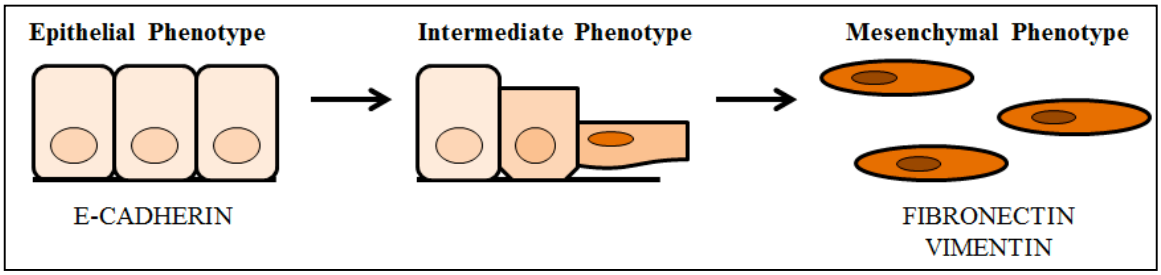

Figure 2. Phenotypic change during epithelial mesenchymal transition. Epithelial mesenchymal transition involves the conversion of a polarized epithelial cell to a mesenchymal one. Phenotype can be assessed using epithelial and mesenchymal markers such as E-CADHERIN and VIMENTIN respectively. Mesenchymal cells have enhanced migratory capacity of invasiveness and elevated resistance to apoptosis[13].

Epithelial mesenchymal transition is triggered in events that necessitate tissue repair and in response to pathological stresses such as inflammation and high-grade carcinomas. It forms mesenchymal cells during the initiation and subsequent metastatic behavior of epithelial cancers[15]. The loss of the E-CADHERIN expression, a transmembrane protein involved in cell adhesion, is one of the indicators of epithelial mesenchymal transition[17]. There is a direct correlation between reduced expression and production of E-CADHERIN and the loss of the epithelial phenotype. Additionally, the reversal of the mesenchymal phenotype is observed as increased production of E-CADHERIN[18]. The production of E-CADHERIN is maintained vigorously in the most differentiated tumors including carcinomas of the skin, head and neck, breast, lung, liver, colon and prostate. Lower E-CADHERIN levels have been seen to indicate high-grade cancers and low patient survival[19, 20].

Excessive cell proliferation along with angiogenesis comprises the early stages of primary epithelial cancer. Coupled with epithelial mesenchymal transition, the resulting invasiveness through the basement membrane leads to dedifferentiation and malignant states with severe consequences[17].

TGFβ suppresses epithelial proliferation and in turn tumor progression but in some contexts, it can aid in tumor advancement and metastasis [21,22,23]. In certain types of cancer cells, epithelial mesenchymal transition can even be induced by TGFβ. One such cell line includes the human lung cancer cell line A549, which demonstrated fibroblast-like shape after treatment with TGFβ. In addition to the morphological change, E-CADHERIN, an epithelial marker, was lost and VIMENTIN and FIBRONECTIN, mesenchymal markers, increased in the A549 cells which indicates epithelial mesenchymal transition[24].

Epithelial mesenchymal transition in human lung adenocarcinomas

Lung cancer is the leading cause of cancer-related mortality in the United States and non-small cell lung cancer makes up approximately 85% of all newly diagnosed cases of lung cancer[25]. The human lung adenocarcinoma cell line A549 is widely used as an in vitro model for testing the outcome of the exposure of volatile compounds[26].

Studies of real-time cell proliferation of A549 revealed no difference following ligand activation of PPARβ/δ with GW0742 or with PPARβ/δ antagonist GSK3787[8]. These data show that activating PPARβ/δ in A549 does not influence cell proliferation or alter expression of putative targets that could modulate cell proliferation or survival. Western blot analysis has also shown that there is no difference in PPARβ/δ expression between lung tumors and normal lung tissue. However, a definitive conclusion cannot be reached; some studies suggest that activating PPARβ/δ promotes proliferation of human lung cancer cell lines, while other studies indicated that PPARβ/δ agonists or antagonists

either inhibit proliferation or have no effect[8]. It should be noted that several papers claiming that PPARβ/δ activation promotes proliferation have been retracted due to data and figure manipulation.

Taking the aforementioned documentation into consideration. We hypothesize that treating A549 cells with TGFβ will induce epithelial mesenchymal transition. We expect to see PPARβ/δ overexpression and ligand activation reverse this transition subsequently showing that PPARβ/δ has the ability to reverse the severity of aggressive cancers by transforming their mesenchymal cells to an epithelial phenotype.

MATERIALS AND METHODS

Cell Culture

A549 cells were purchased from American Type Culture Collection (ATCC, Manassas, VA). They were grown in Ham's F-12K Nutrient Mixture, Kaighn's Mod. With L-glutamine (F12-K, 1X) supplemented with 10% fetal bovine serum (FBS), 100 IU/mL penicillin, and 100 μg/mL streptomycin. The cells were propagated at 37°C and 5% CO_2.

A549 cells carrying empty retroviral vector MigR1 (MigR1) and cells over-expressing PPARβ/δ (hPPARβ/δ) were kindly provided by Dr. Kartick Pramanik, postdoc scholar in Peters' lab, who transfected these cells using previously established methods[14].

Cell Morphology

Post-treatment Study

A549, MigR1 and hPPARβ/δ cells ($1x10^5$) were plated in 12-well plates in serum starved media (F12-K, 1X) supplemented with 100 IU/mL penicillin, and 100 μg/mL streptomycin. The cells were propagated at 37°C and 5% CO_2. Serum starved media was used in order to synchronize the cells. After 24-hours, the serum starved media was

replaced with complete media with 10% FBS and 10 ng/ml TGF-β was added to designated wells. 24-hours later, 5 μM GW0742 was added to designated wells. Morphological changes were observed 24 and 48 hours following the GW0742 treatment using Infinity Analyze software (Lumenera Corporation). Each treatment was controlled for and all trials were completed in triplicates. 10 different views were observed before pictures were taken. The pictures taken are representative of the entire well.

Co-treatment Study

A549, MigR1 and Migr1-hPPARβ/δ ($1x10^5$) cells were plated in 12-well plates in serum starved media, Ham's F-12K supplemented with 100 IU/mL penicillin, and 100 μg/mL streptomycin. The cells were propagated at 37°C and 5% CO_2. Serum starved media was used in order to synchronize the cells. After 24-hours, the serum starved media was replaced with complete media and 10 ng/ml TGFβ and 5 μM GW0742 was added to designated wells. Morphological changes were observed 24 and 48 hours following the co-treatment using Infinity Analyze software (Lumenera Corporation). Each treatment was controlled for and all trials were completed in triplicates.

Protein Isolation

Protein was isolated from cells used for cell morphology study. Cells were trypsinized, then centrifuged into pellets at 8000 rpm for 1 minute. The pellets were re-suspended in 1 mL phosphate buffered saline (PBS) and centrifuged at 8000 rpm for 1

minute. The supernatant was discarded and the cells were re-suspended in 75 μL lysis buffer, consisting of high-salt MENG buffer (25 mM MOPS, 2 mM EDTA, 0.02% NaN_3, and 10% glycerol, pH 7.5) containing 500 mM NaCl, 1% Nonidet P-40, and protease inhibitors. The cells were allowed to sit on ice for 30 minutes, homogenized, then centrifuged at 14,000 rpm for 3 minutes at 4°C. Supernatent was collected and stored for western blot analysis.

Western Blot Analysis

A total of 20 μg of the isolated protein per sample was resolved using SDS-polyacrylamide gels and transferred to a PVDF membrane using an electroblotting method (120 V for 75 min). The membranes were blocked with 5% dried milk in Tris buffered saline/Tween-20 (TBST) and incubated overnight with primary antibodies. The membranes were then washed with TBST and incubated with biotinylated secondary antibody (Jackson ImmunoResearch Laboratories). After washing with TBST, the membrane was incubated with ^{125}I-labeled streptavidin and immunoreactive proteins were detected. Primary antibodies included β-ACTIN (Santa Cruz Biotechnology) (1 μL/mL), PPAR-δ (Abcam) (1 μL/mL), VIMENTIN (Santa Cruz Biotechnology) (2 μL/mL) and E-CADHERIN (BD biosciences) (2 μL/mL). Secondary antibodies included Biotin-SP-Conjugated AffiniPure Donkey Anti-Goat IgG (Jackson ImmunoResearch Laborotories, Inc.) (1 μL/mL) and Biotin-SP-Conjugated AffiniPure Goat Anti-Mouse IgG, Fc_y Fragment Specific (Jackson ImmunoResearch Laboratories, Inc.) (0.5 μL/mL).

RESULTS

Cell Morphology

Morphological changes were observed in order to determine the effect of PPARβ/δ on epithelial mesenchymal transition. TGFβ was used to induce the transition while the cells were treated with PPARβ/δ agonist GW0742 to activate the receptor. Epithelial mesenchymal transition is detected by discerning long, thin cell processes that protrude from a small cell body. This phenotype is consistent with mesenchymal cells.

Post-treatment Study

Post-treatment cells were treated with 5 μM GW0742 24 hours after they were treated with 10 ng/mL TGFβ. Each cell line treated with TGFβ showed epithelial mesenchymal transition. Upon ligand activation of PPARβ/δ, epithelial mesenchymal transition did not seem to be reversed or altered. Morphology pictures were taken such that the picture was a relatively accurate representation of the entire well (Figure 3). Wells could not be accurately quantified due to varying degrees of cell confluency. However, epithelial mesenchymal transition was evident.

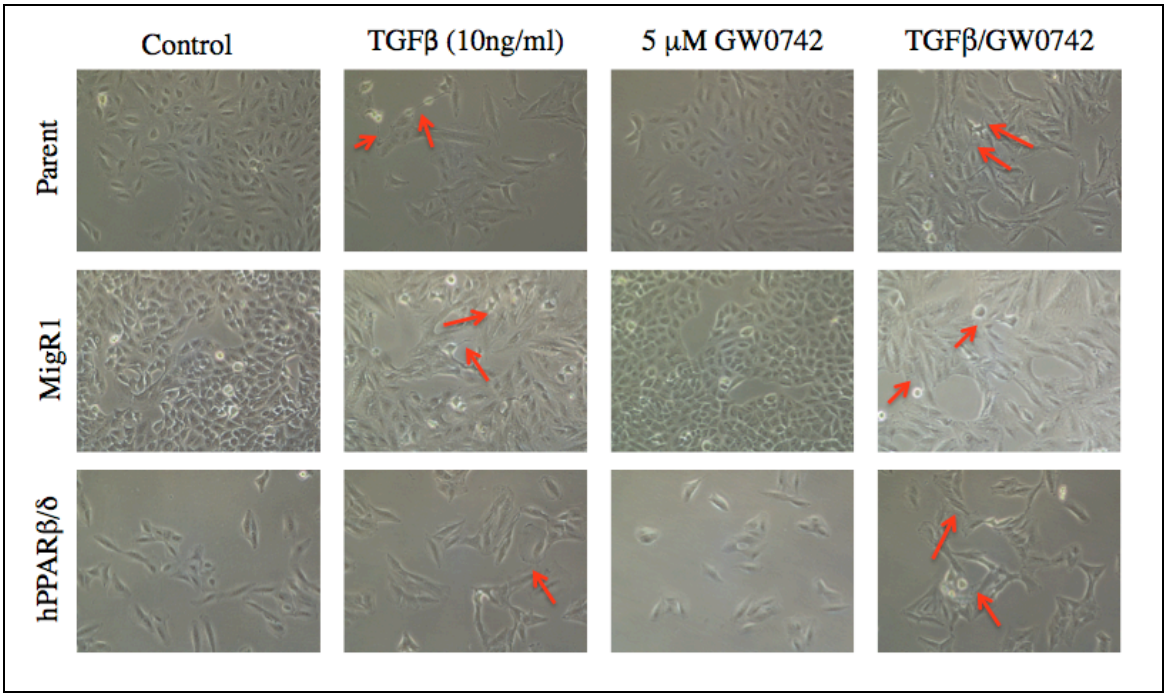

Figure 3. Post-treatment morphology study. Cell were photographed 48 hours after the addition of 5 μM GW0742 (72 hours after the addition of 10 ng/mL TGFβ) in order to observe any effect of ligand activation on epithelial mesenchymal transition in A549 cells. Red arrows point out phenotypic indicators of the mesenchymal call types.

Co-treatment Study

Co-treatment cells were treated with 5 μM GW0742 at the same time they were treated with 10 ng/mL TGFβ. Each cell line treated with TGFβ seemed to undergo epithelial mesenchymal transition. Ligand activation of PPARβ/δ did not seem to reverse or alter the epithelial mesenchymal transition. Morphology pictures were taken such that the picture was a relatively accurate representation of the entire well (Figure 4). Wells could not be accurately quantified due to varying degrees of cell confluency. However, epithelial mesenchymal transition was evident.

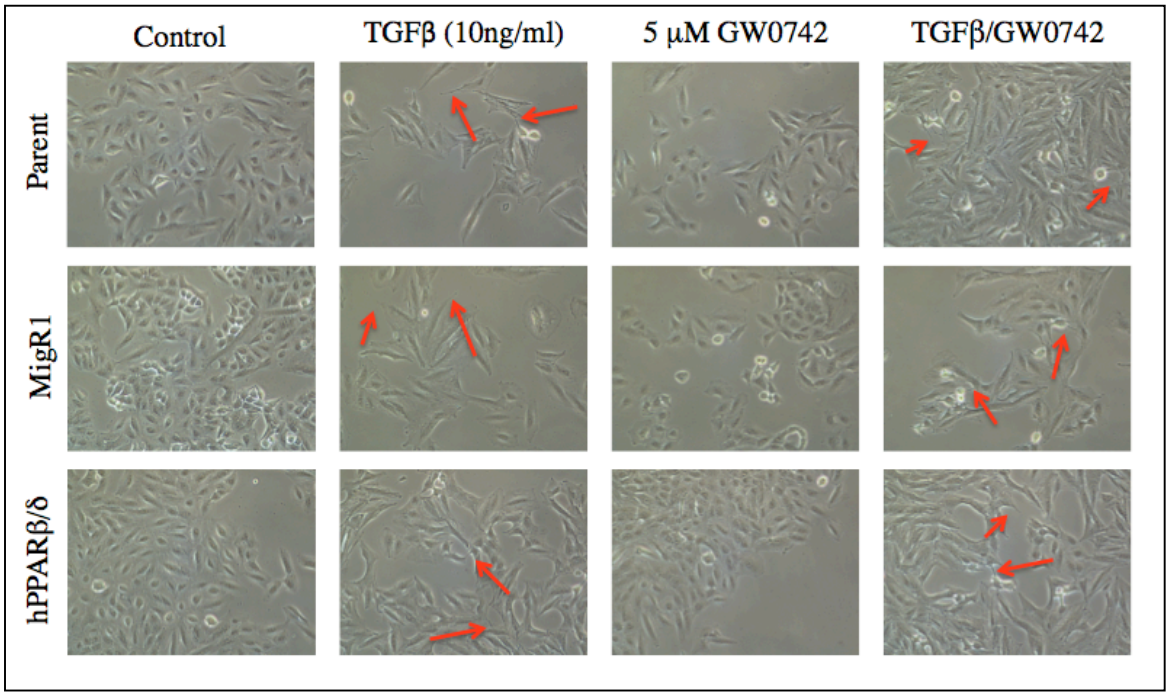

Figure 4. Co-treatment morphology study. Cells were photographed 48 hours after the addition of 5 μM GW0742 and 10 ng/mL TGFβ in order to observe any effect of ligand activation on epithelial mesenchymal transition in A549 cells. Red arrows point out phenotypic indicators of the mesenchymal cell types.

Western Blot Analysis

Western blot analysis was carried out twice in order to confirm and perfect resulting data. Unfortunately, the initial trial was impeded by faulty anti-goat secondary antibody and the second trial was inconsistent due to lack of protein in the wells. This is perplexing because the BCA protein assay was completed in order to ensure each sample contained the same amount of protein (20 μg). Additional trials could not be completed due to lack of enough protein (Figure 5, 6, 7). Figure 5 does not show the overexpression of PPARβ/δ in hPPARβ/δ cells and so subsequent western blot analyses were carried out only with no transfected A549 cells (Figures 6 and 7). This was done in order to observe any change in PPARβ/δ levels and ligand activation effect on epithelial mesenchymal transition.

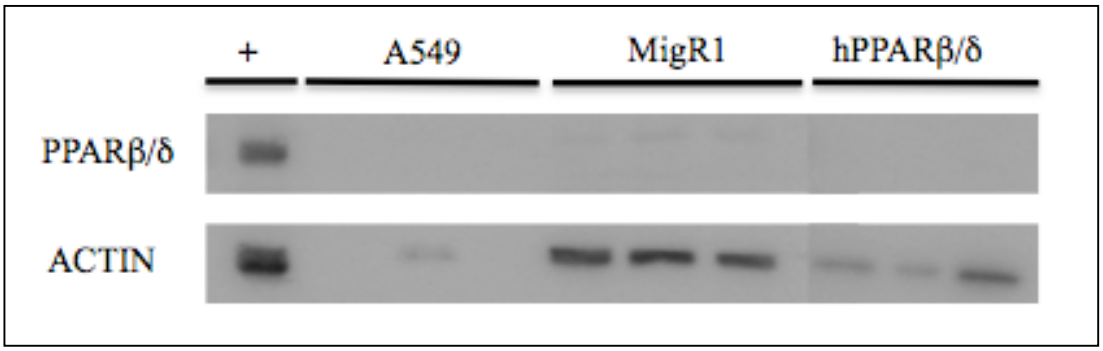

Figure 5. Western blot analysis of A549 and hPPARβ/δ overexpression. Western blot analysis of A549 Parent, MigR1 and hPPARβ/δ cell lines were carried out in order to observe expression of hPPARβ/δ in each cell line. Expression is expected to increase in hPPARβ/δ cell line. This was not observed and means that there was no transduction of the vector or the nuclear receptor and subsequently no over-expression of PPARβ/δ.

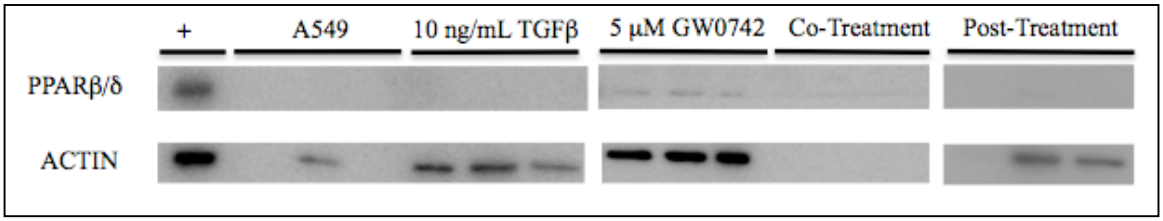

Figure 6. Western blot analysis of A549 parent cells. Western blot analysis of A549 cells were carried out in order to determine if the hPPARβ/δ expression was affected after administration of the PPARβ/δ ligand, GW0742. Many samples did not show ACTIN, which suggests that the protein concentration was too low in those samples. This is perplexing because protein concentration was normalized prior to western blot analysis.

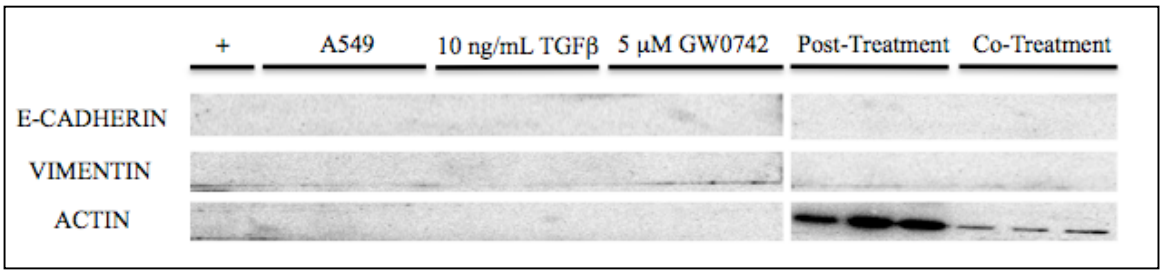

Figure 7. Western blot analysis of epithelial mesenchymal transition markers in A549 cells. Western blot analysis of A549 cells was carried out following treatments with TGFβ, GW0742 along with co-treatment and post-treatment in order to observe the expression of epithelial mesenchymal transition markers. VIMENTIN expression is expected to increase with the treatment of TGFβ, and E-CADHERIN expression is expected to decrease. Due to the absence of ACTIN bands, these blots are inconclusive.

DISCUSSION

The biological role of human PPARβ/δ remains controversial in the scientific community, especially with respect to its role in carcinogenesis. Both pro- and anti-carcinogenic effects have been reported in various cancer models[8]. In this study, a morphology assay and western blot analysis were conducted in order to evaluate the effect of over-expression and/or ligand activation of PPARβ/δ on induced epithelial mesenchymal transition in human lung adenocarcinoma cell line A549.

Previous studies have also used morphological studies to observe possible epithelial mesenchymal transition in A549 cells. One such study has shown that 10 ng/mL of TGFβ successfully induces epithelial mesenchymal transition in A549 cells[26]. The present study yielded similar results. Data showed that the A549 parent cells were of the mesenchymal phenotype 72 and 48 hours after treatment with TGFβ at the same dosage in the post- and co-treatment assays respectively (Figure 3 and 4).

Western blot analysis has also been used to quantitatively measure the effect of 10 ng/mL TGFβ on A549 cells[26]. In the present study, multiple controls were used in order to obtain sound results. First, the expression of PPARβ/δ was measured in A549, MigR1 and hPPARβ/δ cell lines. No ACTIN or PPARβ/δ bands were observed in the A549 parent cells implying that the protein concentration was not sufficient for these samples although equal amounts of protein (20 μg) of each sample were analyzed. The bicinchoninic acid (BCA) analysis was used in order to determine the protein concentration in each sample used in western blot analysis. This suggests that the

problem may have stemmed from the assay. Other possible errors may include, improper transfer, incomplete transfer, or low protein-antibody binding. Improper or incomplete transfer may have resulted from insufficient contact between the membrane and gel while low protein-antibody binding could have resulted from washing the membrane too much.

The protein expression of PPARβ/δ was not detected in either MigR1 or hPPARβ/δ cell lines. These data indicate that PPARβ/δ was not expressed in the MigR1 cell line nor overexpressed in the hPPARβ/δ cell lines since ACTIN and LDH were detected in these two cell lines but PPARβ/δ was not (Figure 5). Both of these cell lines were generated with previously established methods using empty retroviral vector MigR1 (MigR1) and cells overexpressing PPARβ/δ (hPPARβ/δ)[14]. A549 parent and MigR1 cells have very low expression of PPARβ/δ when compared with other cancer cells (breast cancer, colon cancer), which could demonstrate the fact that PPARβ/δ expression is higher in normal tissues but lowers in tumors[14]. This also suggests that PPARβ/δ may have anti-carcinogenic effect. However, hPPARβ/δ cells did not show an increase in PPARβ/δ expression in this study. One possible explanation could be that A549 cells selected against retrovirus-mediated gene transduction, resulting in non-stable expression of target gene (unpublished data). As a result, A549 cells that were previously over-expressing PPARβ/δ may lose the expression with subsequent passes. Meanwhile, the lack of PPARβ/δ expression in these samples could explain the phenotypes seen in the morphological assay. Since hPPARβ/δ was not expressed, the study did not adequately conclude the receptor's effect on epithelial mesenchymal transition.

Due to the lack of hPPARβ/δ expression in the MigR1 and hPPARβ/δ cell lines, subsequent western blot analysis was carried out solely on the post- and co-treatment assays performed on A549 parent cells. Ligand activation showed slightly increased expression of PPARβ/δ, but neither ACTIN nor PPARβ/δ expression were detected in control A549 parent cells; therefore it could not be compared quantitatively to the GW0742 treatment. GW0742 is a well-established PPARβ/δ agonist in the low nanomolar range and should have activated PPARβ/δ[8]. Due to this fact, it was expected that ligand would increase PPARβ/δ expression in cells treated with GW0742. The extent of this activation could not be measured using the data retrieved in this study.

Following the western blot analysis of the expression of PPARβ/δ, epithelial mesenchymal markers including E-CADHERIN and VIMENTIN were analyzed in A549 parent cells. We expected to see the changes in the expression of E-CADHERIN and VIMENTIN, which can further suggest that the cells went through epithelial mesenchymal transition and would be consistent with previous studies[26]. Unfortunately, positive controls and sample bands were not visible on the blot, indicating the transferring issue occurred when performing western blot analysis. Post- and co-treatment samples showed ACTIN but neither VIMENTIN, a mesenchymal cell marker, nor E-CADHERIN, an epithelial cell marker, was observed. At least one of these proteins should have been present because the cells were either in an epithelial state or a mesenchymal state based on the morphology assay. Due to the time point at which the cells were harvested – 72 and 48 hours after the treatment of TGFβ in the post- and co-treatment assays respectively, E-CADHERIN should have decreased in comparison to

control cells and VIMENTIN levels should have increased. Transferring issues in the western blot analysis prevented a quantitative and statistical analysis comparing the post- and co-treatment samples to their controls. Therefore, the experiments performed in this study need to be repeated with optimized western blot conditions in order to confirm epithelial mesenchymal transition at the molecular level. Conditions could be optimized by increasing protein concentration used, reducing the number of times the membrane is washed, increasing antibody concentration and using fresh buffers.

Optimized western analysis would yield compelling evidence regarding the effect of PPARβ/δ on the epithelial mesenchymal transition of A549 cells. We continue to speculate that PPARβ/δ activation will reverse this transition, which may direct future studies in regards to possible treatment. Repeating similar studies with different PPARβ/δ agonists would also allow conclusions to be made with regards to the receptor and epithelial mesenchymal transition. Additional knowledge on the mechanisms of this transition would provide insight as to how cellular identity may be maintained and in turn may aid in keeping diseases made more aggressive by epithelial mesenchymal transition at bay.

Non small-cell lung cancer has few effective treatments and a very poor prognosis. As a result, it is imperative that additional therapies are researched and developed. PPARβ/δ is involved in several metabolic and signaling pathways and its effective manipulation may lead to new treatments[27]. In summary, this study shows that epithelial mesenchymal transition was induced by TGFβ in A549 cells and the role of

PPARβ/δ in TGFβ-induced epithelial mesenchymal transition in A549 cells is not conclusively determined.

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carbon metabolism in lung epithelial cells A549." *Proteomics* 13.21 (2013): 3211-3221.
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Academic Vita

Ayesha Ali
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EDUCATION

The Pennsylvania State University, Schreyer Honors College — **University Park, PA**
Bachelor of Science in Finance, Concentration in Biochemistry — *Class of May 2014*

LEADERSHIP

Presidential Leadership Academy — **University Park, PA**
Member — *2011-Present*

- Enrolled in courses taught by President Rodney Erickson, President of Penn State University and Dean Christian Brady, Dean of the Schreyer Honors College to develop leadership fundamentals and an ethical foundation through exploration of diverse viewpoints, participation in unique experiences, & discussions

Presidential Leadership Academy Student Council — **University Park, PA**
Founder/President — *2013-2014*

- Spearheaded and organized events aiming to bring students together as a cohesive unit
- Addressed student concerns and suggestions with the administration
- Aided in future development of the academy

Ask Big Questions — **University Park, PA**
Fellow — *2012-Present*

- Utilized peer-to-peer engagement strategies to promote civil discourse on campus
- Planned and executed awareness campaigns geared toward expanding perspectives and promoting conversation among youth

Food for Thought — **University Park, PA**
Recruitment Chair — *2012-2013*

- Philanthropy group dedicated to serving children around the world who are denied access to basic living necessities, quality education and proper nutrition
- Planned and executed fundraising efforts as well as recruitment activities

Pakistani Student Association — **University Park, PA**
Fundraising Chair — *2011-2012*

- Planned, pitched and executed a global disaster awareness campaign that raised over $1200 for Pakistani flood victims in 3 weeks
- Became a liaison to Mahvash & Jahangir Siddiqui Foundation in Pakistan for sustaining relief camps after a major natural disaster that displaced 17 million people.

SKILLS

Account management | Market Analytics | Team Development and Supervision | Budget Building | Advanced Excel | Critical Thinking | Customer Relations | Interdisciplinary Discourse | Fluent in Urdu

WORK EXPERIENCE

Office of Governmental Affairs **University Park, PA**
Suite Host *2012*

- Represented Penn State student body as a host to the political guests of the university

College Works Painting **Harrisburg, PA**
Branch Manager *Feb 2011-July 2011*

- Coordinated, budgeted and managed a $40,000 painting business and established a market for building the business from the ground up
- Handled marketing customer relations as well as hired, developed and supervised four staff members while efficiently executing customer orders ranging from $1,500 - $5,000

RESEARCH

Carcinogenesis Research Under Dr. Jeffery Peters **University Park, PA**
Student Investigator *2012-Present*

- Studied the role of a nuclear receptor (PPARβ/δ) on tumor growth, specifically the regulation of carcinogenesis
- Executed biochemistry and toxicology experiments for the completion of Schreyer Honors Thesis

Carcinogenesis Research Under Dr. Alan Lipton **Hershey, PA**
Student Investigator *2006-2010*

- Investigated the effect of cocoa on breast cancer cell lines and analyzed significant data exposing the potential for cocoa's ability to reduce cancer growth in tested cell lines