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Modulation of PPAR-Gamma Signaling in Prostatic Carcinogenesis

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<b>13. SUPPLEMENTARY NOTES</b>						
<b>14. ABSTRACT</b> The long term objective of this work is to elucidate metabolic pathways which can be used to reduce the need for radical surgery in patients at high risk for prostate cancer or with early stage disease. The hypothesis to be tested is that alterations to lipoxygenase (LOX) and cyclooxygenase (COX) activity in early prostate cancer represent distinct druggable pathways which can be treated in conjunction with the PPAR $\gamma$ signaling pathway to slow or prevent the development and progression of prostate cancer. In the third year of funding, we have generated and examined most of the proposed tissue recombinants. We have completed the majority of the experiments proposed. In the last year three papers have been published and as well as two comment pieces which resulted from interest in the primary publications. The second year of work demonstrated that the combination of PPAR $\gamma$ loss with other common genetic insults can cause progression of a PIN phenotype, further studies in this third year have demonstrated that PPAR $\gamma$ loss in human epithelial cells results in phenotypic changes including urothelial differentiation. After long term culture PPAR $\gamma$ KO mouse epithelial cells move from a PIN to an inflammatory malignant phenotype in tissue recombinants supporting our contention that agonizing the PPAR $\gamma$ pathways might be a chemopreventive strategy.						
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Annual Report

PCRP Idea Development Award

W81XWH-07-1-0479

Modulation of PPAR-Gamma Signaling in Prostatic Carcinogenesis

**P.I. Simon W. Hayward, PhD**

### **Introduction**

This project examines the relationship between PPAR $\gamma$  and carcinogenesis. PPAR $\gamma$  sits at a critical juncture in cellular differentiation and metabolism being involved in both differentiation and in the regulation of stress responses mediated through the cyclooxygenase (COX) and lipoxygenase (LOX) pathways of fatty acid metabolism. The basis for this project was the observation that in human prostate cancer there is an early loss of enzymes responsible for the production of the putative endogenous ligands for PPAR $\gamma$ , presumed to result in a decrease in receptor function. We have found that loss of PPAR $\gamma$  function can result in the generation of premalignant prostatic lesions in mice (Jiang et al 2010). We have also shown that there is an associated upregulation of COX pathways which would generate increases in prostaglandin production and oxidative stress, which could underlie such pathology. This project sets out to examine interactions between the PPAR $\gamma$ , COX and LOX pathways and their role in carcinogenesis. We are using predominantly tissue recombination models involving human prostatic epithelial cells. The use of human cells is important in that there are significant differences between the fatty acid metabolic pathways between humans and mice. However we have also generated mouse epithelial cell lines from the transgenic animals and as a result have been able to use their accelerated aging and metabolism as compared to human cells to illustrate malignant transformation in a recombination model. These data provide a strong basis for future studies

## **Body**

### **Status of progress in relation to the original SOW**

*Task 1. Examine the in vivo consequences of suppression of PPAR $\gamma$  signaling in human prostatic epithelium.*

This task is essentially completed, as described in the previous report. The development of new human cell lines opened up the possibility to more thoroughly explore the role of specific genetic targeting of the epithelium than previously possible. As a result we have additionally investigated the effects of loss of PTEN in conjunction with loss of PPAR $\gamma$  in human and mouse prostatic epithelial cells. These studies are currently being written up for publication.

*Task 2. Examine the in vitro and in vivo consequences of overexpression of cyclooxygenase -1 or -2 or 15-lipoxygenase-1 in human prostatic epithelium.*

These studies were modified slightly, as noted in the previous annual report by moving to our new human cell lines, which provide a better model system than those originally proposed. The studies are now completed. The data are undergoing final analysis and a manuscript is in preparation.

*Task 3. Examine protective effects of PPAR $\gamma$  agonists and/or COX/LOX inhibitors against the neogenesis of PIN or progression of prostate cancer.*

This task, which was furthest from completion last year, is broken out in more detail. The work was modified to base experiments on the NHPrE1 and BHPrE1 lines. These are superior models to the original proposal. NHPrE1-GFP- and BHPrE1-GFP-PPAR $\gamma$ 1 or - $\gamma$ 2 shRNA and controls (NHPrE1- or BHPrE1-GFP-pSIREN) have been used to generate recombinants and grafted to SCID mouse hosts. Hosts divided into groups and fed for 7 days prior to grafting, and then throughout experiment on control chow and Avandia chow. These experiments are ongoing, and are due to be harvested through December 2010 and January 2011 for analysis and completion of a manuscript.

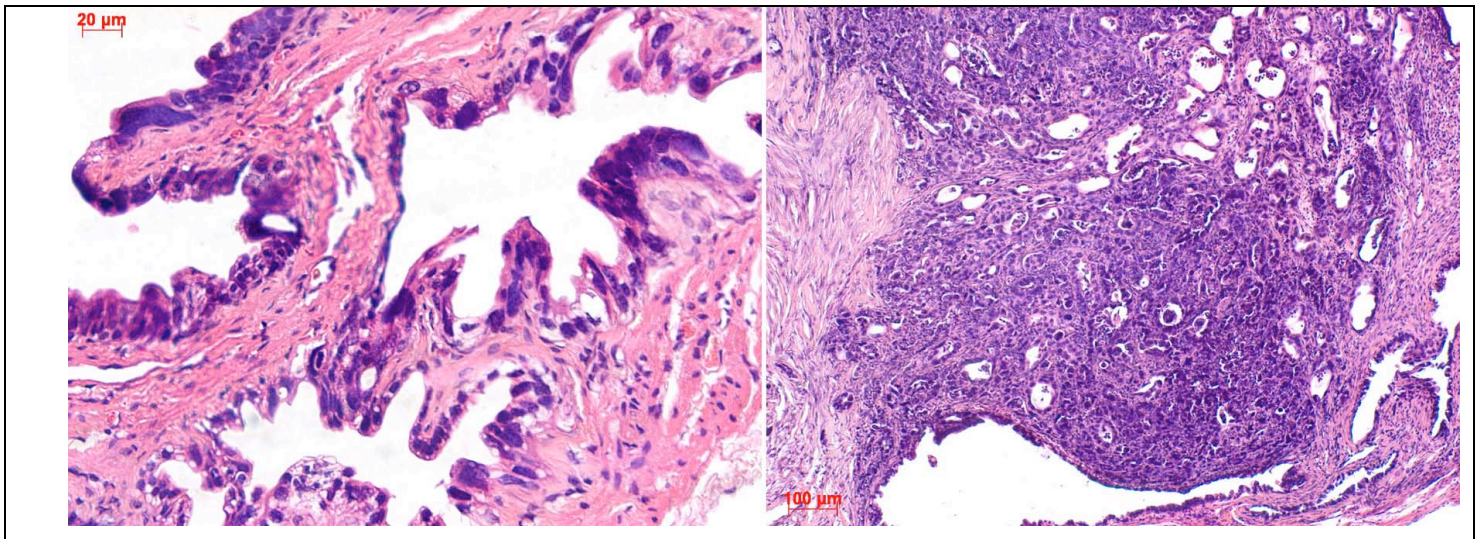
The human prostate tumor tissue xenograft experiments are completed and analysis of the resultant data is ongoing. Tissue samples derived Gleason grade 2+3 or 3+3 tumors were grafted to sets of SCID mice fed for 7 days prior to grafting, and then throughout experiment on control or modified chow. Data analysis are nearly complete and these data will be integrated with the cell line studies for publication.

## Summary of Activity

As listed in the reportable items section below, this period resulted in the publication of papers describing the early production of the project. The principle observations of these were summarized in the second annual report and will not be repeated here.

We have continued to pursue the project as outlined in the statement of work, with some minor modifications as noted above. The project was taken into a no cost extension for one year to allow for completion of outstanding work and to retain funds to attend the forthcoming IMPACT meeting as required under this mechanism.

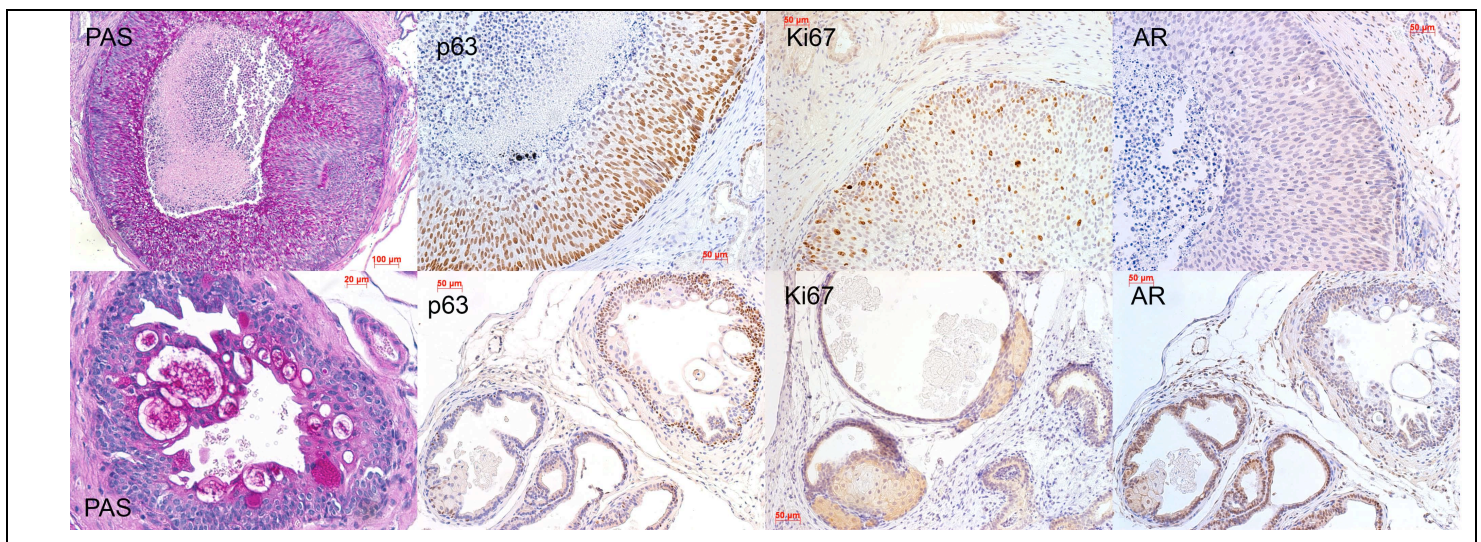
Studies using conditional knockout mouse prostatic epithelium and cell lines derived from this mouse showed that loss of PPAR $\gamma$  gave rise to a PIN phenotype with an extensive inflammatory infiltrate (per Jiang et al 2010 – see reportable outcomes section). However we recently determined that using the derivative cell lines in the same model after extended passage resulted in a wider variety of phenotypes.



Tissue recombinants generated using PPAR $\gamma$ -knockout mouse prostatic epithelium plus rat UGM after two months. Initial studies using low passage epithelial cells resulted in prostatic intraepithelial neoplasia. However in this experiment cells which had been passaged continuously for 6 months were utilized. The resultant grafts showed areas of PIN with occasional giant cells (left panel) there were also widespread malignant lesions and generalized inflammation with many inflammatory cells seen in the malignant lesions.

Specifically we see the widespread emergence of PIN lesions extensive inflammatory infiltrate, which is currently being characterized and large foci of invasive adenocarcinoma. These data suggest that prolonged suppression of PPAR $\gamma$  in these cells leads to the accumulation of further genetic or epigenetic damage that predisposes the cells to malignant transformation. This was one of the initial predicates of the proposal and it is good to see it validated, as this further underlines the expressed possibility of applying PPAR $\gamma$  agonists as chemopreventive agents. These findings also underline the importance of this pathway in regulating inflammatory responses, which is significant in both prostate cancer and benign prostatic disease, as well as in other malignancies.

The effects of PPAR $\gamma$  loss on human epithelial cells have proved to be more complex. We have seen that suppression of PPAR $\gamma$  can give rise to both PIN phenotypes and also transitional phenotypes as shown below.



Effects of deleting total PPAR $\gamma$  (top row) or PPAR $\gamma$ 2 specifically (bottom row) on the morphology of BHP $\alpha$ E1 cells in tissue recombinants with rat UGM.

At 2 months post-xenografting, PPAR $\gamma$ 1/2 deficiency induces transitional cell carcinoma while PPAR $\gamma$ 2 deficiency alone caused urothelial transdifferentiation with associated with glycoprotein accumulation (PAS). Note the difference in p63 pattern between AR-positive prostate glands vs AR-negative urothelium. Urothelium is uroplakin-positive (not shown).

These findings suggest that PPAR $\gamma$  may be playing a role in cellular commitment to adult epithelial lineages. It is well established that prostate, rectal and bladder epithelial morphologies are

interchangeable based upon the cellular microenvironment. The conditions used in these experiments should favor prostatic differentiation however we see that total loss of PPAR $\gamma$  in these cells results in a much more transitional phenotype.

As noted in the previous report we have discontinued study of the tet-inducible PTEN suppression. However the PTEN axis remains of interest, especially in light of the observations on human epithelial cells noted above. PTEN plays a critical role in glucose metabolism regulating aspects of cellular proliferation and differentiation which complement the action of PPAR $\gamma$  (itself acting on the cellular redox balance based upon lipid metabolism. ) Thus studies to examine the complementarity of these pathways would seem indicated. This is especially true since combined PPAR $\gamma$  loss and PTEN suppression in mouse prostatic epithelium gives rise to even more invasive adenocarcinoma than loss of PPAR $\gamma$  alone (not shown). Complementary studies using human cells are also being performed in the laboratory as a part of another project, but should complement the data acquired in this study.

Observations in xenografted human tumors are that Avandia, at least over the relatively short time tested, resulted in increases in cellular differentiation, with associated increased PSA expression (at least based upon immunohistochemical analysis.) These observations are currently being fully analyzed. One of the implications of this is, as we have previously noted, that PPAR $\gamma$  treatment might actually suppress proliferation while increasing patient PSA, an issue which would need to be carefully monitored where these studies translated to the clinic.

## **Key Research Accomplishments**

- Description of new human cell lines published. These represent a powerful tool that can be used to investigate many aspects of both benign and malignant prostatic disease. This is a huge improvement on the previously existing lines and fills a critical need for research by retaining the ability to express all of the key markers of prostate epithelial function (notably androgen receptors and PSA). These cells have already been freely distributed to many laboratories following requests.
- Findings that loss of PPAR $\gamma$  to both autophagy and inflammation were confirmed in tissue recombination models using PPAR $\gamma$ -KO epithelial cell lines. As these lines age they give rise to cancer in tissue recombination models, suggesting that accumulation of insults with time is a potentially transforming event, and further supporting our contention that loss of this pathway can be critical in prostatic carcinogenesis and that activation of PPAR $\gamma$  might be worthwhile chemopreventive approach.
- Observations in the human model demonstrated the key role that PPAR $\gamma$  can play in contributing to epithelial cell differentiation. This work suggests a key role for the pathway in cellular commitment to specific lineages. This unexpected result clearly has significance for a basic understanding of cellular biology, but is of less immediate impact for prostate cancer research.
- Combination of PPAR $\gamma$  loss with loss of the tumor suppressor PTEN, a common locus for LOH or deletion in human prostate cancer can give rise to a carcinoma phenotype.

## Reportable Outcomes

The following publications have been supported in whole or in part from this research grant:

Jiang, M., Strand, D.W., Fernandez, S., He, Y., Yi, Y., Birbach, A., Qiu, Q., Schmid, J., Tang, D.G. and Hayward, S.W. [2010] Functional Remodeling of Benign Human Prostatic Tissues in vivo by Spontaneously Immortalized Progenitor and Intermediate Cells. *Stem Cells* 28, 344-356

Jiang, M., Fernandez, S., Jerome, W. G., He, Y., Yu, X., Cai, H., Boone, B., Yi, Y., Magnuson, M. A., Roy-Burman, P., Matusik, R. J., Shappell, S. B. and Hayward, S. W. [2010] Disruption of PPAR $\gamma$  signaling results in mouse prostatic intraepithelial neoplasia involving active autophagy. *Cell Death and Differentiation* 17, 469-481

Jiang, M., Jerome, W.G. and Hayward, S.W. [2010] Autophagy in nuclear receptor PPAR $\gamma$ -deficient mouse prostatic carcinogenesis. *Autophagy* 6, 175-176

Strand, D.W., Franco, O.E., Basanta, D., Anderson, A.R.A., Hayward, S.W. [2010] Perspectives on Tissue Interactions in Development and Disease. *Current Molecular Medicine* 10, 95-112

[www.UroToday.com](http://www.UroToday.com). Beyond the Abstract - Functional remodeling of benign human prostatic tissues in vivo by spontaneously immortalized progenitor and intermediate cells by Ming Jiang, MD, PhD., Monday, 08 March 2010.

[http://www.urotoday.com/3345/browse\\_categories/beyond\\_the\\_abstract/beyond\\_the\\_abstract\\_functional\\_remodeling\\_of\\_benign\\_human\\_prostatic\\_tissues\\_in\\_vivo\\_by\\_spontaneously\\_immortalized\\_progenitor\\_and\\_intermediate\\_cells\\_by\\_ming\\_jiang\\_md\\_phd03082010.html](http://www.urotoday.com/3345/browse_categories/beyond_the_abstract/beyond_the_abstract_functional_remodeling_of_benign_human_prostatic_tissues_in_vivo_by_spontaneously_immortalized_progenitor_and_intermediate_cells_by_ming_jiang_md_phd03082010.html)

## Conclusions

The third year of this proposal was very productive in terms of publications, we have now published the essential components on which the work was based, demonstrating the link between loss of PPAR $\gamma$  signaling in the prostate and the genesis of premalignant lesions. Work in the current year has also demonstrated that with further cellular aging PPAR $\gamma$ -deficient cells acquire an ability to form frank carcinoma. This progression is presumably due to the acquisition of genetic or epigenetic hits resulting from the induction of inflammation and oxidative stress caused by PPAR $\gamma$  suppression. The observation of autophagic responses to PPAR $\gamma$  loss provides important mechanistic clues, as does the massive induction of inflammation, opening the door to a range of possible approaches which could be applied to early stage tumors.

One of the predicates when we wrote the initial proposal was that, given positive findings, the work would be quickly translatable since there were a number of glitazone drugs on the market specifically designed to agonize the PPAR $\gamma$  pathway. Unfortunately in the intervening period most of these have been pulled from the market due to off target toxicity, including notably bladder cancer – which is interesting given our observations of transitional morphology and uroplakin expression caused by loss of signaling. There is a widespread enthusiasm among clinicians in the field of diabetes where these drugs have been most widely used that there is a pressing need for new drugs targeting PPAR $\gamma$ . Epidemiologic studies at Vanderbilt are also examining the effects of glitazone use in diabetic patients on subsequent diagnoses of BPH/LUTS or of prostate cancer. The outcomes of these studies may also contribute to changing the thought process on whether such approaches can be used to target multiple common comorbidities of diabetes, including BPH/LUTS and possibly also answer the question of whether these compounds are chemopreventive for prostate cancer. Such a finding should spur research in the development of new drugs to directly or indirectly target the pathway.

The final sets of experiments are now in mice and will be harvested for analysis from December through late January. At that point we anticipate writing up the final publications from this work and starting to write proposals to follow up the interesting scientific angles and their translational counterparts. We look forward to presenting the data at the forthcoming IMPACT meeting.