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14. ABSTRACT
This project is an investigation of the involvement of the enzyme arginase type II (AII) in the pathogenesis and growth of prostate cancer. Having cloned the AII gene in our laboratory, we unexpectedly discovered that is expressed at high levels in the normal prostate and even higher in neoplastic prostate samples. The purpose of the present research funded by USAMRMC is to examine the expression of AII in a wider range of benign and malignant prostate specimens and cultured cells to determine its usefulness as a novel marker of prostatic neoplasia and the extent of its involvement in cancer pathogenesis. We are also exploring whether specific chemical and molecular inhibitors of arginase and several related enzymes in the polyamine metabolic pathway might suppress or arrest the growth of prostate cancer cells in vitro or in vivo. This fourth annual report describes our progress over the past year in extending our characterization of arginase and other related enzymes in two new prostate cancer cell lines to address the possibility of AII and androgen susceptibility, correlating polyamine synthesis in all of the prostate cancer cell lines with AII and OAT expression, examining expression levels of polyamine biosynthetic enzymes in these cell lines, creating stable cell lines expressing AII siRNA and overexpression constructs, determining AII expression in the various prostate histological categories of a tissue microarray, and assessing genito-urinary (GU) weights for the proposed in vivo TRAMP studies.

15. SUBJECT TERMS
Prostate cancer, arginase, polyamines

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Introduction

Prostate cancer is the most common and second most lethal cancer among men in the United States, yet despite its high prevalence, relatively little is known about the biochemical and molecular mechanisms controlling benign and malignant prostatic growth. We have proposed to investigate the involvement of the enzyme arginase type II (AII) in this malignancy. We believe that this enzyme plays a pivotal role in the synthesis of polyamines, chemicals involved in cell growth and regulation that are found in high levels in normal prostate tissue and in cancer cells. Having cloned the AII gene in our laboratory, we unexpectedly discovered that it is expressed at high levels in the normal prostate and even higher in neoplastic prostate samples. The purpose of the present research funded by USAMRMC is to examine the expression of AII in a wider range of benign and malignant prostate specimens and cultured cells to determine its usefulness as a novel marker of prostatic neoplasia and the extent of its involvement in cancer pathogenesis. We also are exploring whether specific chemical and molecular inhibitors of arginase and several related enzymes in the polyamine metabolic pathway might suppress or arrest the growth of prostate cancer cells *in vitro* or *in vivo*. The specific aims of the project are to determine: (1) the specific prostate cell types responsible for the high-level expression of arginase AII, (2) the role of arginase in critical pathways of polyamine and nitric oxide synthesis in benign and malignant prostatic growth, (3) the mechanism and efficacy of targeted molecular and biochemical inhibitors of the arginase pathway in blocking the growth of prostate cancer cells, (4) the effect of genetically engineered overexpression of arginase and related enzymes on prostate cancer cell growth, and (5) the significance of arginase AII activity as a potential novel diagnostic marker and/or therapeutic target of prostatic neoplasia *in vivo*. We believe this work will shed new light on the fundamental mechanisms of prostatic neoplasia while at the same time suggesting new directions for diagnosis and therapeutic intervention.

Body of Report

Following a longstanding interest in the first discovered liver isoform of arginase (AI) as the focus of a rare inborn error of metabolism (arginase deficiency; hyperargininemia), our laboratory more recently began to focus on the role of the second, extrahepatic isoform, arginase II (AII) and its potential as a novel marker for prostate cancer. Arginase II is highly expressed in the normal prostate and even more highly expressed in patients with prostate cancer. A major overarching goal of the funded project is to artificially engineer cells, tissues, and model organisms (mice) to achieve overexpression or inhibition of the arginase isozymes and other related genes and enzymes of arginine metabolism and study the effect on prostate cancer cell growth. Much of the initial year of the project was spent developing and testing the necessary reagents for manipulating these genes in the various target milieus specified in the grant proposal. The second year saw the application of these reagents to the *in vitro* experiments proposed and to a variety of benign and malignant human prostate tissue samples. The third year focused on expanding the initial cell culture and prostate tissue experiments while at the same time extended our studies to include the proposed *in vivo* models. During the fourth year, complete expression analysis of AII and other related enzymes was performed in a wide array of prostate cancer cell lines and tissue samples and the data was included in a submitted manuscript. Further progress was made on the *in vitro* studies involving AII overexpression and inhibition while simultaneously acquiring preliminary data from the *in vivo* TRAMP experiments.

The key genes we have chosen to focus on, by virtue of our postulated involvement of them in prostate cancer growth, are arginases I and II, ornithine decarboxylase (ODC), agmatinase (Agm), ornithine aminotransferase (OAT), and arginine decarboxylase (ADC). Each of these enzymes functions within the extended arginine metabolic pathway (Fig. 1), which our group, primarily in a companion grant from the NIH headed by Dr. Stephen Cederbaum, has been working out in the context of a rare inborn error of metabolism, arginase deficiency (hyperargininemia).

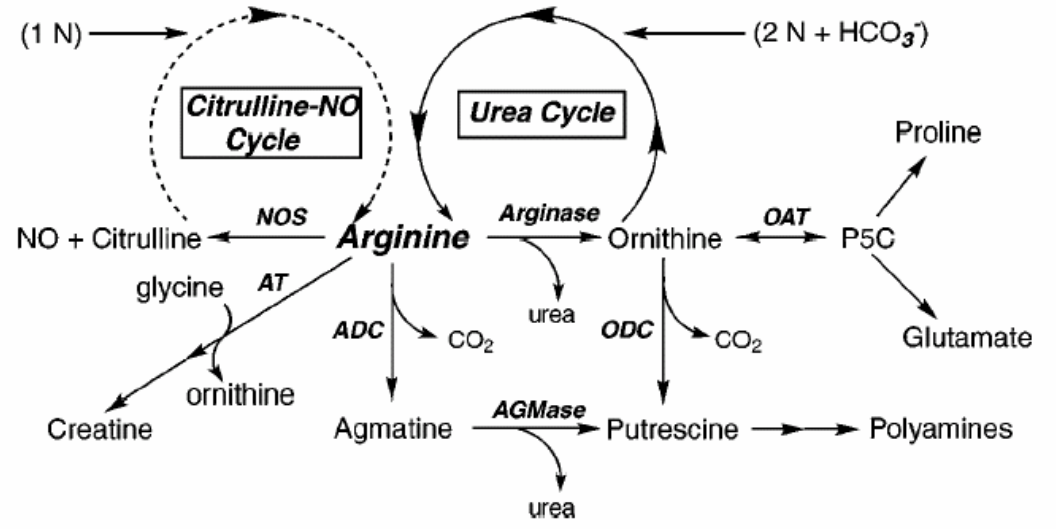

Figure 1. The Arginine Metabolic Pathway, Showing Side Reactions toward Polyamine Synthesis (Morris, 2002).

During the first year we constructed overexpression elements containing GFP (green fluorescent protein) fusions under the control of the CMV constitutive promoter for each of the above genes, and have tested them using a variety of enzymatic assays. In the second year we used these elements to create stable overexpression prostatic tumor cell lines. In addition, interfering RNA (siRNA) constructs (Lieberman et al, 2003) were further optimized using RT-PCR and western blot analysis. We also began to study effects on polyamine synthesis, which according to our hypothesis is the focal point of arginase effect on tumor cell proliferation. Much of the third year was spent gathering more information on the family of genes involved in the arginine and polyamine pathways through quantitative real-time PCR and polyamine studies. We also started our proposed *in vivo* studies, which should mirror the results we have achieved from both the cell culture and tissue experiments conducted thus far. The fourth year was spent completing the expression analysis studies in the prostate cancer cell lines and tissue microarray in order to include the data in a manuscript. Extensive polyamine analysis was performed on all of the cell lines and correlated with the expression profile of AII and other related enzymes. In addition, stable cell lines overexpressing or inhibiting AII expression were generated and functional assays are currently underway to test these cell lines. We have also acquired preliminary data on the *in vivo* TRAMP studies and the results thus far suggest a possible role for AII in tumor progression.

A summary of these accomplishments follows, with reference made to the specific items in the approved Statement of Work to which they apply.

Task 1.a Arginase Isoforms in Prostatic Tissue and Cell Lines

As noted in our last report, we found arginase II expression to be most prominent in the more differentiated, androgen-dependent prostate cancer cell lines (LNCaP, LAPC-4), with lower expression observed in the less differentiated, androgen-independent cell lines (PC3, DU145). To further investigate the issue of AII and hormone-sensitivity, we acquired two new aggressive LNCaP-derived androgen-independent prostate cancer cell lines, CL-1 and CL-2. These cell lines were generated by growing LNCaP cells in androgen-depleted medium for 6 weeks followed by selection of the androgen-independent clones. After selection, the CL-1 cell line was maintained in androgen-free medium, while its counterpart, CL-2, was returned to androgen-containing medium. CL-1 and CL-2 are androgen-independent, PSA negative, and androgen receptor negative cell lines (Freedland et al, 2003). Using both real-time RT-PCR and western blot analysis, we showed a dramatic loss of AII expression in the androgen-independent cell lines, CL-1 and CL-2, compared to the original androgen-dependent LNCaP cell line suggesting that AII is differentially expressed in androgen-dependent versus androgen-independent cell lines. This result is consistent with previous studies that showed that AII activity is dependent on testosterone. In castrated rats, for example, the administration of testosterone has led to enhanced arginase activity (Mendez et al, 2002 and Yamanaka et al, 1975). Currently, we are in the process of determining whether AII is regulated by the presence of androgen or whether it is a marker of degree of differentiation. Preliminary cell culture studies looking at AII expression levels in the presence or absence of dihydrotestosterone suggest that AII may not be a direct target of androgen, but it doesn't rule out the possibility of a secondary mechanism.

In addition to looking at AII expression in the CL-1 and CL-2 cell lines, we also wanted to determine OAT levels in these cells. As described in the last report, we noticed an inverse relationship between AII and OAT among the androgen-dependent and androgen-independent cell lines. When AII expression was high in the LNCaP and LAPC-4 cell lines, OAT expression was low. The opposite trend was observed in the androgen-independent PC3 and DU145 cells which showed low AII expression and high OAT levels. Real-time RT-PCR and western blot analysis revealed statistically higher levels of OAT in the CL-1 and CL-2 cell line compared to the LNCaP cell line. Taken together, the expression analysis studies showed that CL-1 and CL-2 cells were behaving more similarly to the androgen-independent cell lines, PC3 and DU145, with respect to AII and OAT levels, than to the original cell line they were derived from, LNCaP (Fig. 1). The data generated from these studies combined with the colocalization of AII and OAT in the mitochondria and their shared function in producing ornithine, supports a possible compensatory mechanism between these two enzymes. More specifically, we believe that in early stage prostate cancer, AII produces the ornithine necessary for polyamine synthesis, but as the cancer progresses into a more undifferentiated state, OAT seems to compensate for the loss of AII and in turn synthesize the ornithine needed for polyamine production.

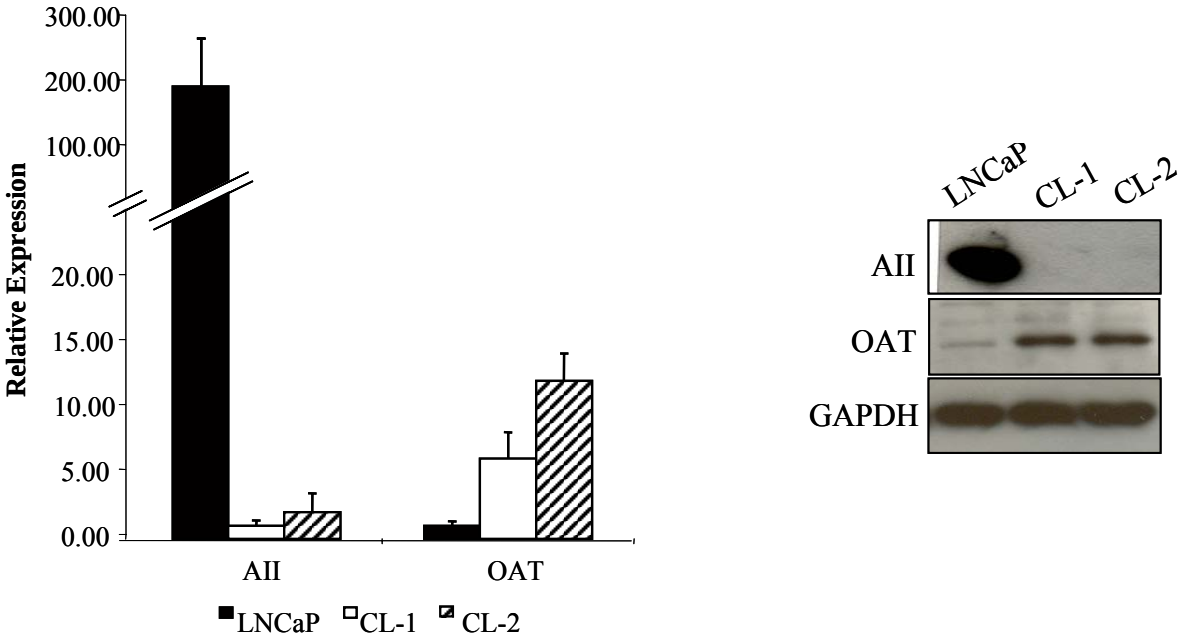

Figure 1. Arginase II (AII) and ornithine aminotransferase (OAT) expression analysis in LNCaP, CL-1, and CL-2 cell lines using real-time RT-PCR and western blot analysis. Inverse relationship between AII and OAT levels among the cell lines.

Task 1.b In Situ Hybridization and Immunohistochemistry

In our last report, we set-up a collaboration with Dr. D. Seligson in the UCLA Tissue Microarray Core, where we obtained a prostate tissue microarray with 1002 different tissue targets containing normal, benign prostatic hyperplasia (BPH), prostatic intraepithelial neoplasia (PIN) and all Gleason grades of prostate malignancy. We performed immunohistochemistry for AII expression on the various prostate tissue targets and after

extensive analysis we found that the BPH (85%) samples had the highest percentage of AII positive cells, followed by PIN (71%), then NL (70%), and the lowest percentage was seen in tumors (61%) (Fig. 2).

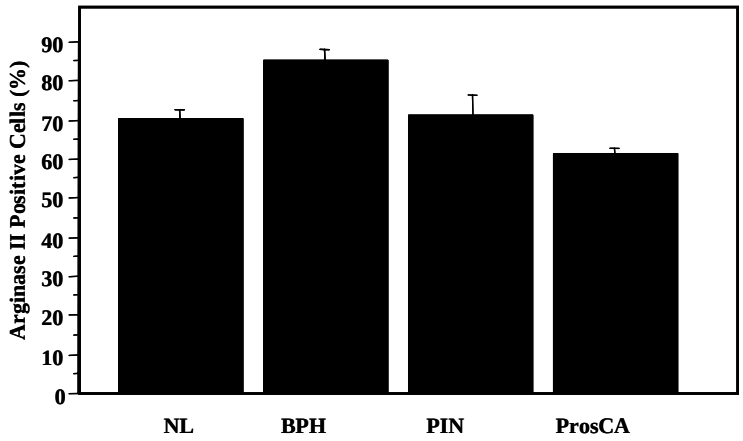

Figure 2. Percentage of arginase II positive cells in normal (NL), benign prostatic hyperplasia (BPH), prostatic intraepithelial neoplasia (PIN), and prostate cancer (ProsCA) tissue spots. BPH had the highest percentage of AII positive cells compared to other prostate histological categories.

Although we had anticipated that the tumor group would have the highest percentage of AII positive cells compared to the other histological categories, an apparent expression gradient across the various tumor Gleason grades could account for this discrepancy. More specifically, we found stronger AII expression in the lowest grade tumors with a decline in expression as the tumors became less differentiated (Fig. 3). This observation was similar to that seen in the cell culture studies, suggesting that high levels of AII are characteristic of more differentiated, early-stage prostate cancer.

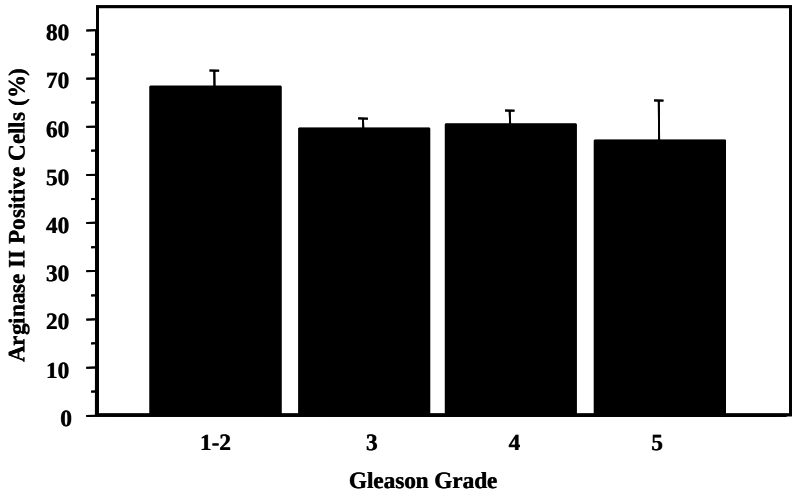

Figure 3. Percentage of arginase II positive cells in Gleason grades 1-5 tissue spots. Gleason grades 1-2 showed a significantly greater percentage of AII positive cells compared to Gleason grade 3.

As mentioned in Task 1.a, it appears that OAT is compensating for the loss of AII in late-stage prostate cancer based on expression levels across several androgen-dependent and androgen-independent cell lines as well as its enzymatic function. To further elucidate whether OAT expression increases due to diminished levels of AII during prostate cancer progression, we are in the process of optimizing the OAT antibody for immunohistochemical analysis. We would like to stain the same prostate tissue microarray for OAT and observe whether OAT is present in the tissue spots/cells with diminished or absent AII expression. From this experiment, we may begin to understand the complex interrelationship that exists between several enzymes present in the arginine metabolic pathway.

We have decided to set aside the *in situ* hybridization experiments and focus our attention on acquiring data from the prostate tissue microarray with respect to arginase and other important enzymes involved in the arginine metabolic pathway (OAT, ODC, polyamine biosynthetic enzymes).

Task 2.a Polyamine Measurements

Since our last report, we have determined polyamine levels in LAPC-4 cells as well as in the new LNCaP-derived androgen-independent clones, CL-1 and CL-2. Based on our polyamine portfolio seen previously for LNCaP, PC3, and DU145 cells we predicted that the LAPC-4 cell line would show a similar pattern of polyamine production to that of LNCaP cells, where CL-1 and CL-2 would behave more similarly to that of the PC3 and DU145 cells. As confirmed in Table 1, total polyamine levels were statistically lower in the androgen-dependent cell lines (LNCaP, LAPC-4) than those measured in the androgen-independent cell lines (PC3, DU145, CL-1, and CL-2). The inverse relationship between AII and OAT seems to correlate with the polyamine levels seen in the prostate cancer cell lines. As suggested earlier, AII may be producing the ornithine necessary for polyamine production in the early stages of prostate cancer progression, but as AII expression diminishes, OAT levels seem to increase and compensate for the loss of AII by continuing to provide the ornithine needed for polyamine synthesis.

TABLE 1. Comparison of Polyamine Levels in Human Prostate Cancer Cell Lines*

Cell Line	Putrescine	Spermidine	Spermine	Total Polyamines
LNCaP	0.82 ± 0.12	15.21 ± 3.57	32.61 ± 5.62	48.63 ± 8.92
LAPC-4	4.01 ± 0.22	21.12 ± 1.08	24.33 ± 0.83	49.45 ± 1.89
PC3	14.20 ± 1.85	31.53 ± 4.06	24.10 ± 2.69	69.83 ± 8.57
DU145	10.58 ± 1.61	38.10 ± 4.29	15.45 ± 0.79	64.13 ± 6.01
CL-1	27.74 ± 1.59	28.12 ± 2.54	26.96 ± 1.60	82.82 ± 5.55
CL-2	14.00 ± 0.92	23.76 ± 0.89	27.94 ± 0.68	65.70 ± 1.56

*Polyamine levels expressed as nmoles/mg of protein ± SD.

The effect of AII inhibition on polyamine production *in vivo* is also an important aspect of this study. We received the polyamine data from our collaborating testing laboratory on a set of prostates from male AII knockout and wild type mice yet there was no significant difference in polyamine levels between the two groups. We suspect that other genes involved in an alternate

pathway (i.e. OAT) are upregulated and may compensate for the depletion of AII and that is why there is no apparent difference in polyamines. Inhibitory studies blocking multiple steps in a pathway are needed to uncover the compensatory mechanism that may be occurring *in vitro* and *in vivo*.

Task 2.b Polyamine Synthetic Enzyme Analysis

We believe that the shift in polyamine production in the prostate cancer cell lines may involve changes in the polyamine biosynthetic enzymes in addition to the supply of ornithine. Antizyme and S-adenosylmethionine decarboxylase (AdoMetDC) are two important biosynthetic enzymes responsible for regulating polyamine levels. Antizyme is involved in the feedback regulation of polyamine levels through its ability to cause degradation of ODC, while AdoMetDC acts as the aminopropyl donor to synthesize spermidine and spermine from putrescine (Hayashi *et al* 1997, Kramer *et al* 1988). Real-time RT-PCR analysis on these two enzymes showed increased mRNA levels in the androgen-dependent cell lines with high AII present, which could explain the reduction seen in putrescine and spermidine levels in these cell lines (Fig. 4).

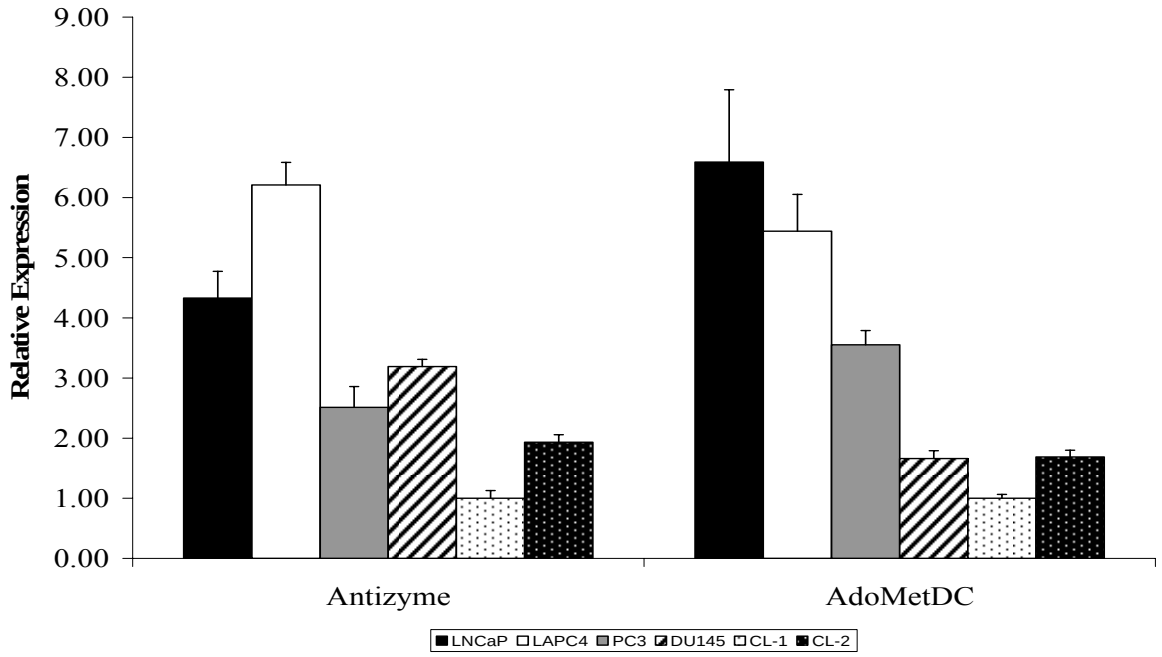

Figure 4. Antizyme and AdoMetDC expression analysis in LNCaP, LAPC-4, PC3, DU145, CL-1, and CL-2 cell lines. Both enzymes revealed higher mRNA levels in the androgen-dependent cell lines compared to the androgen-independent cell lines.

Task 2.c cDNA Miniarrays

As mentioned in our last report, we have decided to use microarray analysis rather than construct cDNA miniarrays to study variations in gene expression. We are in the process of optimizing the microarray technique for our cancer studies. We have been successful in

obtaining significant results from microarray analysis used in other areas of study in our lab so we are confident that we will be able to use this technique to show how alterations in AII influence other biological pathways.

Task 3.a Inhibitors of Gene Expression

As stated in our last report, we had some difficulty creating a stable LNCaP cell line expressing the siRNA against AII. We decided to use a new vector (pQCXIP gfp) with puromycin as the selectable marker and showed 100% knockdown of AII using this siRNA AII vector in HEK cells. Since then, we have been able to transfect the vector into LNCaP cells and after several weeks of puromycin selection generate a pool of cells expressing the AII siRNA. From initial testing, the AII siRNA construct results in approximately 90% knockdown of AII RNA and protein in the LNCaP cells (Fig. 5).

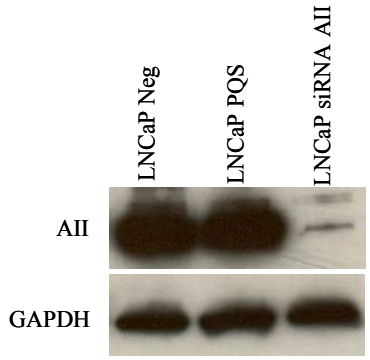

Figure 5. Expression analysis of arginase II in the stable LNCaP siRNA AII cell line compared to two different control cell lines, LNCaP scrambled siRNA (LNCaP PQS) and LNCaP negative (LNCaP Neg). The siRNA AII construct resulted in approximately 90% knockdown efficiency of AII protein.

It is our belief that arginase expression is a necessary factor for promoting the growth advantage of malignant prostatic epithelial cells through its involvement in polyamine synthesis. Since polyamines are important for normal and neoplastic cell proliferation and differentiation, we are in the process of conducting proliferation assays to observe any changes in cell growth between LNCaP scrambled (control) versus LNCaP siRNA AII cells. Cell viability will be measured using the MTT Cell Proliferation Assay over a period of 7 days. In addition, cell growth rates will be determined by the incorporation of radiolabelled thymidine into dividing cells. We predict that reduction in LNCaP AII levels will lead to decreased cell proliferation.

Expression analysis on the LNCaP siRNA AII cell line compared to the control LNCaP scrambled cell line will be performed using real-time RT-PCR and western blot in order to identify changes in gene expression among other enzymes involved in the arginine and polyamine metabolic pathways (i.e. OAT, ODC, and polyamine biosynthetic enzymes). In our last report, we mentioned looking for significant alterations in genes involved in transformation and tumorigenesis with the Human Cancer Pathway Finder PCR Array (SuperArray, Frederick, MD). We analyzed the expression of genes implicated in cell cycle control, apoptosis, adhesion, angiogenesis, invasion and metastasis (i.e. p53, Bcl-X, NFKβ, Myc, MMP) in the presence or absence of AII. More specifically, we used RNA isolated from the LNCaP siRNA AII cell line

and the LNCaP scrambled cell line to perform real-time RT-PCR using 96-well plates that contained primers for a number of genes found in cancer pathways. We found two genes that showed a difference in expression at the RNA level, AKT1 and E2F1, however when we looked at the protein levels of these genes, there was no appreciable difference between the two cell lines.

Task 3.b Inhibitors Using the Tetracycline-Controlled Transactivator

As stated in our last report, no new work with the tetracycline transactivator is planned at this time. We will be focusing most of our attention on testing the stable cell lines expressing the siRNA AII vector mentioned above.

Task 3.c Growth Inhibition and Apoptosis

As discussed in Task 3.a, we are in the process of carrying out proliferation assays on the stable LNCaP siRNA AII cell line that we generated. We would anticipate that depletion of AII in these cells would lead to decreased proliferation. However, our gene expression and polyamine analysis on several prostate cancer cell lines suggests that an alternative pathway does exist for polyamine production. When AII expression is low, other enzymes such as OAT increase in expression and seem to compensate for the reduction in AII by contributing to polyamine production. Therefore, slowing or stopping cell growth may require blocking more than one enzyme in the arginine or polyamine metabolic pathways. To address this issue, changes in cell growth among prostate cancer cell lines manipulated by both AII and OAT siRNAs will also be determined. Preliminary testing of the OAT siRNA has shown an approximate 70% reduction of this gene in HEK cells. Currently we are testing the knockdown efficiency of the OAT siRNA in LNCaP cells.

Task 4.a Arginase Overexpression

We are still in the process of trying to generate a PZ-HPV-7 stable cell line overexpressing AII. We have tried several different methods, but the transfection efficiency for this cell line is extremely low. While we are continuing to try different approaches for overexpressing AII in the benign PZ-HPV-7 prostate cell line, we are also in the process of overexpressing AII in PC3 cells, which is a much easier cell line to transfect. From previous AII expression analysis performed on the prostate cancer cell lines, PC3 cells displayed very low levels of AII. Therefore, overexpressing AII in this cell line may cause the cells to take on a different phenotype. Confirmation of AII overexpression in the PC3 cell line was attained by western blot analysis as seen in Figure 6. Currently, we are determining cell growth rates and assessing invasion potential of the PC3 cell line overexpressing AII compared to a control PC3 cell line. We anticipate that the PC3 cells containing an AII overexpression construct will proliferate at a faster rate than the control cell line due to the suspected role of AII in polyamine synthesis. However, since we have observed differential expression of AII in the androgen-dependent versus androgen-independent cell lines, overexpressing AII in the late-stage PC3 cancer cell line may cause it to take on properties similar to the androgen-dependent cell lines that possess high endogenous AII levels. More specifically, the PC3 cells overexpressing AII may exhibit a slower growth rate and have less invasion potential.

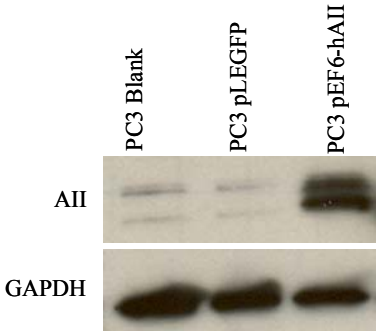

Figure 6. Expression analysis of arginase II in the PC3 cell line overexpressing AII (pEF6-hAII) compared to two different control cell lines, PC3 pLEGFP, and PC3 blank. Significant overexpression of AII in the PC3 cell line stably expressing the pEF6-hAII construct compared to the control cell lines.

Task 5.a Human Serum

As stated in the last report, our accumulation of serum samples from prostate cancer patients has been delayed because our internal source (UCLA Tissue Procurement Core Laboratory) for these samples no longer has access. We are still in the process of searching for an alternate source of serum (an NCI-funded, multi-center biomarker project).

Task 5.b Nude Mice

We have recently begun dissecting prostates from 30-week-old TRAMP mice and recording their weights. We are comparing prostate weights from native TRAMP with those removed from TRAMP/AII knockout mice at 30 weeks of age. In initial studies, we dissected prostates from a number of TRAMP mice at 16, 24, and 30 week time points; however, the heterogeneity in tumor development among these mice prompted us to focus our study on a later time point (30 weeks) in hopes of maximizing our statistical analysis. In order to determine the number of mice necessary to reach a significant conclusion, we utilized the statistical expertise of the Biostatistics, Analytical Support & Epidemiology Unit (BASE) through the Jonsson Comprehensive Cancer Center at UCLA. After careful analysis of our preliminary data, they recommended a sample size of 16 mice per group to reach statistical significance. At this time, we have data for approximately 7 mice in each group and the preliminary results can be seen in Figure 7. This graph shows the genito-urinary (GU) weight (bladder, urethra, seminal vesicles, and prostate) for TRAMP, TRAMP/AII knockout, AII knockout, and wildtype mice. The data generated thus far suggest that depletion of AII in these mice increases the progression of tumor development and ultimately causes a worse phenotype. However, the heterogeneity among tumor size in these animals has generated large error bars, which will hopefully be diminished as our sample size increases.

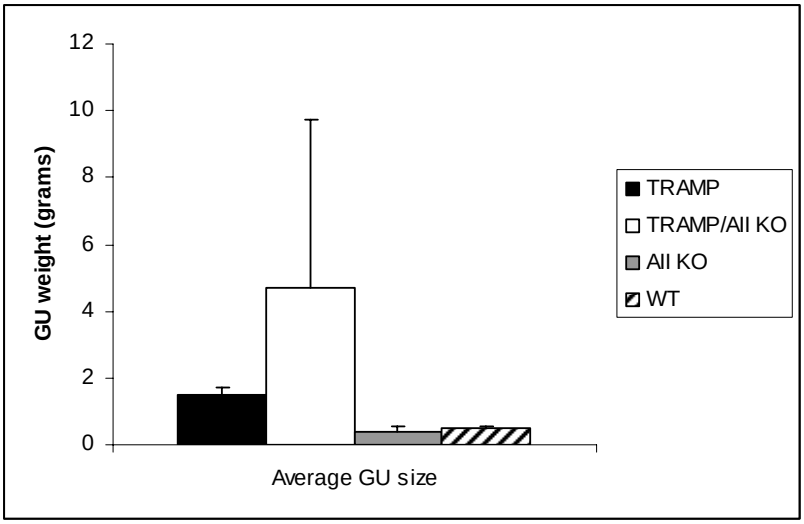

Figure 7. Average GU weight of TRAMP, TRAMP AII KO, AII KO, and WT mice.

The proposed nude mouse studies are dependent upon the development and testing of the stable LNCaP cell lines expressing the AII siRNA. If we see an effect on proliferation rates of the LNCaP siRNA AII cell line compared to a control LNCaP cell line we will begin injecting LNCaP cells transfected with the AII siRNA into the flank of nude mice and compare these experimental tumors with those formed from untransfected control cells. We are aware of the inconsistent nature of LNCaP cells for growth and metastasis in nude mice (Lee *et al*. 1993), so we will also consider using LAPC-4 or PC3 cells for our xenograft studies.

Key Research Accomplishments

- Expanded our prostate cancer cell work to include two new aggressive LNCaP-derived androgen-independent cell lines, CL-1 and CL-2. Assessed the expression levels of arginase II and other related enzymes in these cell lines compared to the LNCaP cell line they originated from using real-time RT-PCR and western blot analysis.

- Determined percentage of AII positive cells across the prostate histological categories (normal, BPH, PIN, tumor) found in a tissue microarray.

- Determined percentage of AII positive cells across the tumor Gleason grades within a prostate tissue microarray.

- Correlated AII and OAT expression with polyamine levels found in the androgen-dependent versus androgen-independent cell lines.

- Performed RNA expression analysis on several polyamine biosynthetic enzymes in the prostate cancer cell lines.

- Created a stable LNCaP cell line expressing a siRNA vector against AII.

- Performed expression analysis on the stable LNCaP siRNA AII cell line using the Human Cancer Pathway Finder PCR Array.

- Generated a stable PC3 cell line overexpressing AII using the pEF6-hAII vector.

- Ascertained GU weights in TRAMP, TRAMP/AII KO, AII KO, and WT mice at 30 weeks of age.

Reportable Outcomes

Publications/Presentations

Mumenthaler, S.M. Yu, H. Tze, S. Pegg, A.E. Cederbaum, S.D. Seligson, D.B. Grody, W.W. Differential expression of arginase II in androgen-dependent versus androgen-independent prostate cancer. The Prostate, *submitted May 2007*.

Mumenthaler, S.M. Pegg, A.E. Cederbaum, S.D. Grody, W.W. Decreased arginase II expression in androgen-independent prostate cancer cell lines. Presented at the American Association for Cancer Research: Frontiers in Cancer Prevention Research Meeting, Boston, November 2006.

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Conclusions

During Year 4, we expanded the previous cell culture experiments to include two new aggressive LNCaP-derived androgen-independent cell lines to further investigate the issue of arginase II and androgen susceptibility. We have shown that AII is differentially expressed in androgen-dependent versus androgen-independent prostate cancer cell lines. We were also able to correlate polyamine levels with the AII and OAT expression profiles determined in the different cell lines. Arginase II expression in a wide array of prostate tissue specimens from a tissue microarray suggest that AII could be a marker of early stage prostate cancer. Stable cell lines manipulating the expression of AII by inhibition or overexpression have been created and biochemical and functional assays are currently underway to test these cell lines. Finally, we looked at the effect of AII inhibition on tumor development in the TRAMP mouse model and preliminary results suggest that AII plays a role in prostate tumor progression.

Despite the high incidence of prostate cancer, relatively little is known about the biochemical and molecular mechanisms controlling benign and malignant prostatic growth. This project sets out a novel and original program that seeks to elucidate fundamental underlying mechanisms linking our surprising observation of elevated prostatic arginase AII levels with promotion and potential inhibition of cancer of the prostate. We are building upon our group's long track record of arginase research in the context of a metabolic disorder, arginase deficiency, and are now applying these resources for the first time to an investigation of what we believe to be this enzyme's fundamental involvement in prostate cancer cell growth. This hypothesis is based on the locus of arginase activity at the convergence of critical urea cycle, polyamine synthetic, and nitric oxide pathways, all of which are key aspects of prostate physiology and cell proliferation. We believe this work will enhance our understanding of the fundamental mechanisms of prostatic neoplasia and also suggest new and specific molecular targets for both diagnosis and therapy.

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