THE PENNSYLVANIA STATE UNIVERSITY
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DEPARTMENT OF BIOLOGY

THE DIFFERENTIAL EFFECTS OF ALL-TRANS RETINOIC ACID ON RETINOIC ACID RECEPTOR (RAR)- AND PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR-β/δ (PPARβ/δ)-DEPENDENT SIGNALING IN THE HUMAN NT2/D1 TESTICULAR EMBRYONAL CANCER CELL LINE

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Abstract

The nuclear hormone receptor peroxisome proliferator-activated receptor-beta/delta (PPARβ/δ) has been shown to promote and inhibit tumorigenesis, suggesting a context-specific role for PPARβ/δ in carcinogenesis. Previous literature suggests that all-trans retinoic acid (atRA) promotes cell proliferation through activation of PPARβ/δ, but more recent literature has been unable to confirm this role for atRA in PPARβ/δ signaling and carcinogenesis. The viability of this hypothesis was tested in the human NT2/D1 testicular cancer cell line by treating cells with atRA and/or PPARβ/δ agonists, and examining the effect on the retinoic acid receptor (RAR) and PPARβ/δ signaling pathways. atRA decreased the intracellular ratio of retinoic acid-shuttling proteins (FABP5:CRABP-II) by increasing levels of CRABP-II, whereas the synthetic PPARβ/δ agonists GW0742 and GW501516 had no effect on the relative expression of FABP5 to CRABP-II. As expected, atRA activated RAR signaling, but had no effect on PPARβ/δ levels, a known PPARβ/δ target gene, or cell proliferation. Interestingly, ligand activation of PPARβ/δ repressed RAR signaling and cell proliferation. Taken together, these data support the hypothesis that atRA is not a PPARβ/δ ligand and that activation of PPARβ/δ signaling inhibits carcinogenesis in testicular cancer.

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Introduction

Testicular cancer is the most common malignant solid tumor in Caucasian males between the ages of 15-39 (1). Moreover, testicular cancer incidence has been rising for decades, a trend that persists with 8,430 new cases of testicular cancer expected in the United States in 2015 (2). Current chemotherapies against testicular cancer have produced high survival rates for patients (1:5000 risk of death), but post-treatment complications are common, prompting further investigation into alternate therapeutics (2-5). This need is particularly acute for mixed testicular germ cell tumors, which metastasize aggressively and, thus, exacerbate complications associated with chemotherapy. Thus, embryonal carcinoma, as the most common subset of cells found in mixed testicular germ cell tumors, represents a logical starting point for the testing and discovery of novel therapeutics for testicular cancer (6).

Peroxisome proliferator-activated receptors (PPARα, β/δ, and γ) and retinoic acid receptors (RARα, β, and γ) are part of a family of transcription factors called nuclear hormone receptors (NRs), which regulate multiple biological processes including growth, differentiation, and metabolism (7). To alter transcription, NRs recruit obligatory partner retinoid X receptor (RXR) and bind response elements consisting of two direct repeat (DR) DNA motifs separated by one to five nucleotides (DR1-DR5) (8-9). Because there are more than 20 known NRs that compete for RXR, and subsequently, the same 5 response elements, competition known as "cross talk" interferes with NR signaling. For example, a study reported that excess of liver X receptor (LXR) or its ligand inhibited the association of RXRα-PPARα with PPAR response element (PPRE) (9). Endogenous ligands and transcriptional corepressors and coactivators that assemble on the heterodimer upon DNA-binding further complicate the molecular picture (8-9).

Some aspects of PPAR and RAR signal-activation have been elucidated. First, RXR-PPAR dimers activate transcription via binding to DR1 or DR2, while RXR-RAR dimers block transcription upon binding to DR1, DR2, or DR5 (9). Interestingly, assembly of RXR-RAR on DR-5 can be modulated by ligands, which promote transcription upon binding, whereas its assembly on DR-1 is ligand-independent and constitutively represses transcription. Cross-talk between these receptors has also been observed. One study found that transfection of RARα decreased ligand-dependent RXR-PPARα binding to DR1 and subsequent transcriptional activation (7). The same study also reported that RAR binding prevented ligand-dependent recruitment of coactivators to RXR (7). These findings provide context for the study outlined below, which examined the effects of RAR and PPARβ/δ ligands on downstream signaling and cell proliferation.

By regulating growth, differentiation, and metabolism, NRs play a role in diseases like diabetes and cancer (8, 12). Retinoic acid (RA), a known RAR ligand, has been shown to promote differentiation and, consequently, is being tested as a cancer therapeutic (10). Though PPARα and PPARγ are also being evaluated as potential drug candidates for cancer, the role of PPARβ/δ in the disease's progression is controversial due to conflicting studies that reported tumorigenic and anti-tumorigenic effects of the receptor in multiple cancer-types, including colon, breast, skin and lung cancers (11-14). Contradictory data have even been reported in the same cancer-type. For example, knockdown of PPARβ/δ inhibited proliferation of ER+ and ER- human breast cancer cells in vitro in one instance (15), whereas another report demonstrated suppression of tumor growth in ER+ and ER- human breast cancer cells overexpressing PPARβ/δ (16). A third study suggested that ligand activation of PPARβ/δ only promoted growth of ER+ cells, further complicating the role of the receptor in breast cancer (17). Due to these

conflicting results, researchers have called for collaboration among labs and comprehensive analyses of the PPARβ/δ signaling pathway that use quantitative methods and correlate RNA levels with protein expression (13-14).

One hypothesis attributed the opposing effects described for PPARβ/δ in cancer to the differential shuttling of ligands to the receptor. Specifically, it has been reported multiple times that RAR agonist all-trans retinoic acid (atRA) is also a PPARβ/δ agonist (18-22). Furthermore, these studies reported that atRA is delivered to PPARβ/δ in the presence of a high intracellular ratio of fatty acid-binding protein 5 (FABP5) to cellular retinoic acid-binding protein II (CRABP-II), and that PPARβ/δ promotes cell proliferation in an atRA-dependent manner given this high relative ratio (19-20). Importantly, intracellular expression levels of FABP5 and CRABP-II have never been quantitatively examined to date (14).

Yet, more recent experiments have failed to support these conclusions (10, 15, 23-24). The first such report concluded that atRA is not a ligand for PPARβ/δ. It was shown that atRA had no effect on PPARβ/δ transcriptional activity, did not activate its established target genes, *ADRP* and *ANGPTL4*, and did not compete with PPARβ/δ synthetic agonists for the ligand-binding domain (LBD) (10). A second study demonstrated that HaCaT keratinocytes had a high FABP5:CRABP-II ratio, but found that atRA did not activate PPARβ/δ target genes *PDK1* and *ANGPTL4*. Additionally, ligand activation of PPARβ/δ inhibited HaCaT keratinocyte proliferation, contradicting the notion that PPARβ/δ acts as a proliferator in cells with the described high ratio of FABP5:CRAPBII (23). The third study reported anti-tumorigenic effects of the overexpression and ligand activation of PPARβ/δ in human breast cancer cells, but found that the demonstrated effects were independent of the FABP5:CRABP-II ratio (15). Finally, a

recent report demonstrated that atRA activity was not mediated by the overexpressed PPAR in the NT2/D1 testicular embryonal cancer cells, a model used in the current study, despite a high FABP5:CRABP-II ratio. Moreover, hPPARβ/δ overexpression decreased cell proliferation, migration, and invasion of NT2/D1 cells, reduced tumor mass and volume of NT2/D1 xenografts, and competed with RXR/RAR-dependent signaling (24).

Though the role of PPARβ/δ in cancer remains unclear, these studies contradict the aforementioned hypothesis that atRA is a PPARβ/δ ligand and is pro-tumorigenic in cells exhibiting a high relative ratio of FABP5:CRABP-II. The present study focuses on (1) characterizing the relationship between atRA, the ratio of FABP5- and CRABP-II-shuttling proteins, and the activity of nuclear receptors PPARβ/δ and RARα, and (2) testing the hypothesis that PPARβ/δ exhibits anti-tumorigenic activity, independent of the ratio of shuttling proteins. To examine this notion, human NT2/D1 testicular embryonal cancer cells were treated with atRA or synthetic PPARβ/δ agonists and the effects were evaluated by analyzing the expression of relevant protein, target gene expression, and cell proliferation, all compared to controls.

Materials and Methods

Cell Culture

The human testicular embryonal carcinoma cell line, NTERA-2 cl. D1 (NT2/D1), was purchased from American Type Culture Collection (CRL-1973; ATCC, Manassas, VA) and cultured in Dulbecco's Minimal Essential Medium (DMEM) 1x (30-2002; ATCC) with 10% fetal bovine serum (26140-079; GIBCO-Invitrogen) and 1% penicillin-streptomycin (15140-122; GIBCO-Invitrogen). The cell line was incubated at 37°C and 5% carbon dioxide and the media changed every 2-3 days.

Treatment

Multiple treatments were applied to the NT2/D1 cell line and treatment response measured with western blotting and quantitative real-time PCR. atRA was purchased from Sigma-Aldrich (St. Louis, MO). Cells were treated with 0, 1, or 10 μM atRA for 0-48 h. PPARβ/δ agonists GW0742 and GW501516 were purchased from Glaxosmith-Kline (Research Triangle Park, NC) and Sigma-Aldrich (St. Louis, MO), respectively. Cells were treated with 1 μM GW0742 or 1 μM GW501516 for 24 h. Response was also measured using a cell proliferation assay. Cells were treated with 10 μM atRA, 1 μM GW0742, or 1μM GW501516 for 72 h, or co-treated with atRA and GW0742 or GW501516.

Western blotting

Whole-cell protein extracts were prepared using a homogenization solution containing radioimmunoprecipitation assay (RIPA) buffer (50 mM Tris-HCl, 500 mM NaCl, 1% NP-40, 0.5% Sodium deoxycholate, 0.1% SDS, and ddH_2O, pH 7.5) and protease inhibitors (Roche, Indianapolis, IN). Extract concentrations were determined using the BCA Protein Assay kit

(Thermo Scientific, Rockford, IL) and a spectrophotometer. Twenty micrograms of protein per sample was resolved using SDS-PAGE (10 or 15%), depending on the size of the protein of interest, and applied using the Bio-Rad (Hercules, CA) electroblotting technique. Membranes were blocked with a solution of 5% dried milk in Tris-buffered saline with Tween-20 (TBST) for 30 minutes, washed three times for 10 minutes with TBST, and incubated with primary antibody overnight at 4°C. Primary antibodies were applied against RARα (1:200; Santa Cruz Biotechnologies), RXRα (1:200; Santa Cruz Biotechnologies), PPARβ/δ (1:1000; Abcam, Cambridge, MA), ACTIN (1:200; Santa Cruz Biotechnologies), FABP5 (1:1000; Biovendor, Asheville, NC), and CRABP-II (1:1000; Abcam). Membranes were then washed three times for 10 minutes with TBST, incubated in biotin-conjugated secondary antibody for 1-2 hours at room temperature, washed three times for 10 minutes with TBST, and incubated in ^{125}I-streptavaidin for 15-30 minutes. The signal was detected using filmless autoradiographic analysis and quantified with Optiquant 4.0 (Packard Instrument Co., Meriden, CT).

Quantitative real-time polymerase chain reaction

mRNA expression was measured via quantitative real-time polymerase chain reaction (qPCR). Total mRNA was extracted with RiboZol RNA Extraction Reagent (AMRESCO, Solon, OH) per the manufacturer's protocol. cDNA was generated with Multi-Scribe Reverse Transcriptase (Applied Biosystems, Foster City, CA) and three micrograms per sample were prepared in SYBR Green PCR Supermix (Quanta Biosciences, Gaithersburg, MD). Relative mRNA expression levels of glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*), cytochrome P450- 26A (*CYP26A1*), and angiopoietin-like protein 4 (*ANGPTL4*) were measured using the

qPCR program—95°C for 10s, 60°C for 30s, 72°C for 30s—repeated 45 times. *CYP26A1* and *ANGPTL4* levels were normalized to *GAPDH* levels.

Cell proliferation assay

Cell proliferation was monitored in real time using the xCELLigence system (Roche, Indianapolis, IN). 2000 cells were plated and treatments (Control, atRA 10 μM, 1 μM agonist, and combinatorial) applied in replicates of 4 twenty-four hours after plating. Adherent cell growth was measured by electrical impedance every 15 minutes from treatment (t=0) for 72 hours with the RTCES System (ACEA Biosciences, San Diego, CA). Measurements were expressed by the arbitrary unit "Cell Index," defined as (Rn-Rb)/15 where Rn is the impedance of wells containing cells and media and Rb is the impedance of wells containing only media.

Data analyses

Statistical significance was determined using one-way analysis of variance (ANOVA) and the Bonferroni's multiple comparison tests or Student's t-test as noted in figure legends. Significant difference was assessed when $p<0.05$. Tests were performed by Prism 6.0 (GraphPad Software Inc., La Jolla, CA).

Results

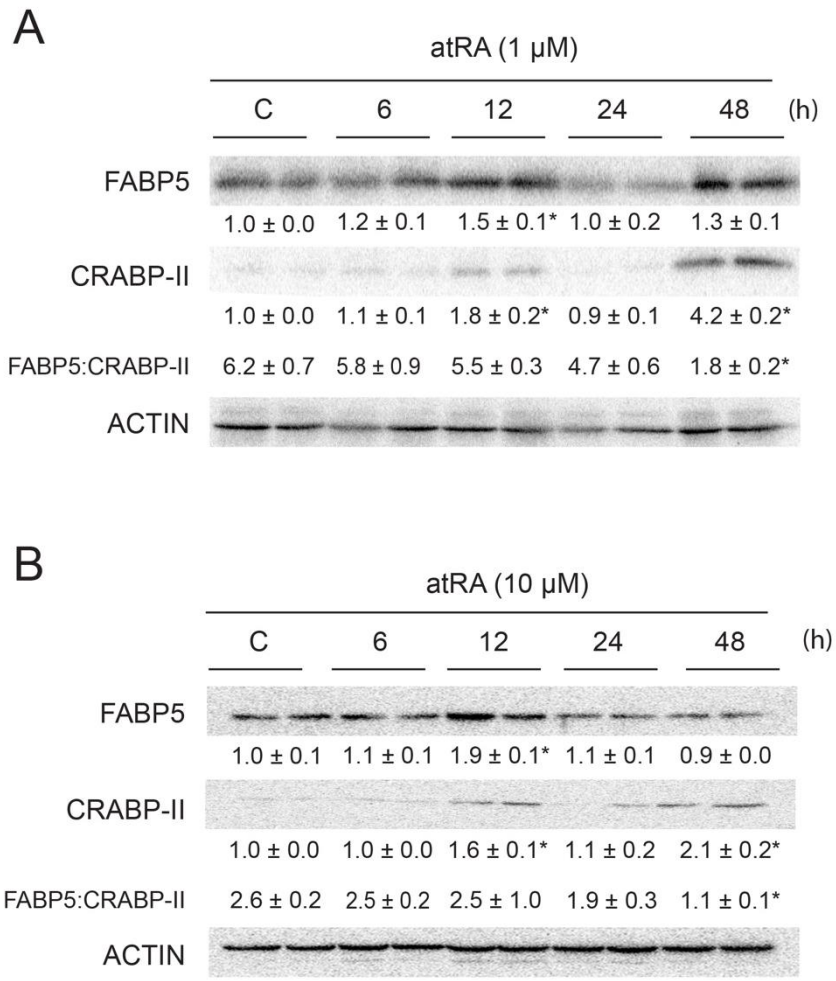

Figure 1. atRA decreases the relative expression of FABP5 to CRABP-II through induction of CRABP-II. NT2/D1 cells were treated with 1 μM atRA (A) or 10 μM atRA (B) for 0-48 hours and whole cell extracts prepared. 20 μg of protein was resolved in a 15% SDS-polyacrylamide gel and transferred to a membrane by electroblotting. The membranes were blocked with 5% milk, incubated in a primary antibody overnight at 4°C, and incubated in biotinylated secondary antibody at room temperature for one hour. Protein was labeled with ^{125}I-streptavaidin and the signal detected via autoradiographic analysis. Protein levels were quantified

using Optiquant 4.0 software and normalized to ACTIN. “C” represents the control treatment and values are presented as the mean ± standard error of the mean (SEM). Significant difference from the control was measured using Student’s t-test, indicated with an asterisk.

Western blot analysis was used to measure the temporal change in expression of a putative retinoic acid- and PPARβ/δ agonist-shuttling protein, FABP5, and an established retinoic acid-shuttling protein, CRABP-II, in response to 1 μM and 10 μM atRA. Similar changes were observed at both concentrations with significantly higher levels of CRABP-II observed at 48 hours (Figure 1). Importantly, CRABP-II induction at 48 hours also caused a significant decrease in the ratio of FABP5 to CRABP-II at this time point (Figure 1). Although significant differences were observed for both proteins at 12 hours, the relative increase was similar and, therefore, resulted in no difference in relative expression from the control (Figure 1). 24 h samples should be disregarded due to issues with whole cell extract preparation.

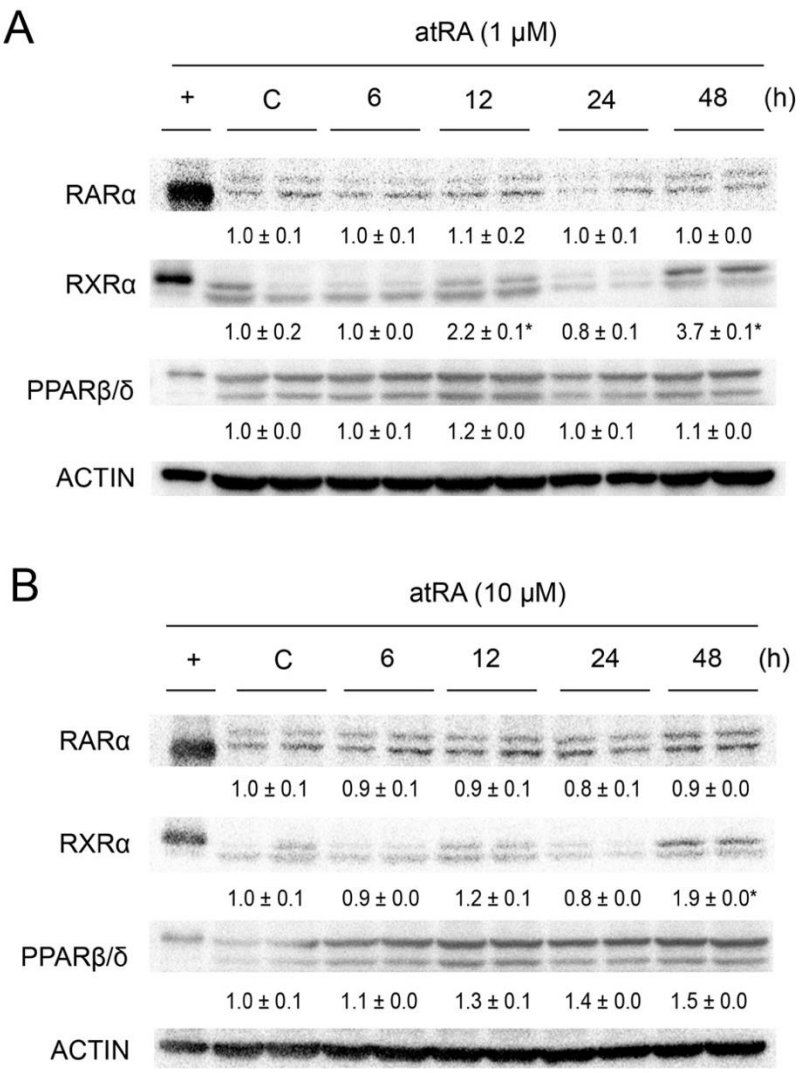

Figure 2. The effect of atRA on temporal expression of target receptor proteins. NT2/D1 cells were treated with 1 μM or 10 μM atRA for 0-48 hours and whole cell extracts prepared. 20 μg of protein was resolved in a 10% SDS-polyacrylamide gel and transferred to a membrane by electroblotting. The membranes were blocked with 5% milk, incubated with a primary antibody overnight at 4°C, and incubated in biotinylated secondary antibody at room temperature for two hours. Protein was labeled with ^{125}I-streptavaidin and the signal detected via autoradiographic analysis. Protein levels were quantified using Optiquant 4.0 software and normalized to ACTIN.

"C" represents the control treatment and values are presented as the mean ± S.E.M, N=2. Multiple controls were pooled for statistical analysis and significant difference from the control was measured via one-way analysis of variance (ANOVA) and the Bonferroni's multiple comparison tests, indicated with an asterisk.

Western blot analysis was used to analyze the effect of atRA on protein expression of its known target receptor, RAR, a putative target receptor, PPARβ/δ, and both receptors' heterodimer, RXR. Significant increases in RXR protein were observed at 48 hours at both 1 μM and 10 μM concentrations when compared to the untreated control, with the change beginning at 12 h. atRA had no significant effect on RAR or PPARβ/δ protein levels (Figure 2). Though an increasing PPARβ/δ trend for 10 μM atRA treatment was observed, it should be disregarded as an incomplete transfer for the control side of the blot is evident (Figure 2B). 24-hour samples should also be disregarded due to issues with whole cell extract preparation.

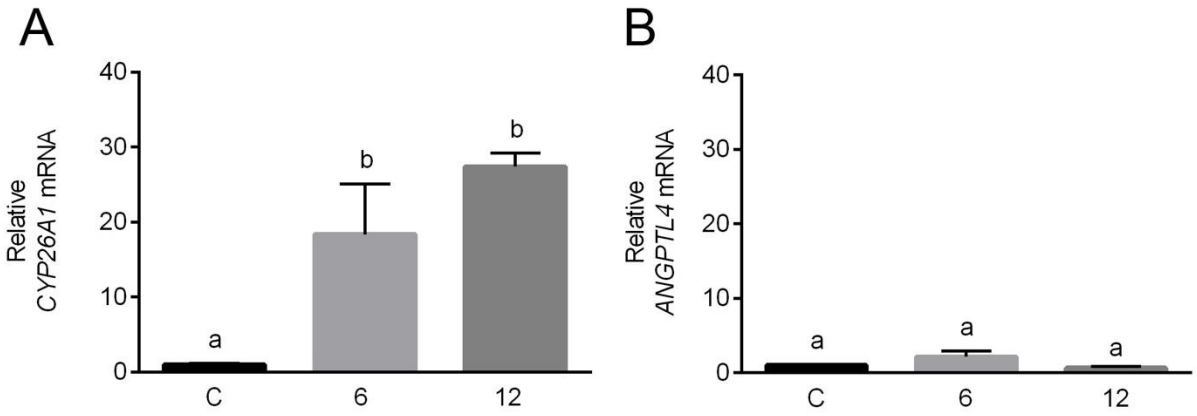

Figure 3. The effects of atRA on RAR and PPARβ/δ target genes. NT2/D1 cells were treated with 10 μM atRA for 0-12 hours and harvested for mRNA extraction. cDNA was reverse transcribed from 3 μg of mRNA and expression levels measured for *CYP26A1* (A), a known

RAR target gene, and *ANGPTL4* (B), a known PPARβ/δ target gene, by qPCR. Ct values were then normalized to the internal control *GAPDH*. "C" represents the control treatment and levels are presented as the mean ± S.E.M., N=4. Statistical significance was assessed via ANOVA and the Bonferroni's multiple comparison tests. Significant difference between two means is indicated by different letters.

atRA induced significant increases in *CYP26A1* expression after 6 hours (~20 fold), indicating activation of the RAR signaling pathway (Figure 3A). After 12 hours, atRA had no effect on *ANGPTL4* expression when compared to untreated cells, indicating that the PPARβ/δ signaling pathway was not induced by atRA (Figure 3B).

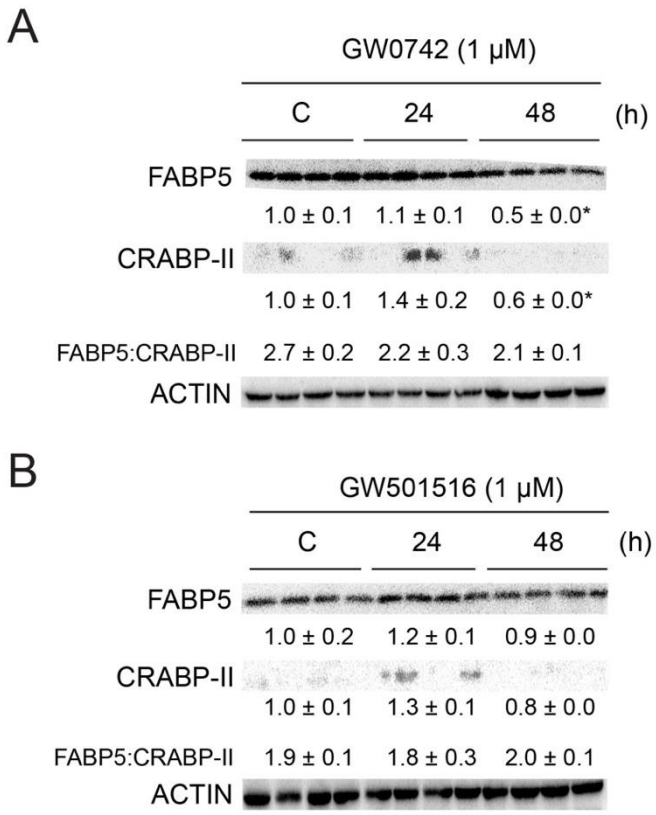

Figure 4. Ligand activation of PPARβ/δ has no effect on the relative expression of putative PPARβ/δ agonist-shuttling proteins. NT2/D1 cells were treated with 1 μM GW0742 or 1 μM GW501516 for 0, 24, or 48 hours and whole cell extracts prepared. 20 μg of protein was resolved in a 15% SDS-polyacrylamide gel and transferred to a membrane by electroblotting. The membranes were blocked with 5% milk, incubated in a primary antibody overnight at 4°C, and incubated in biotinylated secondary antibody at room temperature for one hour. Protein was labeled with ^{125}I-streptavaidin and the signal detected via autoradiographic analysis. Protein levels were quantified using Optiquant 4.0 software and normalized to ACTIN. "C" represents the control treatment. Values are presented as the mean ± S.E.M, N=4 and significant difference from the control measured using the Student's t-test, indicated with an asterisk (*).

Western blot analysis was used to analyze the effects of two PPARβ/δ synthetic agonists, GW0742 and GW501516, on the levels of retinoic acid-shuttling proteins FABP5 and CRABP-II (Figure 4). FABP5 and CRABP-II decreased significantly from the control after 48 hours of treatment with GW0742. The parallel decrease in the proteins resulted in no significant change in the relative expression of the two proteins, however, compared to the relative expression of the two proteins in untreated cells (Figure 4A). GW501516 had no effect on FABP5 or CRABP-II levels, or the relative expression of the two proteins (Figure 4B)

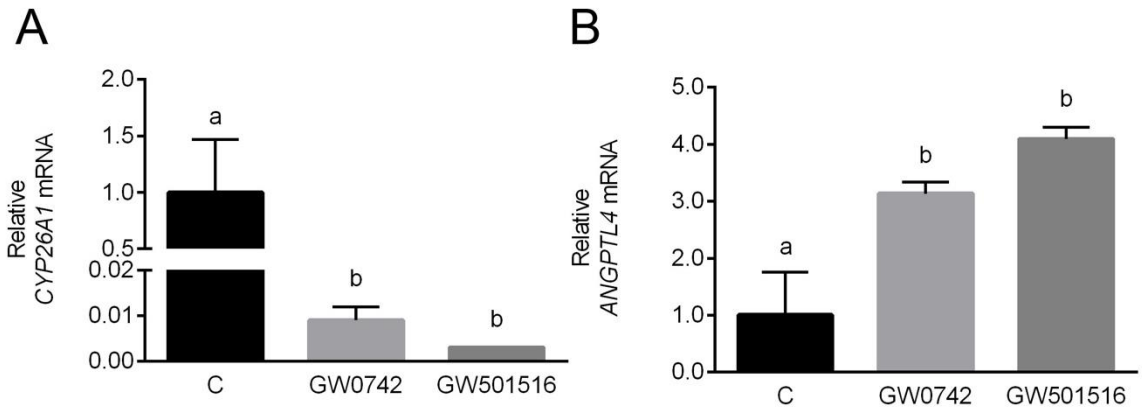

Figure 5. The effect of PPARβ/δ agonists on RAR and PPARβ/δ target gene expression. NT2/D1 cells were treated with 1μM GW0742 or 1 μM GW591516 for 24 hours and harvested for mRNA extraction. cDNA was reverse transcribed from 3 μg of mRNA and expression levels measured for *CYP26A1* (A), a known RAR target gene, and *ANGPTL4* (B), a known PPARβ/δ target gene, by qPCR. Ct values were then normalized to the internal control *GAPDH*. "C" represents the control treatment and levels presented as the mean ± S.E.M., N=4. Statistical significance was assessed via ANOVA and the Bonferroni's multiple comparison tests. Significant difference between two means is indicated by different letters.

qPCR was used to examine if synthetic PPARβ/δ agonists, GW0742 and GW501516, activated the RAR signaling pathway by measuring mRNA levels of known RAR-target gene *CYP26A1*. Interestingly, GW0742 inhibited target gene expression 100-fold and GW501516 inhibited expression ~400-fold, suggesting downregulation of the RAR signaling cascade (Figure 5A). mRNA expression of PPARβ/δ target gene *ANGPTL4* was also examined to confirm activation of PPARβ/δ signaling. As expected, the synthetic agonists significantly increased expression of the target gene, verifying that the agonists were active in the cells (Figure 5B).

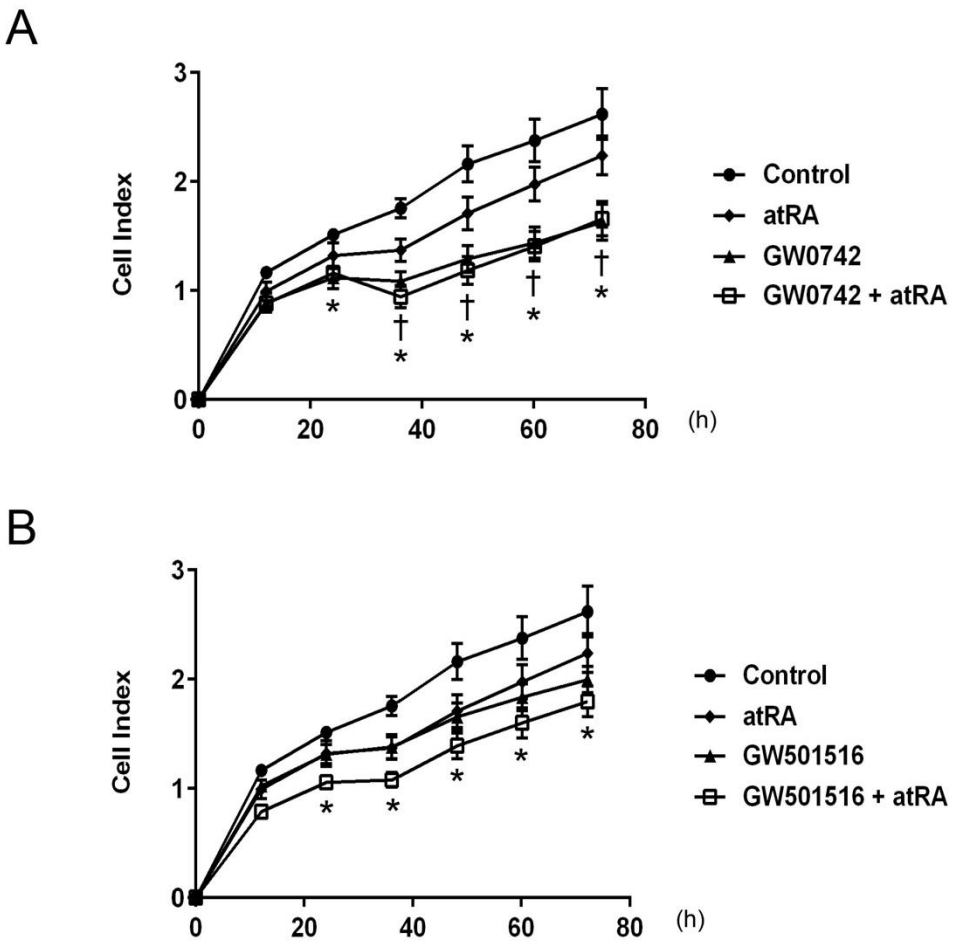

Figure 6. Ligand activation of PPARβ/δ inhibits NT2/D1 cell proliferation. 2000 cells were plated per well for 24 hours and then treated with 10 μM atRA, 1 μM GW0742, or 1 μM GW501516, or co-treated with atRA and GW0742 or GW501516 in replicates of 4. Proliferation was monitored for 72 hours after treatment using the xCELLigence system. Values are presented as the mean ± S.E.M, N=4. Significant difference from the control is indicated with a cross (†) for GW0742 (A) and with an asterisk (*) for GW0742+atRA (A) and GW501516+atRA (B).

atRA did not significantly affect proliferation of NT2D1 cells over 72 hours, but an inhibitory trend was observed (Figure 6A, B). Treatment with PPARβ/δ agonist GW0742 inhibited cell proliferation after 24 hours. GW0742 co-treated with atRA also significantly decreased cell proliferation. As the growth curve of GW0742 treatment and that of co-treatment with atRA run in parallel, inhibition of cell proliferation can be attributed to GW0742 (Figure 6A). An inhibitory trend was also observed with GW501516 treatment, but the difference from the control was not significant. The inhibitory trends of GW501516 and atRA combined to significantly decrease cell proliferation.

Discussion

Classically, all-trans retinoic acid (atRA) is shuttled to RAR by CRABP-II and activates transcription of its downstream target genes (10). Given a high intracellular ratio of FABP5 to CRABP-II, however, atRA was putatively described as a PPARβ/δ agonist (18-22). Thus, atRA was applied to human NT2/D1 testicular embryonal cancer cells to characterize its effect on the proposed signaling system, defined as the proteins involved in ligand-shuttling to RAR and PPARβ/δ and their downstream targets.

In the absence of atRA, a high relative ratio of FABP5 to CRABP-II was observed. The precise ratio was not consistent among controls, but a high relative ratio was detected in all samples and the system thus treated as one which fit the parameters for the described hypothesis. Interestingly, atRA increased the expression of CRABP-II, thereby reducing the FABP5:CRABP-II ratio. This increase is not surprising as CRABP-II serves as a shuttle for atRA, likely furthering delivery of excess atRA to its target receptors. atRA also had no effect on the levels of known target receptor, RARα, and putative target receptor, PPARβ/δ, but increased RXRα protein. Given reports that RXR is limiting in certain cell lines (9), this effect is plausible, as an increase in RXR favors the formation of RXR heterodimers to which atRA can bind. Logically, it was shown that atRA promoted the expression of proteins that participate in its signaling cascade.

Given the high relative ratio of FABP5:CRABP-II, the effect of atRA on PPARβ/δ signaling was examined. Even at higher concentrations (10 μM), atRA did not stimulate mRNA expression of the PPARβ/δ target gene *ANGPTL4.* Unsurprisingly, atRA promoted the expression of *bona fide* RAR target *CYP26A1*. These results confirm the established RAR-

dependent activity of atRA, but lend no support to the notion that atRA can be differentially shuttled to PPARβ/δ by FABP5. The same analyses were then performed using synthetic PPARβ/δ ligands GW0742 and GW501516 to differentiate the activity of atRA in NT2/D1 cells from that observed upon activation of PPARβ/δ. As expected, ligand activation of PPARβ/δ enhanced mRNA expression of *ANGPTL4*, confirming the activation of PPARβ/δ-dependent signaling. Interestingly, the agonists also attenuated expression of *CYP26A1*. This result supports recent data which showed that PPARβ/δ interferes with RAR-dependent transcriptional activity (24). Finally, treatment with PPARβ/δ ligands had no effect on the ratio of FABP5 to CRABP-II. This result offers no further insight, but suggests that the aforementioned effects were not dependent on upstream changes in available protein to deliver PPARβ/δ ligands to target receptors. Taken together, these data suggest opposing activities for atRA and PPARβ/δ ligands, despite the high relative ratio of FABP5 to CRABP-II, further contradicting the hypothesis that this ratio switches atRA from an RAR- to a PPARβ/δ-activator.

Attempts to elucidate the function of PPARβ/δ in cancer have produced diverging results (11-14). However, multiple recent studies using quantitative methods have demonstrated an anti-tumorigenic effect in non-melanoma skin, breast, and testicular embryonal cancers *in vitro* and *in vivo* (14-15, 24-25). Ligand activation of PPARβ/δ with GW0742 attenuated cell proliferation and an inhibitory trend was observed with GW501516, supporting these results. Though atRA was reported as a proliferator through modulation of PPPARβ/δ (19), no effect was shown here. Interestingly, the small inhibitory trend produced by atRA, when added to that produced by GW501516, decreased proliferation significantly. This trend falls in line with reported RAR-

dependent activities of atRA, which include promoting differentiation, cell-cycle arrest, and apoptosis (19).

Given a high relative FABP5:CRABP-II ratio, there is no evidence that atRA is a PPARβ/δ ligand or that it promotes cell proliferation in a PPARβ/δ-dependent manner in testicular embryonal cancer cells, consistent with multiple studies which reexamined this hypothesis (10, 15, 23-25). Moreover, ligand activation of PPARβ/δ opposed the downstream effects of atRA and decreased cell proliferation, supporting a recent study in the same cell line (24). Alas, the present study did not confirm all observed effects at both the RNA and protein level, a shortcoming that should be addressed in future studies.

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24. Yao P, Chen LP, Dobrzanski TP, Phillips DA, Zhu B, Kang B-H, Gonzalez FJ, Peters JM. Inhibition of testicular embryonal carcinoma cell tumorigenicity by peroxisome proliferator-activated receptor-β/δ- and retinoic acid receptor-dependent mechanisms. Oncotarget. 2015 Sep 26 [Epub ahead of print].
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TOMASZ (TOMEK) DOBRZANSKI

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EDUCATION

The Pennsylvania State University — **University Park, PA**
The Eberly College of Science — *Anticipated December 2015*
The Schreyer Honors College

- Bachelor of Science in Biology with honors

ACADEMIC AWARDS AND ACHIEVEMENTS

The Pennsylvania State University — **University Park, PA**
Dean's List Honors — *Fall 2011- Present*

- Awarded to all students who attain a GPA of 3.5 or higher

Pennsylvania State University Academic Excellence Scholarship — *Fall 2011-Present*

- Awarded to Schreyer Honors College students for their dedication to academics

United States Collegiate Ski and Snowboard Association (USCSA) Academic All-American Nominee — *2012-2015*

- Awarded to athletes who attain a 3.3 GPA or higher and qualify for national events

RELEVANT COURSES AND EXPERIENCE

Bryn Mawr Rehab Hospital — **West Chester, PA**
Volunteer — *Summer 2010*

- Delivered patients to activities, check-ups, and treatments

University of Pennsylvania Memory Center/The Veteran's Affairs Medical Center — **Philadelphia, PA**
Intern — *April 2011*

- Shadowed Dr. Mitchel Kling observing mental health tests and meetings to discuss treatment options

Teva Pharmaceuticals — **West Chester, PA**
Intern — *Summer 2013*

- Generated multiple xenograft mouse models of cancer from cell propagation to injection
- Administered drugs, performed pharmacodynamic (PD) analyses, and extracted tumors
- Performed lung perfusions for quantitative analysis of lung metastasis

Dr. Jeffrey Peters Research Group — **University Park, PA**
Undergraduate Researcher — *June 2014-Present*

- Protein and RNA analysis of PPARβ/δ biological pathway in the NT2D1 testicular cancer cell line

Pennsylvania Literacy Corps Internship — **University Park, PA**
ESL Tutor — *Fall 2014*

- Worked with an adult learner from Rwanda to improve proficiency in the English language

Tussey Mountain Alpine Racing Team — **Boalsburg, PA**
Assistant Coach — *Winter 2014-2015*

- Instructed 6-18 year olds in junior alpine ski racing

Mt. Nittany Medical Center — **State College, PA**
Emergency Department Volunteer — *Sept. 2015-Present*

- Delivered patients to cat scans, ultrasounds, x-rays, and MRIs; assisted nurses in immediate patient care

LEADERSHIP

Alpha Sigma Phi **University Park, PA**
Executive Board *Jan. 2013- May 2014*

- Alumni Relations Director: Designed annual alumni newsletter, maintained website, planned alumni events
- Recruitment Director: Promoted fraternity at involvement fairs, reached out to potential members, coordinated multiple events
- New Member Education Director: Organized brotherhood events to acquaint new members, rituals, and chapter meetings
- House Manager: Managed vendors, placed weekly supply orders, maintained the fraternity residence up to required standards

Penn State Ski Team **University Park, PA**
Men's Team Captain *2011, 2013*
Vice President *2014-2015*

SKILLS

- Conversational Polish

PUBLICATIONS

1. Yao P, Chen LP, **Dobrzanski TP**, Phillips DA, Zhu B, Kang B, Gonzalez FJ, & Peters JM. (2015, September). Inhibition of testicular embryonal carcinoma cell tumorigenicity by peroxisome proliferator-activated receptor-β/δ- and retinoic acid receptor-dependent mechanisms. *Oncotarget, Advance Publications 2015* [Epub ahead of print].

2. Zhao H, Jan M, Camp F, Gotchev D, **Dobrzanski T**, Ruggeri B, Hudkins R, Dobrzanski P. Antitumor and antimetastatic efficacy of the new class of FASN inhibitors (in preparation)