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13. ABSTRACT (Maximum 200 Words)

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 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 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 second annual report describes our progress over the past year in characterizing the overexpression and inhibitory vector constructs used for these experiments, our optimization of small inhibitory RNA transfer techniques in prostatic cancer cells, the construction and study of stable arginase-overexpressing prostatic cell lines, the correlation of polyamine synthesis levels in these lines, and our measurement and localization of arginase expression in benign and malignant prostate tissue samples.

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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 recently 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 will also explore 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 may shed 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 has begun 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 and properties. 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 has seen the application of these reagents to the *in vitro* experiments proposed and to a variety of benign and malignant human prostate tissue samples.

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, 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
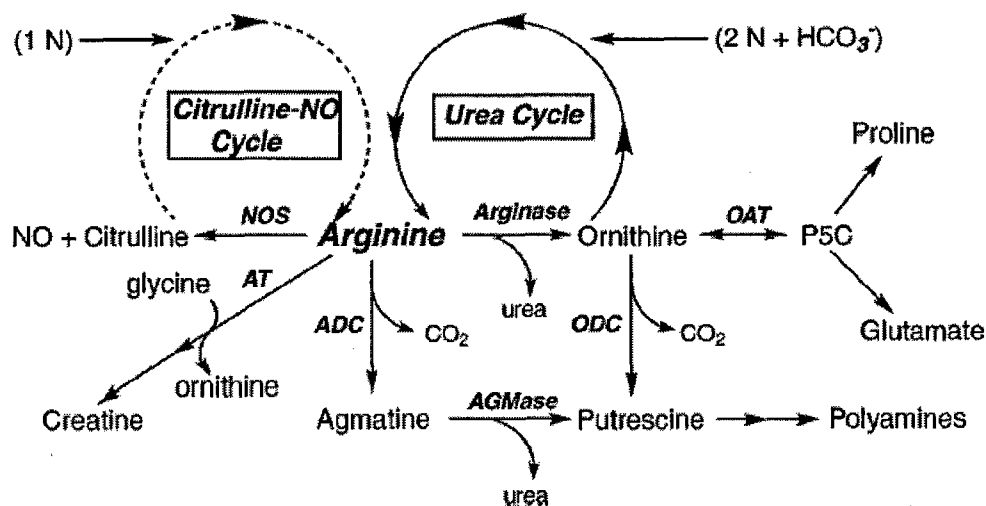

Fig. 1. The arginine metabolic pathway, showing side reactions toward polyamine synthesis (Morris, 2002).

NIH headed by Dr. Stephen Cederbaum, has been working out. During the first year we constructed overexpression elements containing GFP (green fluorescent protein) fusions under the control of the CMV constitutive promoter for some of the above genes, and have tested them

using a variety of enzymatic assays. In the second year we have used these elements to create stable overexpression prostatic tumor cell lines. In addition, interfering RNA (siRNA) constructs (Lieberman *et al.* 2003) have been further optimized using RT-PCR and western blot analysis. We have also begun to study effects on polyamine synthesis, which according to our hypothesis is the focal point of arginase effect on tumor cell proliferation.

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

Since our last report, we have created several stable LNCaP and DU145 cell lines that overexpress arginase-I (AI), arginase-II (AII), agmatinase (Agm), and ornithine decarboxylase (ODC). We are still in the process of creating a stable PZ-HPV-7 normal prostate cell line that overexpresses AII, and validation is in progress. This line is well-differentiated and grows quite slowly. Table 1 lists all of the stable cell lines that have been established to date. Throughout this report we refer to the stable clones as GBhAI, for example, where the 'G' represents GFP, the 'B' represents Blasticidin (selectable marker), and 'hAI' represents the enzyme being overexpressed in the construct (human Arginase I). We prioritized AI and AII as the most important enzymes to focus on initially. Therefore, most of our data from the past year were generated using the LNCaP stable cell lines that overexpress human AI and mouse AII. The GFP fusion gene overexpression constructs reported last time were transfected into both LNCaP and DU145 cell lines and, through antibiotic selection using Blasticidin, we were able to generate several stable clones. The GFP fusion protein present in the construct allowed us to visualize our transfection efficiency in the respective prostate cancer cell lines using a fluorescent microscope as well as assess any differences in expression between transiently transfected and stably integrated genes (Fig. 2).

Once the stable cell lines were created, RT-PCR and western blot analysis were used to confirm the presence of increased RNA and protein levels in the overexpression cell lines (Figs. 3 and 4, respectively). Our results show a significant increase in the RNA level of AI and AII in the stable cell lines compared to native LNCaP cells. AI RNA levels are known to be low in native LNCaP cells, but when overexpressed in the stable cell lines, the AI levels are dramatically higher. For the AII RT-PCR, we used mouse primers because the overexpression construct contained mouse AII. There was no mouse AII RNA detected in native LNCaP cells because it is a human cell line; however, it was seen in our stable AII cell line, confirming that AII is being overexpressed.

It was also necessary to confirm the overexpression of AI and AII at the protein level. After validating several AI and AII antibodies, we were able to show increased protein levels of AI and AII present in our stable cell clones. We determined the best arginase I antibody to be a rabbit polyclonal AI antibody generated in our lab several years ago, and for AII we purchased a commercially available rabbit polyclonal antibody from Santa Cruz Biotechnology. We also purchased a rabbit polyclonal anti-GFP antibody that was used to confirm our results seen with the AII antibody from Santa Cruz. The AI protein is approximately 35 kD and the AII protein

runs slightly higher at 40 kD. The native GFP fusion protein is approximately 27 kD, which makes the size of our overexpression AI and AII fusion proteins approximately 62 and 67 kD, respectively. As seen in Fig. 4A, native LNCaP cells produce trace amounts of AI that are undetectable by western blot analysis even though they were seen by RT-PCR. When comparing lysate from native LNCAP cells to that of GBhAI-LNCaP cells, there is a strong band around 62 kD, consistent with the size of the hAI-GFP fusion protein. In the AII western blot (Fig. 4B), native LNCaP cells produce a large amount of endogenous AII protein. When comparing native LNCaP cells with GBmAII-LNCaP cells, the endogenous levels of AII (40 kD) are relatively equal; however, a strong band around 67 kD is present in the overexpression cell lysate consistent with the size of the mAII-GFP fusion protein. When we created these stable cell lines, we generated 5 clones of each type. Our reasoning behind this approach was that different clones may be expressing AI and AII at varying levels depending on where the overexpression construct inserted itself. It is apparent that some of our stable clones overexpress AI and AII at higher levels than others. The higher overexpressing clones were used in most of the subsequent experiments.

As seen in Fig. 5, native DU145 cells do not contain any AI protein as determined by Western analysis. However, one of the stable AI transfected cell lines is shown to have an increase in arginase I expression. This is confirmed by expression of the GFP fusion protein as determined by its detection using both our anti-AI antibody and a commercially available anti-GFP antibody.

As noted, we are in the process of creating a stable PZ-HPV-7 normal prostate cell line that overexpresses AII. Validation of the stable clones is in progress. Our plan is to perform metastatic and proliferation assays comparing native PZ-HPV-7 cells with PZ-HPV-7 cells overexpressing AII. The hypothesis would be that overexpressing AII in a normal prostate cell line may cause the cells to take on cancerous properties including increased cell proliferation and metastatic potential.

Table 1: Stable Cell Lines Overexpressing Enzymes Involved in the Arginase Pathway

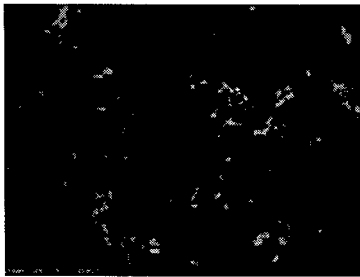| LNCAP	DU145
Mouse AI	Mouse AI
Human AI	Human ODC
Mouse AII	
Human ODC	
Human Agm	

Fig. 2. Overexpression vector validation using fluorescence intensity. Stable LNCaP clone overexpressing mouse AII GFP fusion vector.
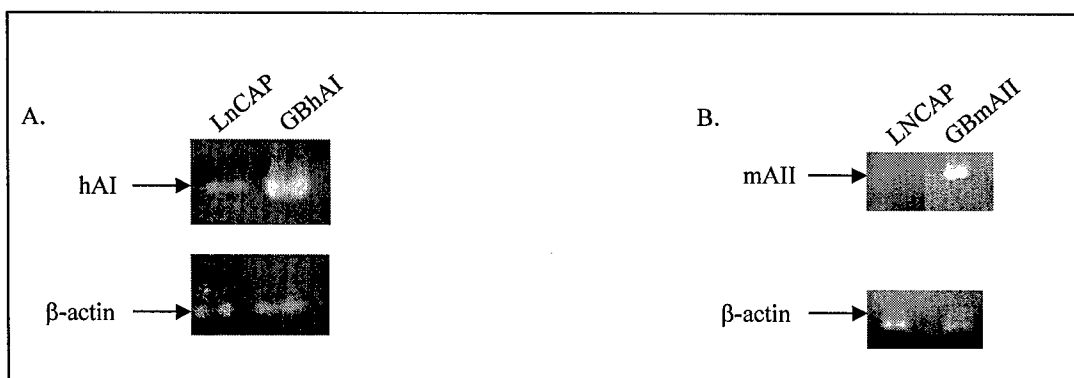

Fig. 3. Overexpression vector validation using RT-PCR. (A) RT-PCR comparing hAI RNA levels from native LNCaP cells to the levels observed when hAI is overexpressed in LNCaP(GBhAI). (B) RT-PCR comparing mAII RNA levels from native LNCaP cells to the levels observed when mAII is overexpressed in LNCaP (GBmAII).
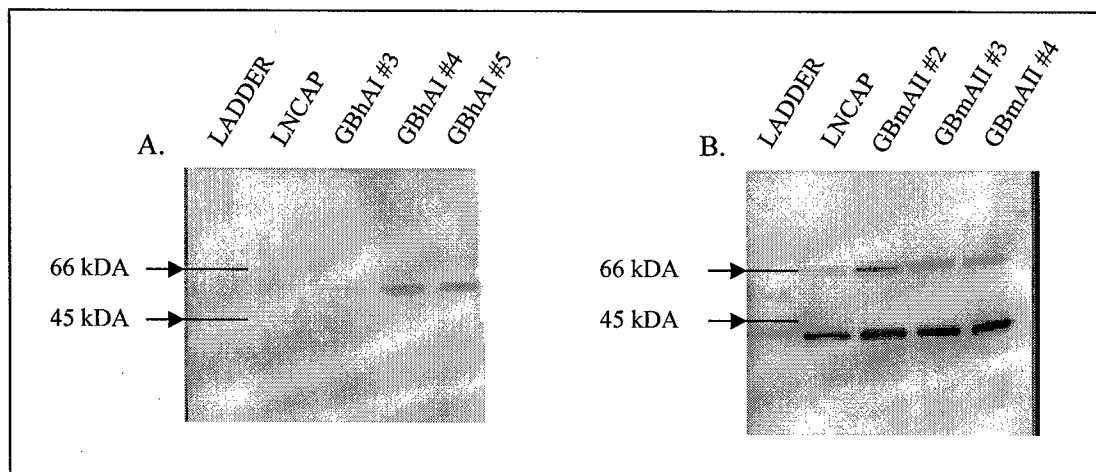

Fig. 4. Overexpression vector validation using western blot analysis. (A) AI levels in native LNCaP cells and in three different stable LNCaP clones overexpressing the human AI GFP fusion vector. (B) AII levels in native LNCaP cells and in three different stable LNCaP clones overexpressing the mouse AII GFP fusion vector.

62 kD →

DU145   D GFP-AI #1   D GFP-AI #2   D GFP-AI #3

anti-AI

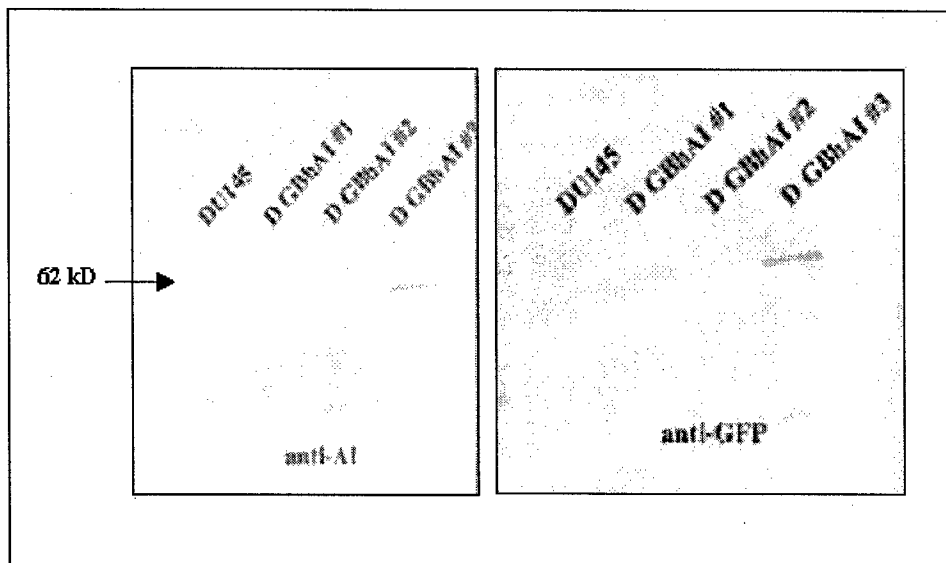DU145   D GFP-AI #1   D GFP-AI #2   D GFP-AI #3

anti-GFP

Fig. 5. Protein levels of the GFP-AI fusion protein are increased in the stable cell lines. DU145 clone #3 is shown to have overexpression of the fusion protein as confirmed by its ability to be detected on a Western blot using both our anti-AI and anti-GFP polyclonal antibodies.

Task 1.b   In Situ Hybridization and Immunohistochemistry

After obtaining IRB approval for the use of human prostate tissues, we were able to acquire normal, benign hyperplasia (BPH), and malignant (of various Gleason grades) tissue samples from the Tissue Procurement Core Facility at UCLA. We received frozen sections prepared on slides along with hematoxylin-and-eosin-stained sections to view morphology. We used the AII antibody mentioned in our previous work to localize the cells of origin of AII expression. Fig. 6 shows some representative immunohistochemistry work done on the various prostate tissue samples. We observed AII activity in the glandular epithelial cells as predicted; however, to our surprise we also saw activity in the stroma of the prostate. When comparing the different prostate tissue types, we observed a gradient in glandular AII expression where normal tissue expresses the least, benign hyperplasia shows slightly higher levels of AII, and the strongest AII signal was seen in the malignant tissue. An interesting observation was made when we looked at tissue from the individual Gleason grades. We noticed that AII expression is significantly stronger in the lower Gleason grade tissues compared to the higher Gleason grades. This result is consistent with the observation seen in native prostate cancer cell lines. LNCaP is the more differentiated prostate cancer cell line and it expresses AII at a higher level than DU145 and PC3, which are more undifferentiated cell lines. This suggests that arginase expression is highly organotypic and characteristic of more differentiated prostatic tissues; as tumors lose their differentiation, AII expression diminishes. It also suggests that elevated arginase activity may be an important marker for early stage carcinoma rather than late stage metastatic cancer.
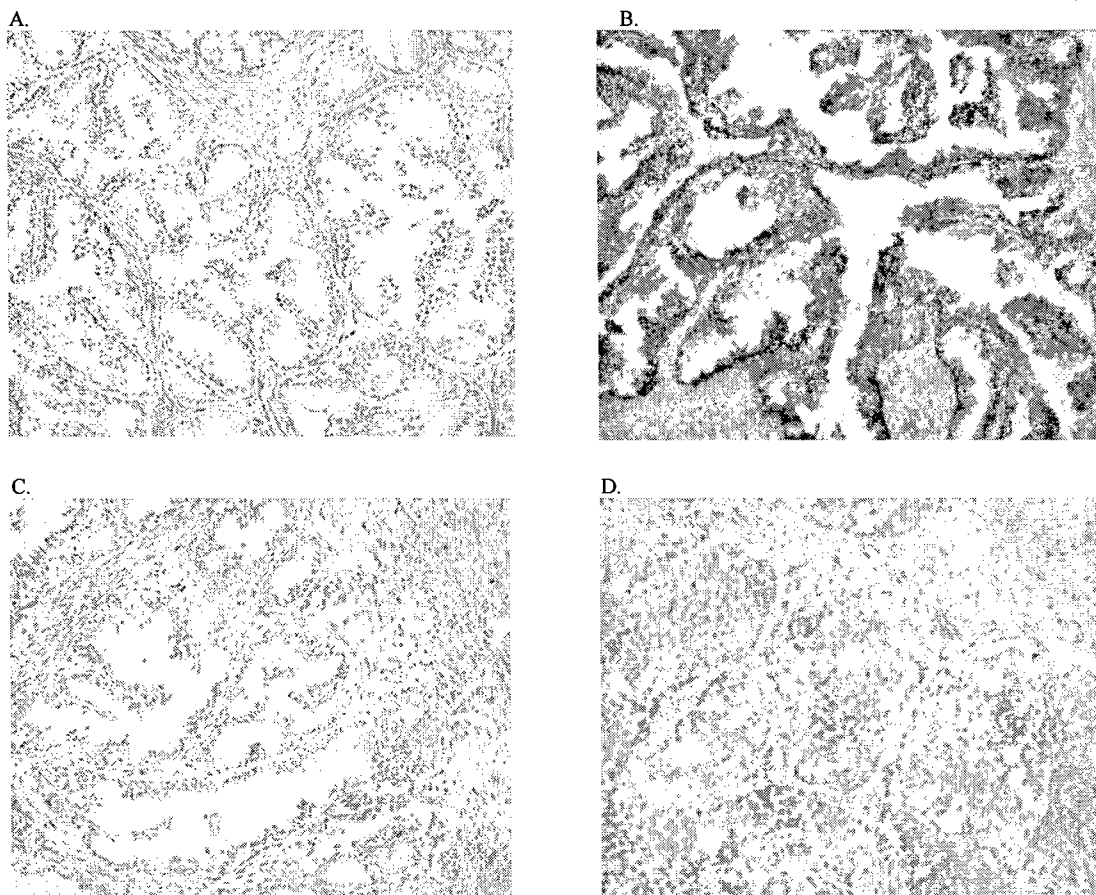
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Fig. 6. Immunohistochemical detection of arginase-II expression in human prostate tissue samples. (A) benign (normal) prostate; (B) prostatic adenocarcinoma, Gleason grade 7; (C) prostatic adenocarcinoma, Gleason grade 9; (D) negative control of prostatic adenocarcinoma, grade 10 (no primary antibody applied).

Due to some unexpected delay in the IRB approval for release of human prostate tissues, we have not made more progress on the *in situ* hybridization experiments. We are beginning these studies now and will have results shortly. It is our belief that the *in situ* hybridization studies will mirror the results we have observed by immunohistochemistry. But they will be helpful in defining the differences as due to gene expression rather than protein stability.

Task 2.a   Polyamine Measurements

Since the last annual report, we were able to overcome our technical hurdles and set up a polyamine collaboration with Dr. Anthony Pegg's laboratory at Penn State College of Medicine. In our previous update we mentioned a possible collaboration with Dr. Bill O'Brien at Baylor College of Medicine, but he was unable to set up a protocol in a reasonable amount of time. The Pegg laboratory provided the protocol we are currently using to harvest cells for polyamine analysis by HPLC. Putriscine, spermidine (Spd), and spermine (Spm) levels are tested for all of our samples. Currently, we have polyamine measurements for LNCaP native cells and for stable LNCaP cells overexpressing AI and AII (Fig. 7). In the pathway shown in Fig. 1, arginase acts as

a precursor to polyamine synthesis. According to our hypothesis, we would predict that overexpressing arginase would lead to an increase in polyamine levels which are known to support the growth of rapidly proliferating cells and tumors (Schipper *et al*. 2003; Gerner & Meyskens 2004). Our initial studies show that one overexpressing AI clone has statistically higher levels of putriscine than native LNCaP cells. More importantly, one overexpressing AII clone shows a significant increase in both putriscine and spermidine levels compared to native LNCaP cells. If one compares the expression levels between the different clones shown previously in our western blot analyses (Fig. 4), there is a direct correlation between arginase expression levels and polyamine measurements. Thus, our hypothesis appears to be correct.
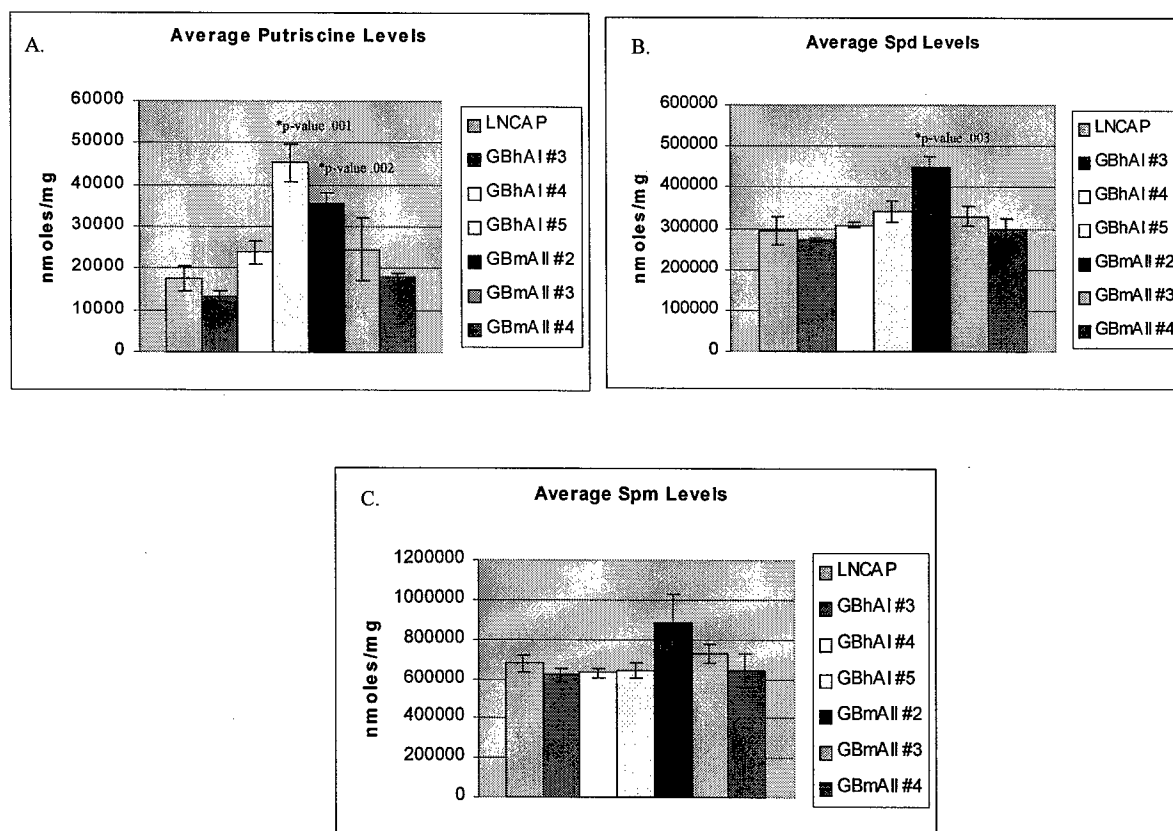

Fig. 7. Polyamine levels for native LNCaP cells and for stable LNCaP overexpression clones. (A). GBhAI #4 and GBmAII #2 clones had statistically higher levels of putriscine when compared to native LNCaP cells. (B) GBmAII #2 clone had statistically higher levels of spermidine when compared to native LNCaP cells. (C) No clone showed significantly higher levels of spermine when compared to native LNCaP cells.

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Task 2.b   Polyamine Synthetic Enzyme Analysis

As shown previously (Figs. 3 and 4), we have RT-PCR data and Western blot analysis on AI and AII levels in native LNCaP cells and in AI and AII overexpressing LNCaP stable clones. Data provided in Fig. 8 shows arginase enzymatic activity in cells using a colorimetric enzyme activity assay (Corraliza *et al.*, 1994) which we have set up over the previous year. Our lab had for many years been using a radioactive arginase enzymatic assay (Spector *et al.* 1980), but we wanted to move away from the use of radioactivity for obvious reasons and have found this spectrophotometric assay to be much more manageable. We compared arginase activity in native LNCaP cells to the activity seen in our stable LNCaP overexpressing hAI clone. The overexpressing GBhAI clone expresses twice the level of AI compared with native LNCAP cells. The enzymatic levels of arginase seen here are consistent with the RT-PCR and western data shown earlier.
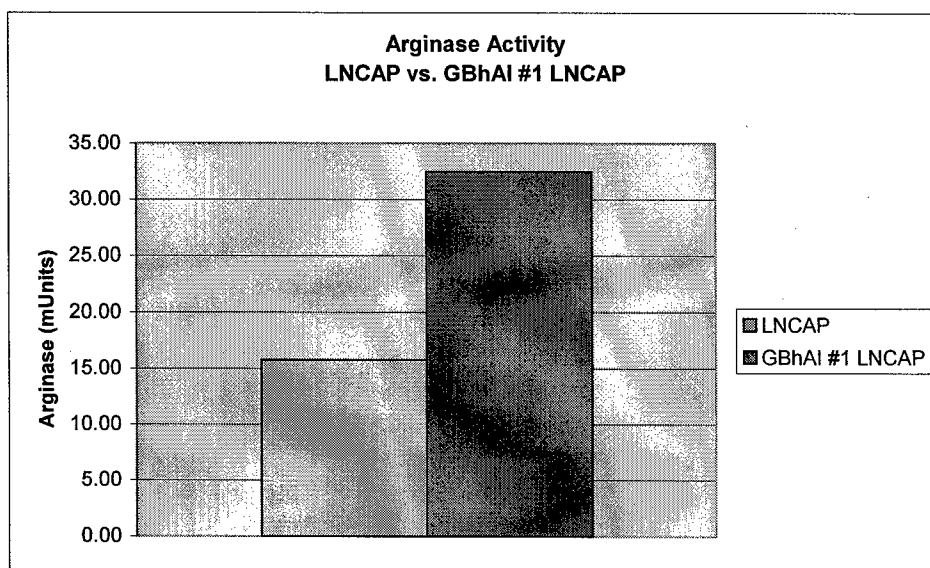

Fig. 8. Arginase enzymatic activity assay. The stable LNCaP overexpression hAI cell line expresses twice the level of AI compared with native LNCaP cells.

In related work in our laboratory that has important implications for this part of the project, we have during Year 2 obtained accurate polyamine measurements in several tissues of our arginase-I knockout mice. While some minor increases and decreases of the three polyamines compared to wild type and heterozygous knockout mice were noted, none reached statistical significance. This is not surprising, and in fact is consistent with our theory that it is AII, and not AI, that is the critical arginase isoform involved in polyamine synthesis. Of greater interest for such studies will be the AI/AII double-knockout mouse strain that we have developed through cross-breeding of the two lines during Year 2 (Deignan *et al.* 2005). This mouse strain is unique in the world and will likely possess a number of properties of relevance to this project. We are presently harvesting tissues from these mice for measurement of polyamine levels, and will naturally be particularly interested in prostate. We are working with one of the research urologists at UCLA who will assist us in dissecting this organ.

Task 2.c   cDNA Miniarrays

We have decided that rather than construct cDNA miniarrays containing the self-defined repertoire of relevant genes involved in the arginine, polyamine, and nitric oxide pathways, we would rather address all the genes in the mouse genome. Therefore, we have begun the task of setting up microarray analysis in our laboratory. Parameters and conditions have already been worked out, and several sets of mouse tissues and cell lysates have been analyzed for other projects being conducted by our group. It is our intent to use microarray technology to expand our knowledge of how alterations in arginase II and other relevant genes influence the expression of other genes in ancillary pathways. We would first apply this technology to our cancer studies by analyzing the arginine metabolism pathway gene expression patterns in our TRAMP transgenic mouse prostate cancer model, as this *in vivo* model is a better reflection of the true tumor environment than our *ex vivo* cell culture lines.

Fig. 9 shows an example of the microarray technique we have established, the substrate in this case being RNA from neural stem cells isolated from day 13 embryos of our AI knockout mie and corresponding wildtype controls. These were processed with a universal mouse reference RNA (Stratagene 740100) and applied to a cDNA Riken Fantom-1 21,000 mouse gene microarray slide (UCSF-MMC). The results inticated that several genes involved in proliferation and stress response including TGF-β family receptors (inhibin-β-b) and some integrin subunits (integrin βv1) were overexpressed in the knockout cells. We hope to apply this same technology to our arginase overexpressing and knockdown constructs, both *in vitro* and *in vivo*.
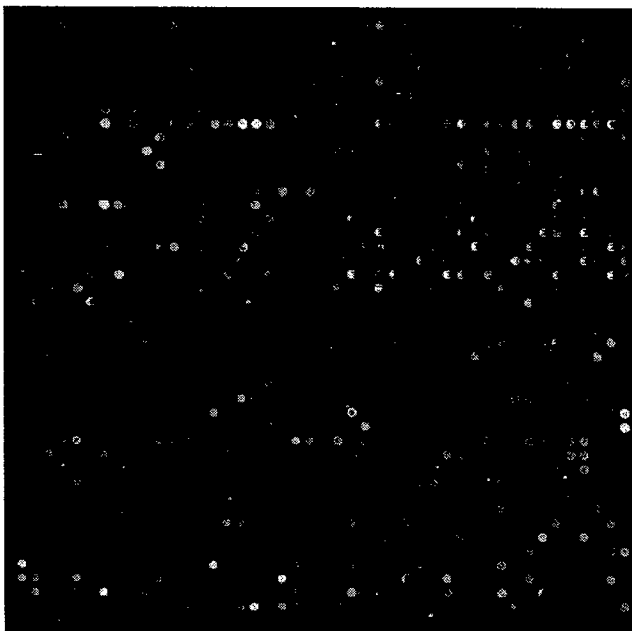

Fig. 9. Sample knockout and wildtype microarray showing the intensity of gene expression.

Task 3.a   Inhibitors of Gene Expression

In our last report, we mentioned several siRNA vectors that had been created to knock down the expression of AI, AII, and other enzymes involved in the pathway. Unfortunately, we were never able to get these siRNAs to function as well as we had hoped. Instead of utilizing a majority of our time trying to get these siRNAs to work, we decided to purchase an AII siRNA from Dharmacon to guarantee specific knockdown of AII. We obtained a pool of 4 siRNA oligonucleotides specific to AII for testing. We transfected the AII siRNA pool into LNCaP cells along with two controls (siControl and water) and performed a western blot using an AII antibody. Fig. 10 shows the validation of the siRNA, and Fig. 11 demonstrates an approximate 50% knockdown in AII expression in the cells transfected with siRNA AII compared with the controls.

Our next step is to separate the individual siRNA oligonucleotides present in the pool and test them individually for knockdown efficiency. Once we have determined the siRNA with the highest AII knockdown percentage, we will clone it into a viral vector in order to make stable cell lines expressing the AII siRNA. Producing a stable prostate cancer cell line containing an AII siRNA vector will benefit several aspects of this project. We will examine the effect of AII siRNA on polyamine levels in both native LNCaP and overexpressing AII LNCaP cell lines. The stable AII siRNA cell line will also prove useful in proliferation studies. We hypothesize that a reduction in AII levels in LNCaP cells will lead to decreased proliferation. These studies will be well underway within the next couple of months.
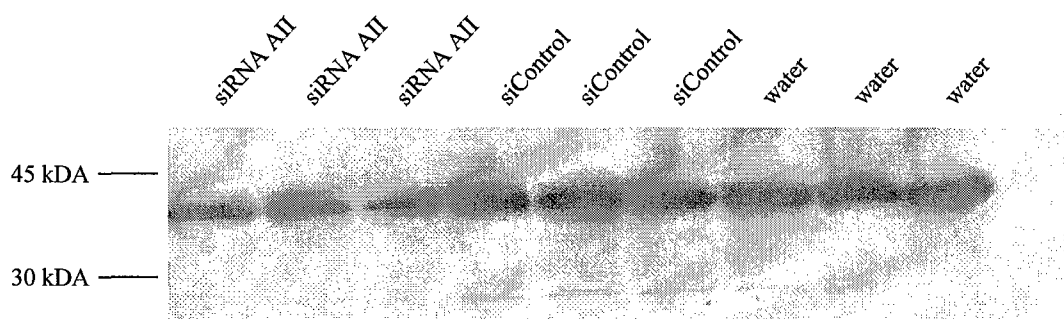

Fig. 10. Dharmacon siGENOME siRNA AII pool validation. The western blot shows an approximate 50% knockdown in AII expression in LNCaP cells when transfected with the siRNA compared to the controls. The experiment was done in triplicate.
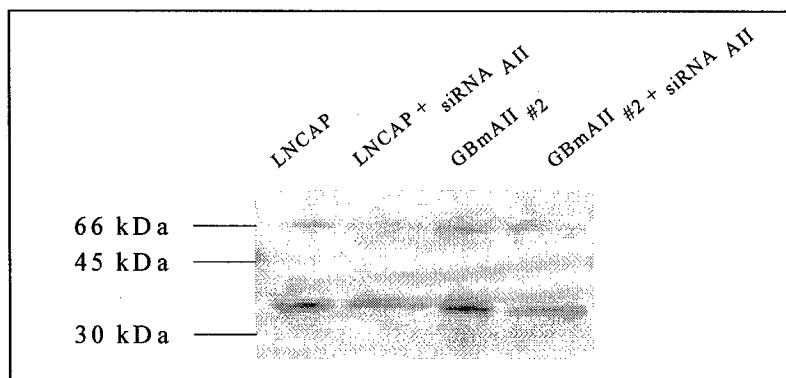
Fig. 11. Western blot analysis on native cells compared with AII siRNA transfected cells. A 50% reduction in endogenous AII levels is observed when the cells are transfected with AII siRNA.

Task 3.b   Inhibitors Using the Tetracycline-Controlled Transactivator

Unfortunately, since most of our previously constructed siRNA vectors were not able to silence gene expression to the extent sufficient enough for our purposes, we currently do not have any such inhibitors under the control of the tetracycline transactivator. As stated above, we will be cloning our new validated human AII siRNA into a vector for the construction of stable cell lines, but no new work with the tetracycline transactivator is planned at this time.

Task 3.c   Growth Inhibition and Apoptosis

Our initial attempt to inhibit the growth of LNCaP cells with a chemical inhibitor was done using NOHA (*N*-hydroxyarginine), a compound known to block arginase. We examined native LNCaP cell proliferation alone compared with LNCaP cells in the presence of 1mM NOHA. Using a hemocytometer, we counted the number of cells over a 5-day period. Unfortunately, there was no apparent difference in LNCaP cell proliferation in the presence of NOHA (Fig. 12). This result did not come as a complete surprise since NOHA has been shown to have no effect in certain cell lines (R. Singh, personal communication). Chemical inhibitors of arginase are useful tools to have, but specific molecular targeting of AII such as siRNA is more advantageous. As mentioned earlier, we will be looking at growth inhibition studies using LNCaP cells in the presence of our AII siRNA.
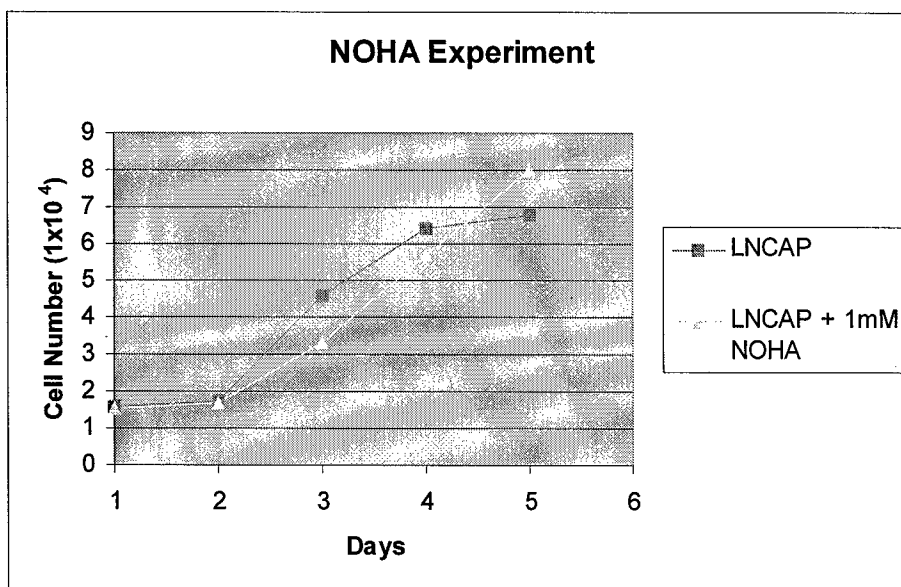
NOHA Experiment

Fig. 12. Affect of chemical arginase inhibitor on proliferation rates. Compared cell numbers over a period of 5 days between untreated LNCaP cells and LNCaP cells in the presence of 1mM NOHA. No apparent difference in proliferation rates between the two samples was seen.

We are in the process of obtaining ORI 1202 from Oridigm Corporation, a polyamine transport inhibitor compound previously shown to be functional in LNCaP, PC3, and DU145 cells for reducing the rate of cell proliferation when combined with DFMO (difluoromethylornithine, an inhibitor of ornithine decarboxylase, the rate-limiting enzyme in the polyamine synthetic pathway) (Devens et al. 2000; Weeks et al. 2000). Interestingly, their results showed that DFMO or ORI 1202 alone had no effect on cell growth, but had a very significant effect when used in combination. It is our hypothesis that NOHA and ORI 1202 may show a similar effect when used in combination on various cell lines and would be highly interesting for the purposes of this project. If silencing arginase expression inside the tumor cells decreases the intracellular production of polyamines, their growth may be unaffected if they are able to simply transport exogenous polyamines from the media into the cell. Therefore, the blockage of polyamine transport is a necessity for our cell culture studies.

Task 4.a   Arginase Overexpression

As already stated, we have created, validated and studied several stable arginase overexpression prostate cancer cell lines. We believe that AII plays a significant role in cell proliferation in prostate cancer cells. Initial proliferation studies were performed to compare the growth properties of native LNCaP cells with LNCaP cells overexpressing AII. Cell numbers were counted using a hemocytometer over a period of 5 days. We observed a modest increase in cell proliferation when AII was overexpressed; however, the result was not dramatic. We feel this observation is due to the rapid basal rate of proliferation of native LNCaP cells under no manipulation and the already high levels of endogenous AII. Therefore, overexpressing AII to the degree possible with this approach may not be enough to show a significant increase in cell

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proliferation. However, knocking down AII using the AII siRNA or NOHA plus ORI 1202 may be more effective in such proliferation assays. We will also perform similar proliferation and inhibition studies using the DU145 cell lines we have created.

Perhaps most interesting will be the PZ-HPV-7 cell lines which overexpress AII that we are creating. Validation and expression analysis of the stable clones are currently in progress. We will soon be performing proliferation and invasion assays comparing native PZ-HPV-7 cells with PZ-HPV-7 cells overexpressing AII. Our hypothesis is that overexpression of AII in a normal prostate cell line may cause the cells to become transformed and they may take on more cancerous properties such as an increase in cell proliferation and an increased invasive capacity. The latter will be studied using Matri-Gel invasion assays which are being established at this time.
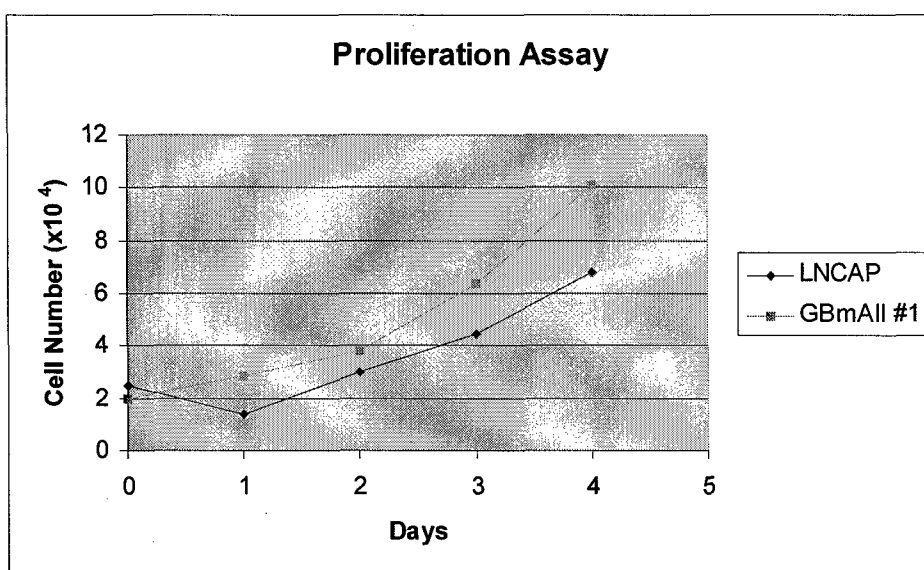

Fig. 13. Proliferation assay. Cell numbers were compared over a period of 5 days between untreated LNCaP cells and LNCaP cells overexpressed with mAII. A modest increase in LNCaP proliferation rate was observed when AII is overexpressed.

Task 5   Nude Mice and Human Samples

As stated previously in Task 1.b, our immunohistochemistry data on human tumor samples suggest that elevated arginase activity may be an important marker for early-stage carcinoma rather than late-stage metastatic cancer. We also plan to study whether this is reflected by the arginase levels in serum of early-stage and metastatic prostatic cancer patients. This has been delayed somewhat because our internal source of serum samples which we planned to use (UCLA Tissue Procurement Facility) no longer has access to these samples, and we must obtain them from outside (an NCI-funded, multi-center biomarker project).

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Our proposed breeding studies with the TRAMP transgenic prostate cancer mouse model were delayed over this past year due to a persistent *Helicobacter* infection in the UCLA Vivarium which has necessitated re-derivation of the mouse strains. After this, we were able to acquire a new TRAMP mouse from Jackson Laboratories and have mated it to a wildtype male mouse and already have pups; therefore, we should have several clean TRAMP mice with which to continue our crossbreeding studies with the AII knockout mouse and also to cross with the AI/AII double knockout. We would like to have *in vivo* results to support our hypothesis that arginase II is important for prostate cancer progression and that the lack of AII expression using our knockout mice would have a significant effect on the rate of formation and/or invasive behavior of prostate tumors in the TRAMP mice. In fact, one recent report suggests a dependence on polyamines for tumor growth in these mice (Kee *et al.* 2004), and our studies would established the mechanism involved.

Our proposed studies involving transplantation of arginase overexpressing and knockdown cell lines into nude mice were not planned until Year 3, and will thus be developed in the next few months.

Key Research Accomplishments

• Construction of stable arginase-overexpressing prostatic cell lines, characterized at both the RNA and protein levels

• Localization of arginase-II expression to specific cell types in human prostate tumors, and correlation of expression levels with tumor grade

• Validation of small interfering RNAs (siRNA) for arginases I and II, and demonstration of significant expression knockdown in prostate cancer cell lines

• Initial cell growth/proliferation assays to measure the effect of these alterations

• Establishment of an effective approach for measurement of cell and tissue polyamine levels, and initial correlation with arginase expression

• Experience with microarray hybridization technology which can be applied to these studies

• Initiation of cross-breeding experiments between the prostate cancer-prone TRAMP mouse and our AI, AII, and AI/AII double knockout mouse strains.

Reportable Outcomes

We plan to submit an abstract for the upcoming American Society of Human Genetics meeting describing the arginase expression results in the various prostate samples, cell lines, and tumor grades. This will be followed by a publication once the remainder of the knockdown studies are completed. We have also had a poster accepted for presentation at a conference this summer of the Stop Cancer Foundation. We anticipate several other abstracts and papers to be developed as data accrue during Year 3.

Conclusions

Our progress during Year 2 has served to further validate and now apply the unique molecular reagents in our hands to the questions of arginase expression in prostate cancer tissues and cell lines. We have localized expression of the critical arginase-II isozyme to glandular elements and stroma of the prostate, and shown that it increases in lower-grade tumors before diminishing with evolution to higher grades. We have developed the means to manipulate AII to overexpression or knockdown in prostatic cell lines, and related the effects to cell proliferation and polyamine synthesis. And we are embarking on the proposed Year 3 *in vivo* studies in mouse models.

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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