The Role of Peroxisome Proliferator-Activated Receptor-β/δ (PPARβ/δ) in the Human MCF7 Breast Cancer Cell Line

Dylan A. Phillips, McNair Scholar
The Pennsylvania State University

McNair Faculty Research Advisors:
Dr. Jeffrey M. Peters, Ph. D
Distinguished Professor of Molecular Toxicology & Carcinogenesis
Department of Veterinary and Biomedical Sciences
and The Center of Molecular Toxicology and Carcinogenesis,
The Pennsylvania State University

Dr. Pei-Li Yao, Ph. D
Postdoctoral Researcher:
Department of Veterinary and Biomedical Sciences
and The Center of Molecular Toxicology and Carcinogenesis,
The Pennsylvania State University

Abstract

The role of PPARβ/δ in breast carcinogenesis remains unclear because some studies show that activating this receptor promotes breast cancer cell growth, while other studies show that activating this receptor inhibits breast cancer cell growth. Further, some studies suggest that the function of PPARβ/δ can be altered by the presence of fatty acid binding protein 5 (FABP5) and cell retinoic acid binding protein-II (CRABP-II) by diverting atRA or PPARβ/δ agonists to PPARβ/δ and promote cell proliferation by preventing activation of retinoic acid receptors (RARs). This study examined the effects of PPARβ/δ on cell proliferation in the presence of GW0742 (a PPARβ/δ agonist), GSK0660 (a PPARβ/δ antagonist) and all-trans retinoic acid (atRA a putative PPARβ/δ agonist) in a genetically engineered human MCF7 breast cancer cell line. Control cells (MCF7), cells infected with a control retrovirus (MCF7-MigR1), or cells infected with a retrovirus expressing PPARβ/δ (MCF7-MigR1 hPPAR) were treated with either atRA, GSK0660 and/or GW0742 and gene expression and cell proliferation were examined. This allowed for examining the hypothesis that the activity of PPARβ/δ can be altered by FABP5/CRABP-II ratio in a human breast cancer cell line. The mRNA levels of the PPARβ/δ target gene, adipose differentiation-related protein (*ADRP*) were increased by treatment with GW0742 but not by atRA. In contrast, atRA increased expression of the RAR target gene *CYP26A* but GW0742 had no effect on expression. While GSK0660 inhibited GW0742-induced *ADRP* mRNA, it had no effect on *ADRP* or *CYP26A* mRNA expression. The FABP5/CRABP-II ratio was measured by western blot analysis and relatively high. However, over-expression of PPARβ/δ did not influence the effect or lack of effect of GW0742 or atRA. Cell proliferation in response to GW0742, GSK0660 and atRA in MCF7 cells was not different between treatment

groups. Results from these preliminary studies show argue against a role of FABP5 delivering ligands to PPARβ/δ in human breast cancer cells and altering gene expression by activating this receptor. Whether PPARβ/δ affects cell proliferation in these models must be further examined because of high variability.

Introduction

Peroxisome proliferator-activated receptor (PPAR) is a member of the nuclear hormone receptor superfamily. These receptors function as transcription factors to regulate gene expression and are activated by endogenous ligands or synthetic compounds which have been thought to behave similar to natural ligands. Discovered back in the early 1980's, PPAR has been divided into three separate isoforms including PPARα, PPARβ/δ and PPARγ. Each isoform may vary with its effects on the growth of different types of cells. With PPARγ the most known because it has been used for cancer treatment today (1). The least known PPAR is PPARβ/δ. Since its original discovery by Krey et. al. in 1993 (2), there has been no clear determination of the characteristics and effects PPARβ/δ has on the human body. Unclear determination is mostly due to opposite conclusions drawn on proliferation properties of PPARβ/δ (3, 4).

On the other hand, the role of retinoic acid and its receptor, retinoic acid receptor (RAR), in cancers has been the subject of many reports. Retinoic acid's inhibitory effect on cell proliferation has been mostly agreed upon (3, 5, 6). According to their similar binding pockets for the receptors, there may be a competitive interaction between the RAR and PPARβ/δ (7). This relation has been in major discrepancy lately involved two retinoic acid transports, fatty acid binding protein 5 (FABP5) and cellular retinoic acid binding protein-II (CRABP-II). One theory is: retinoic acid predominately operates through RAR inhibiting cell growth, but when a high FABP5/ CRABP-II ratio is present, retinoic acid binds with PPARβ/δ and activates cell proliferation (7, 8, 9). Another theory is: There is no interaction between retinoic acid and PPARβ/δ, even in high FABP/CRABP-II ratios (3, 6, 10, 11).

The first theory is assuming PPARβ/δ is a cell proliferator which has not been clearly determined. Also, human breast cancer cell line MCF7 has been shown to express a low FABP5/CRABP-II ratio (8), which will be useful in determining the effect PPARβ/δ has on the cell line alone. Therefore, in the present study, I will examine the effects of the PPARβ/δ agonist (GW0742) and the PPARβ/δ antagonist (GSK0660) on cell proliferation. I will also look at the effect the RAR agonist all-trans retinoic acid (atRA) has on MCF7 cells. I will be measuring the mRNA levels of known PPARβ/δ target genes adipose differentiation-related protein (*ADRP*) along with RAR target gene cytochrome P450-26A (*CYP26A*) using qPCR. I will also examine the protein levels of FABP5 and CRABP-II which have been mentioned before. Cell proliferation will be detected in real-time after activating or inhibiting PPARβ/δ in MCF7 cells. Dr. Peters' laboratory previously generated MCF7 hPPARβ/δ cells which are stably over-expressing human PPARβ/δ. This cell line allows me to estimate the effect high expression of PPARβ/δ has on cell proliferation compared to control MCF7 cell line. Therefore, GW0742, GSK0660 and atRA will also be used in MCF7 hPPARβ/δ cells.

Materials and Methods

Cell culture

Human breast cancer cell line MCF7 was purchased from American Type Culture Collection (ATCC, Manassas, VA). Cells were maintained in Dulbecco's minimal essential medium (DMEM) with 10% non-essential amino-acids, 10% fetal bovine serum and 1% penicillin/streptomycin at 37°C and 5% CO_2. The parent MCF7 cells, MCF7 cells with the retroviral empty vector (MCF7 MigR1), or MCF7 cells over-expressing PPARβ/δ (MCF7 hPPARβ/δ) were used.

Treatment

PPARβ/δ antagonist GSK0660 and agonist GW0742 were synthesized by GlaxoSmithKline (Research Triangle Park, NC) (Sznaidman et al., 2003). MCF7 cells were treated with 0, 0.1 and 1 μM of GSK0660 or GW0742 for 24 h, or co-treated with GSK0660 and GW0742. All-trans retinoic acid (atRA) was purchased from Sigma-Aldrich (St. Louis, MO). Cells were treated with atRA for 8 and 72 h.

Western blot analysis

Protein was isolated using radioimmunoprecipitation assay buffer (5% Tris-HCl pH7.5, 3% NaCl, 1% NP-40 and 0.5% Sodium deoxycholate) and protease inhibitors. Ten μg of protein per sample was resolved in 12% SDS-polyacrylamide gels. Proteins were transferred onto polyvinylidene fluoride membrane using an electroblotting method. The membranes were blocked with 5% dried milk in Tris-buffered saline/Tween 20 (TBST) and incubated at 4°C overnight with primary antibodies against CRABP-II (1:1000, Abcam, Cambridge, MA), FABP5 (1:1000, BioVision, Milpitas, CA) and ACTIN (1:500, Santa Cruz Biotechnology Inc., Santa Cruz, CA). After incubation with biotinylated secondary antibody (1:2000, Jackson ImmunoResearch Laboratories, West Grove, PA), immunoreactive proteins on the membrane were detected by ^{125}I-labeled streptavidin (GE Healthcare, Chalfont St. Giles, Buckinghamshire, UK). ACTIN was used as the loading control.

Quantitative polymerase chain reaction (qPCR)

Total RNA was extracted using RiboZol RNA Extraction Reagent (AMRESCO, Solon, OH) and the manufacturer's recommended protocol. The expression levels of glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*), adipose differentiation-related protein (*ADRP*), and cytochrome P450-26A (*CYP26A*) were measured using qPCR. cDNA was generated using MultiScribe Reverse Transcriptase (Applied Biosystems, Foster City, CA).The MyiQ Real-time PCR Detection System (Bio-Rad Laboratories, Hercules, CA) was used to detect the quantitative signals from SYRB© Green PCR Supermix (Quanta Biosciences, Gaithersburg, MD) in the iCycler. The following cycle was used for all PCR run: 95 ºC for 10s, 60 ºC for 30s, and 72 ºC for 30s, repeated 45 times. The relative mRNA was normalized according to the *GAPDH* gene.

Cell proliferation

MCF7 parent cells, MCF7 MigR1 cells and MCF7 hPPARβ/δ cells (1,200 cells) were plated onto 16-well Eplate. Changes in cell index were measured using an xCELLigence RTCA DP (ACEA

Biosciences, Inc., San Diego, CA), real-time cell monitoring instrument. During the first 24 hours, cell index was counted once every 15 minutes. After 24 hours, the PPARβ/δ agonist GW0742 and/or the PPARβ/δ antagonist GSK0660 was added into cells. Cell index was then counted once every hour for an additional 48 hours. Triplicate samples for each treatment were used for each time point, and each replicate was counted three times.

Statistical analysis

All experimental groups were performed in triplicate and repeated using three sets of cells. The data were subjected to a parametric one-way analysis of variance (ANOVA) followed by Tukey test for post hoc comparisons. Statistical significance was considered to be achieved when $p<0.05$.

Results

PPARβ/δ antagonist GSK0660 suppressed GW0742-induced *ADRP* expression in MCF7 cells

Over-expression of PPARβ/δ induced ~2-fold expression of *ADRP* in MCF7 cells (Fig. 1). The activation of PPARβ/δ by GW0742 further induced *ADRP* mRNA level in MCF7 hPPARβ/δ cells (Fig. 1). PPARβ/δ antagonist GSK0660 treatment suppressed GW0742-induced *ADRP* expression in MCF7 hPPARβ/δ cells (Fig. 1); however, GSK0660 alone did not affect *ADRP* mRNA level (Fig. 2).

PPARβ/δ agonist GW0742 decreases GSK0660-induced *CYP26A* expression in MCF7 cells

Over-expression of PPARβ/δ decreased *CYP26A* expression in MCF7 cells (Fig. 3). Interestingly, the high dose of GSK0660 treatment induced *CYP26A* expression in MCF7 MigR1 and MCF7 hPPARβ/δ cells, while the low dose of GSK0660 induced *CYP26A* in MCF7 parent cells (Fig. 3). This suggests that the inhibition of PPARβ/δ activity may increase the RAR signaling. *CYP26A* expression was decreased in MCF7-MigR1 cells after GW0742 treatment while increased in MCF7 hPPARβ/δ cells (Fig. 4). Additionally, in MCF7 MigR1 cells, GSK0660-induced *CYP26A* expression was inhibited by GW0742 treatment (Fig. 4).

The effect of atRA on *CYP26A* and *ADRP* expression remains unclear

After treatment with the MigR1 vector, there was a decrease in the *CYP26A* expression after treatment with atRA. Without the vector, 1μM atRA treatment greatly increased the *CYP26A* expression; however, 10 μM atRA treatment greatly decreased *CYP26A*. The decrease in *CYP26A* after 10 μM treatment went down in all cell types. No significant change occurred in the *ADRP* expression no matter the atRA treatment.

Activation or inhibition of PPARβ/δ has no participation in cell proliferation in MCF7 cells

By using xCELLigence system, we were able to monitor cell growth in real-time. No significant difference in cell proliferation had been observed among MCF7, MCF7 MigR1, and MCF7 hPPARβ/δ cells. The PPARβ/δ agonist GW0742 and/or the PPARβ/δ antagonist GSK0660 were also applied to cells; however, there is no significant change in any cell treatments (Fig. 7).

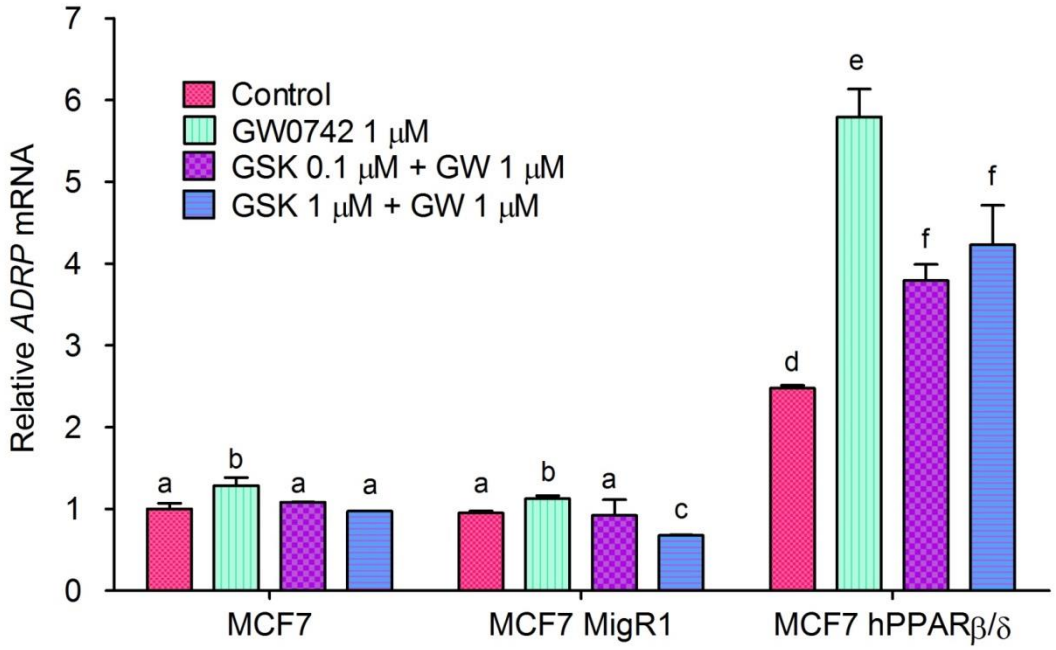

Fig 1. The expression of PPARβ/δ target gene *ADRP* in human breast cancer cell MCF7 following the activation and/or inhibition of PPARβ/δ. MCF7 parent cells, MCF7 MigR1 cells and MCF7 hPPARβ/δ cells were treated with PPARβ/δ agonist GW0742 in the absence or presence of PPARβ/δ antagonist GSK0660 for 24 h. The mRNA levels of *ADRP* and *GAPDH* were determined by qPCR. Over-expression of PPARβ/δ caused the increase in mRNA level of *ADRP*. The activation of PPARβ/δ by GW0742 significantly induces *ADRP* mRNA level in MCF7 hPPARβ/δ cells. GSK0660 treatment suppressed GW0742-induced *ADRP* expression in MCF7 hPPARβ/δ cells. Values represent mean ± SEM of *ADRP* expression normalized to *GAPDH* expression as compared to parent cell line. Significant differences ($p < 0.05$) between groups are denoted by bars with different letters.

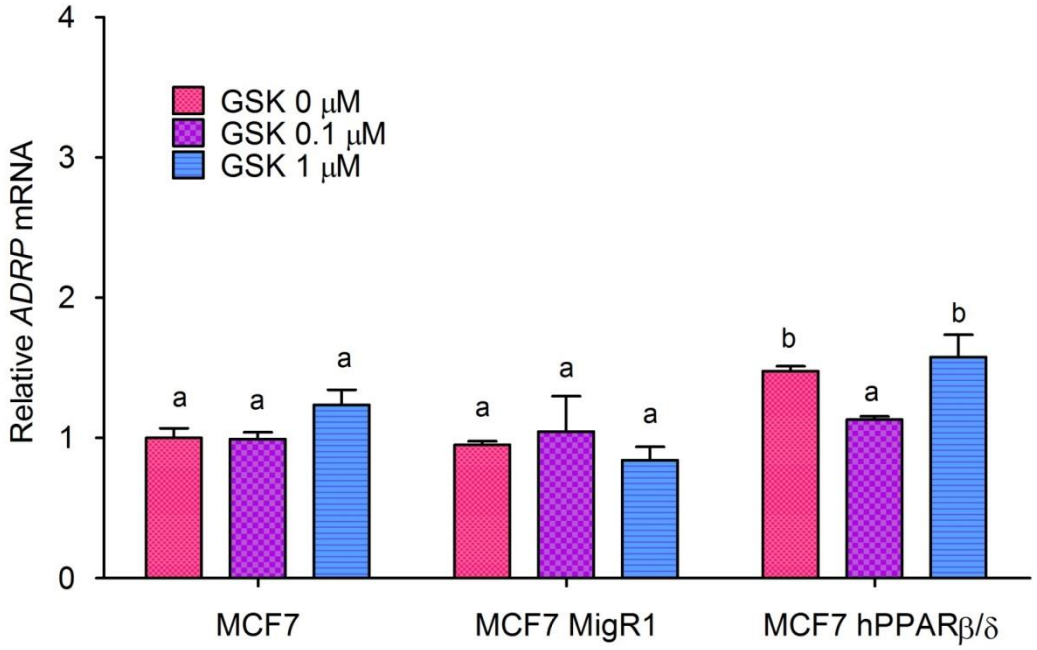

Fig 2. The effect of PPARβ/δ antagonist GSK0660 on the expression of PPARβ/δ target gene *ADRP* in human breast cancer cell MCF7. MCF7 parent cells, MCF7 MigR1 cells and MCF7 hPPARβ/δ cells were treated with PPARβ/δ antagonist GSK0660 for 24 h. The mRNA levels of *ADRP* and *GAPDH* were determined by qPCR. Antagonist GSK0660 did not affect *ADRP* mRNA level. Values represent mean ± SEM of *ADRP* expression normalized to *GAPDH* expression as compared to parent cell line. Significant differences ($p < 0.05$) between groups are denoted by bars with different letters.

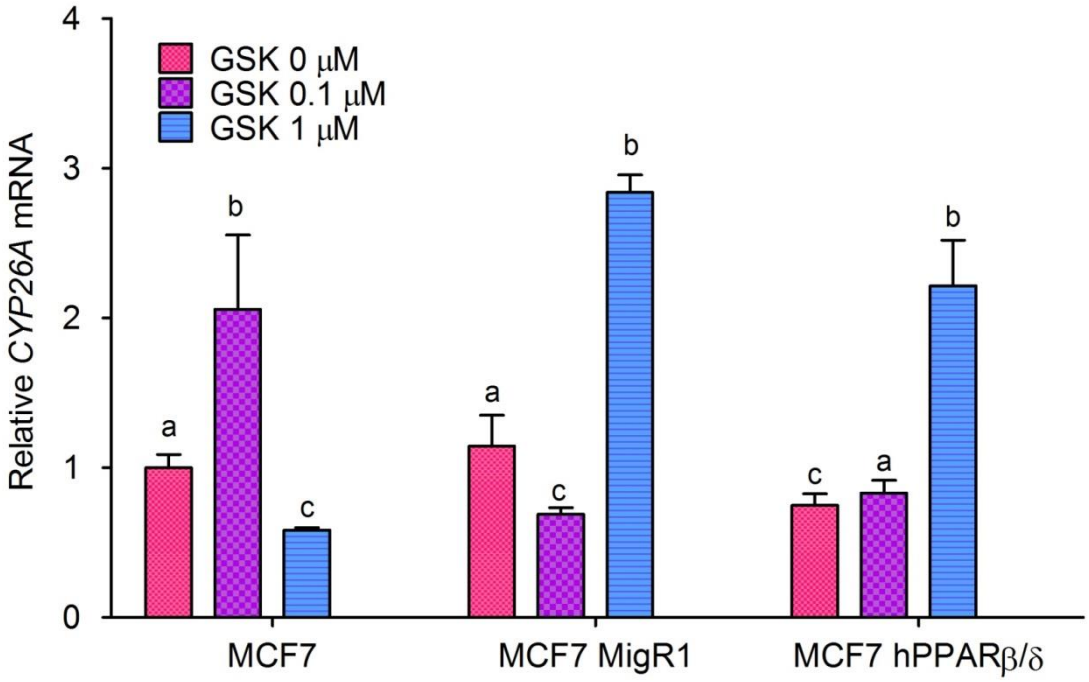

Fig 3. The effect of PPARβ/δ antagonist GSK0660 on the expression of RAR target gene *CYP26A* in human breast cancer cell MCF7. MCF7 parent cells, MCF7 MigR1 cells and MCF7 hPPARβ/δ cells were treated with PPARβ/δ antagonist GSK0660 for 24 h. The mRNA levels of *CYP26A* and *GAPDH* were determined by qPCR. Over-expression of PPARβ/δ decreased *CYP26A* expression. High dose of GSK0660 treatment induced *CYP26A* expression in MCF7 MigR1 and MCF7 hPPARβ/δ cells, while low dose of GSK0660 induced *CYP26A* in MCF7 parent cells. This suggests that the inhibition of PPARβ/δ activity may increase the RAR signaling. Values represent mean ± SEM of *ADRP* expression normalized to *GAPDH* expression as compared to parent cell line. Significant differences ($p < 0.05$) between groups are denoted by bars with different letters.

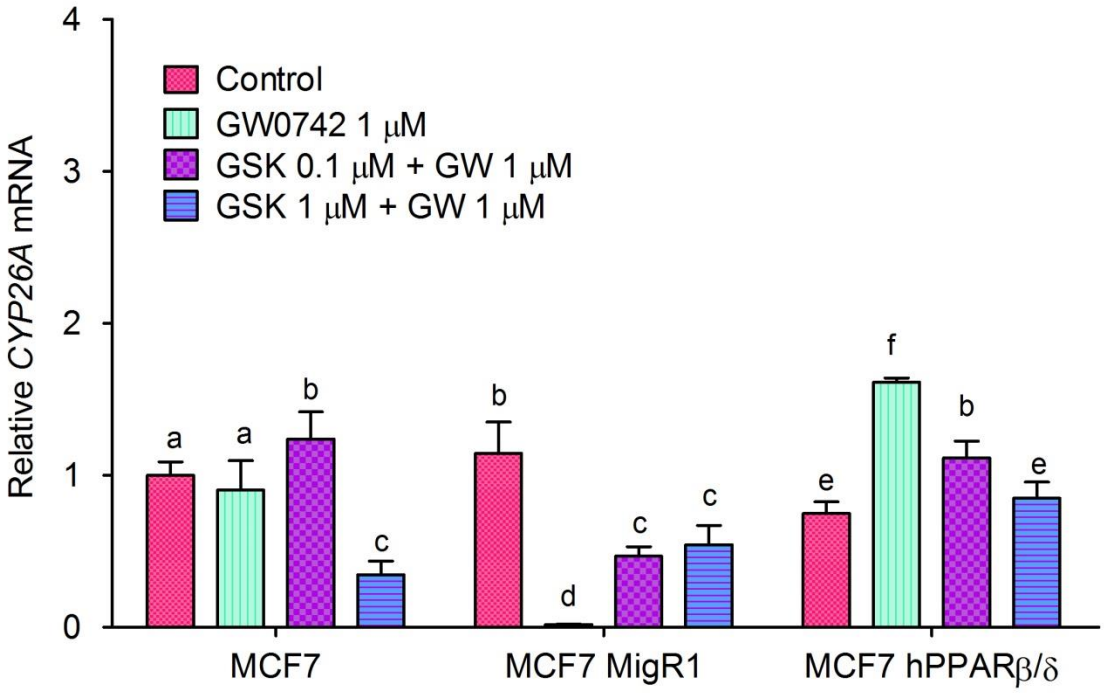

Fig 4. The expression of RAR target gene *CYP26A* in human breast cancer cell MCF7 following the activation and/or inhibition of PPARβ/δ. MCF7 parent cells, MCF7 MigR1 cells and MCF7 hPPARβ/δ cells were treated with PPARβ/δ agonist GW0742 in the absence or presence of PPARβ/δ antagonist GSK0660 for 24 h. The mRNA levels of *CYP26A* and *GAPDH* were determined by qPCR. Over-expression of PPARβ/δ decreased *CYP26A* expression. *CYP26A* expression was decreased in MCF7-MigR1 cells after GW0742 treatment while increased in MCF7 hPPARβ/δ cells. In MCF7 MigR1 cells, GSK0660-induced *CYP26A* expression was inhibited by GW0742 treatment. Values represent mean ± SEM of *CYP26A* expression normalized to *GAPDH* expression as compared to parent cell line. Significant differences ($p < 0.05$) between groups are denoted by bars with different letters.

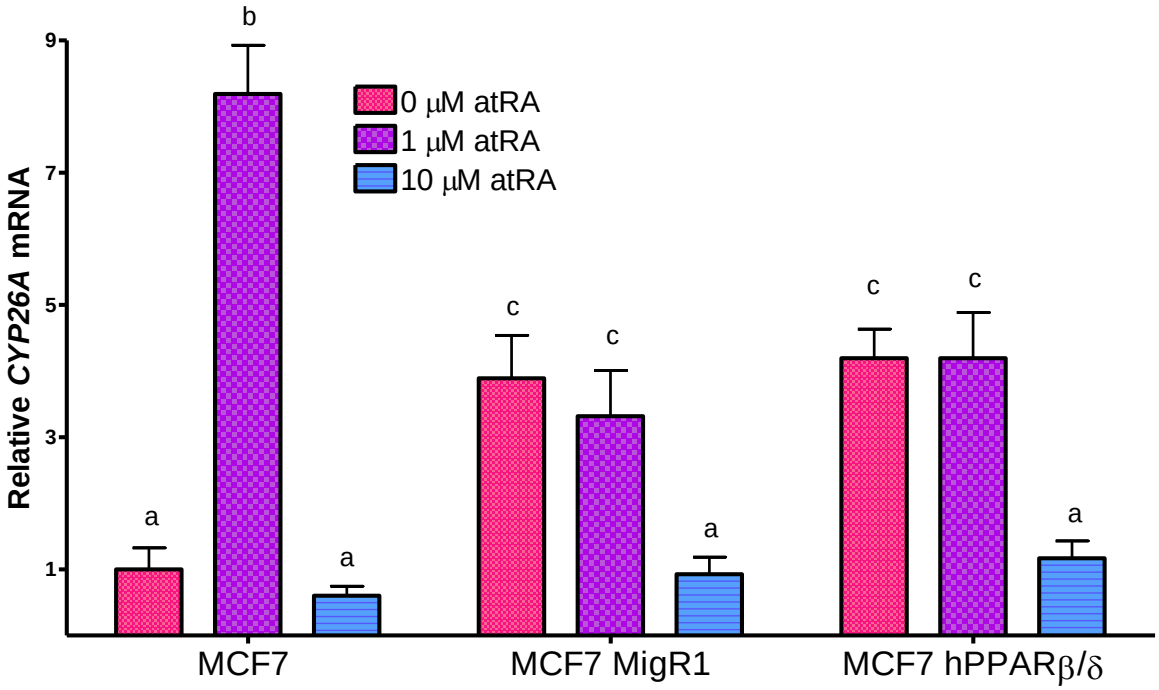

Fig 5. The effect of atRA on the expression of RAR target gene *CYP26A* in human breast cancer cell MCF7. MCF7 parent cells, MCF7 MigR1 cells and MCF7 hPPARβ/δ cells were treated with atRA for 8 h. The mRNA levels of *CYP26A* and *GAPDH* were determined by qPCR. Over-expression of PPARβ/δ decreased *CYP26A* expression. The initial treatment of atRA showed a significant increase in *CYP26A* expression at 1μM concentration in MCF7 parent cells; however 10μM of atRA decreased *CYP26A* expression in both MCF7 cells with low and high expression of PPARβ/δ. Values represent mean ± SEM of *CYP26A* expression normalized to *GAPDH* expression as compared to parent cell line. Significant differences ($p < 0.05$) between groups are denoted by bars with different letters.

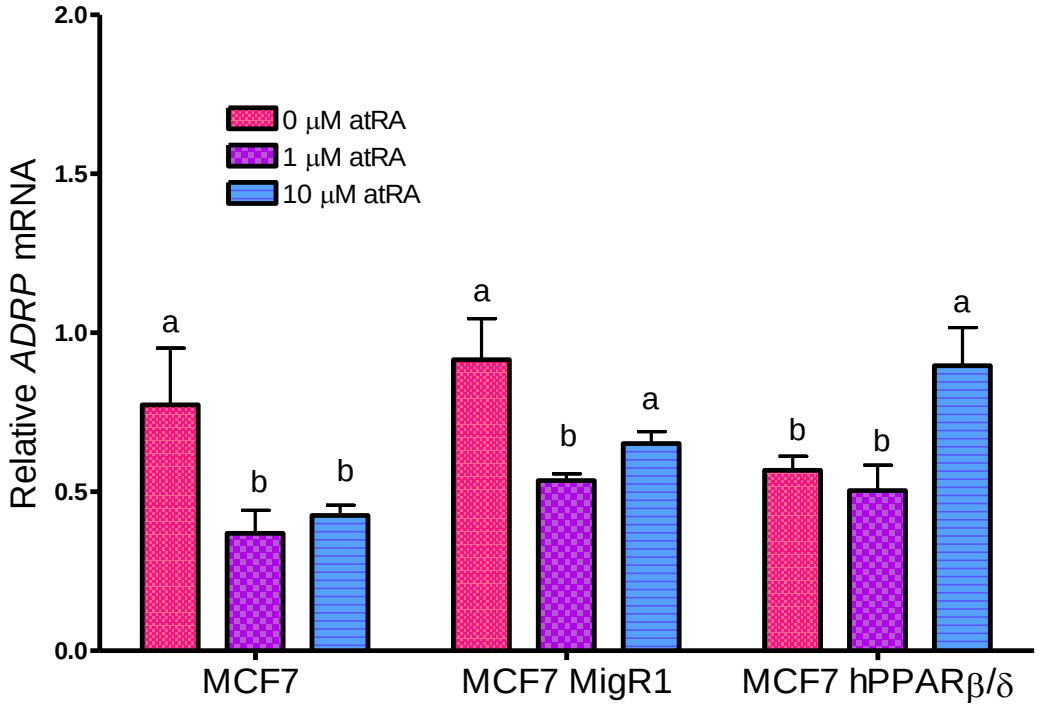

Fig 6. The effect of atRA on the expression of PPARβ/δ target gene *ADRP* in human breast cancer cell MCF7. MCF7 parent cells, MCF7 MigR1 cells and MCF7 hPPARβ/δ cells were treated with atRA for 8 h. The mRNA levels of *ADRP* and *GAPDH* were determined by qPCR. Over-expression of PPARβ/δ decreased *CYP26A* expression. In the parent control cell line and MigR1 cell line, *ADRP* expression went down after atRA treatment; however 10 μM of atRA increased *ADRP* expression in both MCF7 cells with low and high expression of PPARβ/δ. Values represent mean ± SEM of *ADRP* expression normalized to *GAPDH* expression as compared to parent cell line. Significant differences ($p < 0.05$) between groups are denoted by bars with different letters.

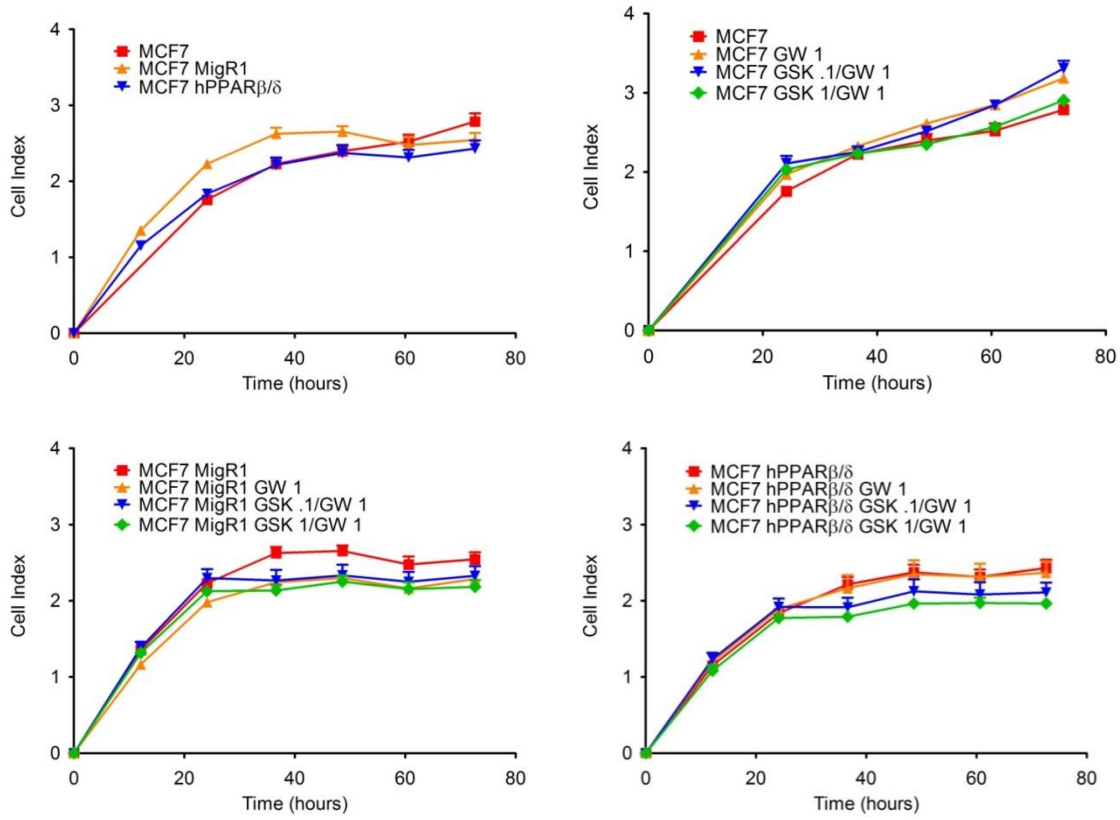

Fig 7. The effect of activation or inhibition of PPARβ/δ on cell proliferation in MCF7 cells. Cell proliferation in MCF7 parent cells, MCF7 MigR1cells and MCF7 hPPARβ/δ cells was measured by xCELLigence System. No significant difference in cell proliferation had been observed among MCF7, MCF7 MigR1, and MCF7 hPPARβ/δ cells. The PPARβ/δ agonist GW0742 and/or the PPARβ/δ antagonist GSK0660 was applied to cells. There is no significant change in any cell treatments

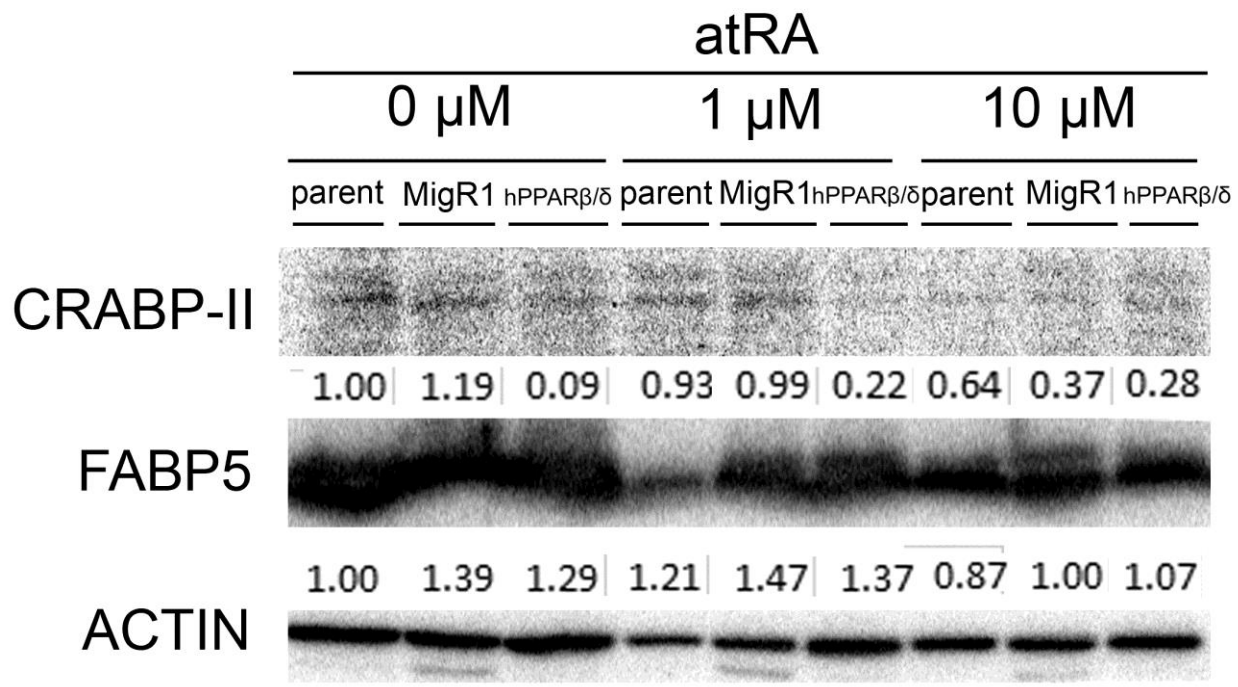

Fig 8. The effect of atRA on the production of CRABP-II and FABP5. There is no significance in the difference in FABP5 or CRABP-II in any of the treatments.

Discussion

To date, the actual function of PPARβ/δ in regulating cancer cell proliferation is still unclear. (1) Here we show that both over-expression of PPARβ/δ and ligand activation of PPARβ/δ by GW0742 increase the expression of the PPARβ/δ target gene, *ADRP,* in the breast cancer cell line MCF7. PPARβ/δ antagonist GSK0660 is able to negate GW0742-induced *ADRP* expression. *ADRP* seems to be a good marker to determine the activity of PPARβ/δ in MCF7 cells. Further using PPARβ/δ agonist GW0742 or agonist GSK0660, we can determine how activation or inhibition of PPARβ/δ influences the growth of human breast cancer cells.

In the xCELLigence test, we can observe whether the increase in PPARβ/δ expression and/or the treatment with GW0742 ± GSK0660 has effects on cell growth in a real-time manner. Other studies in our lab have shown that the over-expression of PPARβ/δ inhibits cell growth in various cancer cell lines, including MCF7 cells. However, our current results showed that no changes in cell proliferation between MCF7 cells expressing either low or high level of PPARβ/δ. In addition, our current results showed the activation of PPARβ/δ by agonist GW0742 has no effect on cell growth, consistent with previous results from our lab. These observations may be due to the fact that the natural ligands present in MCF7 cells compete with the binding site of GW0742 and PPARβ/δ. Our study also showed there was no change in PPARβ/δ activity after strict treatment with GSK0660. The natural ligands of MCF7 already were competing with GW0742 to bind to PPARβ/δ could explain the lack of change in cell growth after the addition of GSK0660.

Surprisingly, the addition of high concentration of atRA has an inhibitory effect on *CYP26A* expression. This chemical imbalance may be responsible for the decrease in *ADRP* expression after high dose of atRA treatment. However, the inconsistent results led to uncertain conclusion whether or not the interaction between PPARβ/δ and RAR occurs in response to atRA. The variability may be resulted from different passages of cells.

Without more than one sample per treatment, the western data needs to be repeated for consistency purposes. Previous studies have examined the effect of atRA on PPARβ/δ at a low ratio and have theorized atRA will take its normal path to the RAR receptor (8). However, after the addition of high concentration of atRA to the cells, the excessive atRA in the cells may have been able to bind to the possibly lower affinity PPARβ/δ. This could explain the increase in *ADRP* expression of the PPARβ/δ over-expressed cell line. Further studies should use an RAR knock-out/hPPARβ/δ over-expressed cell line, using RAR siRNA, to further prove this theory.

Also, our current results showed the activation of PPARβ/δ by agonist GW0742 has no effect on cell growth, consistent with previous results from our lab. These observations may be due to the fact that the natural ligands present in MCF7 cells compete with the binding site of GW0742 and PPARβ/δ. Our study also showed there was no change in PPARβ/δ activity after strict treatment with GSK0660. The lack of change in cell growth after the addition of GSK0660 could be explained by natural ligands of MCF7 competing with the GW0742.

The affect GW0742 and GSK0660 have on the RAR's target gene *CYP26A* are vague. *CYP26A* expression appears to increase when treated with GSK0660, suggesting that RAR signaling has been activated. This cannot be confirmed because the increased expression of *CYP26A* is also apparent in the MCF7 MigR1 vector treated cell line. Without further testing, it cannot be determined whether the over-expression of PPARβ/δ is affecting *CYP26A* expression. The increase in *CYP26A* expression in the MCF7 hPPARβ/δ cell line after treatment with GW0472 indicates the PPARβ/δ agonist also activates RAR. The activation of RAR by GW0742

is also apparent when GSK0660 decreases the GW0742-induced *CYP26A* expression.

Surprisingly, the addition of high concentration of atRA has shown an inhibitory effect on *CYP26A* expression. This chemical imbalance of high levels of atRA may be responsible for the increase in *ADRP* expression after 10μM of atRA treatment.

In conclusion, the role of PPARβ/δ in regulating cell proliferation is still unclear. Instead of directly affecting cell proliferation, the possible change in RAR signaling by PPARβ/δ agonist and antagonist may lead to promoting cell differentiation. Therefore, the differentiation markers should be tested in the future.

Acknowledgements

Special thanks to the Ronald E. McNair Postbaccalaureate Achievement Program for providing the means and funds for this research project.

References

1: Peters JM, Shah YM, Gonzalez FJ. (2012) The role of peroxisome proliferator-activated receptors in carcinogenesis and chemoprevention. *Nat Rev Cancer*. 12(3):181-95.

2: Grigorios Krey, Hansjörg Keller, Abderrahim Mahfoudi, Jeffrey Medin, Keiko Ozato, Christine Dreyer, Walter Wahli. (1993) Xenopus peroxisome proliferator activated receptors: Genomic organization, response element recognition, heterodimer formation with retinoid X receptor and activation by fatty acids. *The Journal of Steroid Biochemistry and Molecular Biology*. 47(1–6): 65-73

3: Borland MG, Foreman JE, Girroir EE, Zolfaghari R, Sharma AK, Amin S, Gonzalez FJ, Ross AC, Peters JM. (2008) Ligand activation of peroxisome proliferator-activated receptor-beta/delta inhibits cell proliferation in human HaCaT keratinocytes. *Mol Pharmaco*. 74(5):1429-42

4: Han S, Ritzenthaler JD, Sun X, Zheng Y, Roman J. (2009) Activation of peroxisome proliferator-activated receptor beta/delta induces lung cancer growth via peroxisome proliferator-activated receptor coactivator gamma-1alpha. *Am J Respir Cell Mol Biol.* 46(3):414

5: Donato LJ, Suh JH, Noy N. (2007) Suppression of mammary carcinoma cell growth by retinoic acid: the cell cycle control gene Btg2 is a direct target for retinoic acid receptor signaling. *Cancer Res*. 67(2):609-615

6: Peters JM, Foreman JE, Gonzalez FJ. (2011) Dissecting the role of peroxisome proliferator-activated receptor-β/δ (PPARβ/δ) in colon, breast, and lung carcinogenesis. *Cancer Metastasis Rev*. 30(3-4):619-640

7: Kittler R, Zhou J, Hua S, Ma L, Liu Y, Pendleton E, Cheng C, Gerstein M, White KP. (2013) A comprehensive nuclear receptor network for breast cancer cells. *Cell Rep*. 3(2):538-51

8: Schug TT, Berry DC, Shaw NS, Travis SN, Noy N. (2007) Opposing effects of retinoic acid on cell growth result from alternate activation of two different nuclear receptors. *Cell*. 29(4):723-33

9: Schug TT, Berry DC, Toshkov IA, Cheng L, Nikitin AY, Noy N. (2008) Overcoming retinoic acid-resistance of mammary carcinomas by diverting retinoic acid from PPARbeta/delta to RAR. *Proc Natl Acad Sci U S A*. 105(21):7546-51

10: Borland MG, Khozoie C, Albrecht PP, Zhu B, Lee C, Lahoti TS, Gonzalez FJ, Peters JM. (2011) Stable over-expression of PPARβ/δ and PPARγ to examine receptor signaling in human HaCaT keratinocytes. *Cell Signal*. 23(12):2039-2050

11: Rieck M, Meissner W, Ries S, Müller-Brüsselbach S, Müller R. (2008) Ligand-mediated regulation of peroxisome proliferator-activated receptor (PPAR) beta/delta: a comparative analysis of PPAR-selective agonists and all-trans retinoic acid. *Mol Pharmacol*. 74(5):1269-1277

12: Palkar PS, Borland MG, Naruhn S, Ferry CH, Lee C, Sk UH, Sharma AK, Amin S, Murray IA, Anderson CR, Perdew GH, Gonzalez FJ, Müller R, Peters JM. (2010) Cellular and pharmacological selectivity of the peroxisome proliferator-activated receptor-beta/delta antagonist GSK3787. *Mol Pharmacol*. 78(3):419-430