Role of Pigment-Epithelial-Derived Factor in Metastatic Prostate Cancer

A Paper Presented for Completion of Honors Project

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To the Honors Committee:

I am submitting this paper by Melodie Blackmon entitled "Role of Pigment-Epithelium-Derived Factor in Metastatic Prostate Cancer". I have read the final form and content of this manuscript and recommend that it be accepted in partial fulfillment of the requirements for an Honors Project with a major in Biology.

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Abstract

Prostate cancer is a hormone-sensitive cancer influenced by testosterone and is the second leading cause of cancer-related death in men living in western countries. Approximately 241,740 men in the United States were diagnosed with prostate cancer in 2012, and, of those, ca. 28,170 died from this cancer[1]. A combination of Docetaxel/Prednisone is the current chemotherapeutic standard of treatment for patients with metastatic prostate cancer; most patients have an initial response to the treatment but within 16-18 months, in one-half of the patients, cancer develops a resistance to the treatment. Pigment-Epithelial-Derived Factor (PEDF) is a secreted glycoprotein that is a potent antiangiogenic factor and is widely expressed in normal tissue, but, is down regulated in prostate cancer tissue. Pigment-Epithelial-Derived Factor initiates apoptosis, blocks angiogenesis, and initiates differentiation of cancer cells. It has been shown that PEDF limits Interleukin-8 production, and it is thought that expressing PEDF in cancer cells in combination with a low dose of Docetaxel is a more effective method of treatment of prostate cancer than Docetaxel alone.

Anatomy of Prostate Gland

The prostate gland, the largest accessory gland in the body of the human male, is a walnut shaped gland 4 cm x 1.5 cm x 3 cm, and has a mass of 30-40 g[1]. The prostate surrounds the proximal portion of the urethra, just inferior to the urinary bladder (Fig.1). The prostate is composed of a connective tissue framework, smooth muscle, and glandular tissue.

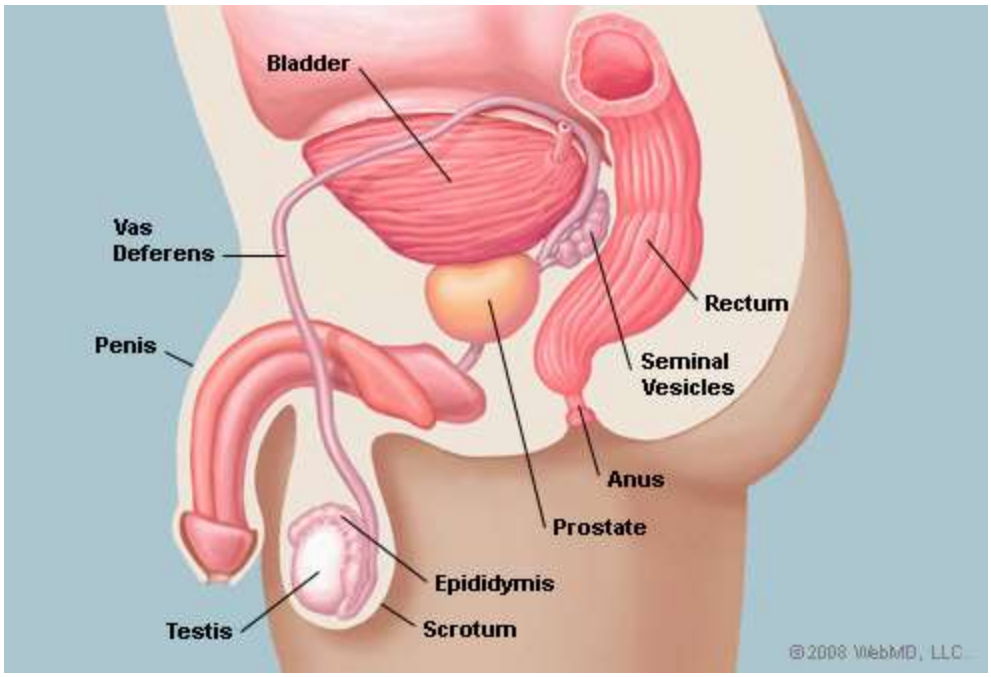

Figure 1. Diagram of male reproductive anatomy depicting the prostate. Accessed from men.webmd.com 14 Nov. 2012

Surrounding the prostate gland is a framework of supportive connective tissue with bundles of involuntary muscle. The connective tissue framework has an external fibroelastic envelope, the capsule proper, and a medium septum which extends inward from the capsule separating the glandular tissue from the muscular tissue. (Fig.2).

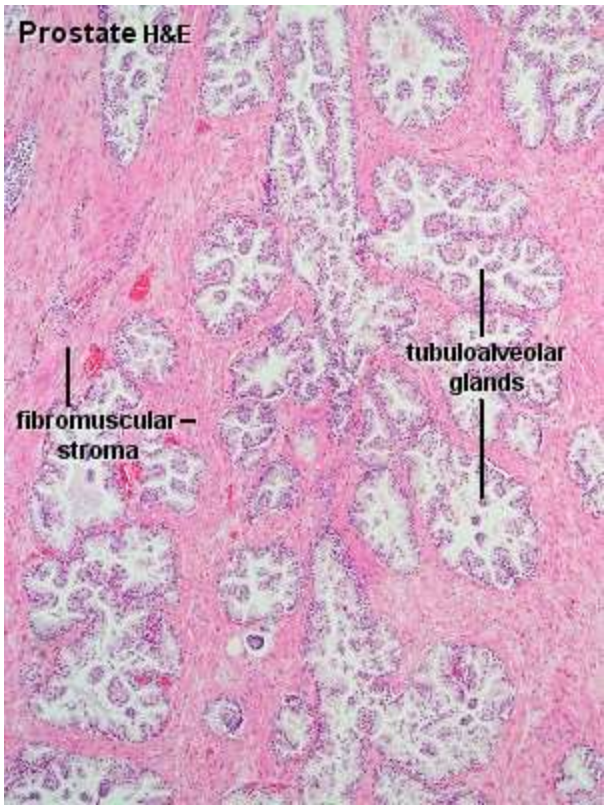

Figure 2. Prostate tissue, depicting the tubuloalveolar glands and fibromuscular stroma. Accessed from: http://www.lab.anhb.uwa.edu.au/mb140/corepages/malerepro/malerepro.htm Accessed 14 Nov. 2012

The prostate gland is composed of 30-60 smaller tubuloacinar prostatic glands. The prostatic glands are arranged in three concentric glands: mucosal gland, submucosal gland, and peripheral gland. The mucosal glands secrete directly into the prostatic urethra, while the submucosal and peripheral glands deliver their secretions through ducts that open into the prostatic sinuses on the posterior wall of the urethra (Fig.3). The secretions of these glands include acid phosphatase, citric acid, fibrinolysin, amylase, and other proteins. An identifying

characteristic of prostatic glands is prostatic concretions. Prostatic concretions are formed from aggregations of dead epithelial cells and precipitated secretory products[3].

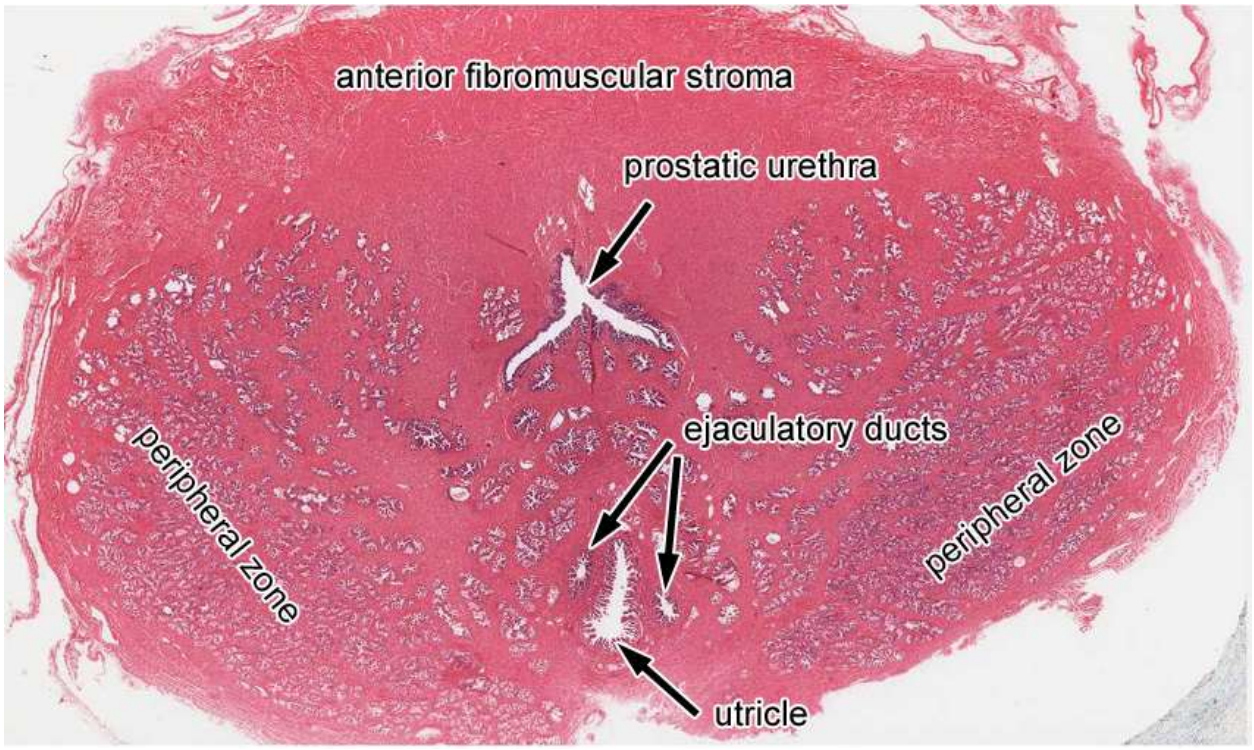

Figure 3. prostate tissue depicting the prostatic urethra. Accessed from: http://histology.med.umich.edu/medical/male-reproductive-system Accessed 14 Nov 2012

While each layer of the prostatic glands is primarily made up of pseudostratified columnar epithelium, the cells that make up the glandular tissue do vary. In some regions, the glandular tissue may be squamous or cuboidal epithelium[1]. The epithelium of the prostatic glands contains distinct cell types, including the secretory and basal cells. Neuroendocrine cells, cells that integrate the nervous system and the endocrine system, are also found, but are rare and scattered throughout the acini and ducts of the glands.

Secretory cells comprise the exocrine portion of the prostatic epithelium. These cells are characterized by columnar-luminal cells that synthesize and secrete proteins into the glandular lumen. The apical portion of these cells projects into the lumen, and the basal portion rests on the basal cells and basement membrane. These cells are androgen dependent, thus requiring testosterone for their survival[5]. Except for testosterone, derived from adrenal androgens, I am not aware of any information concerning the effect of other adrenal androgens on the prostate gland.

Basal cells lie beneath the secretory cells and appear as squamous epithelial cells. Basal cells can be distinguished in that they range from small squamous cells (area of 52-62 μm^2) with condensed amounts of chromatin and small amounts of cytoplasm, to cuboidal cells with larger amounts of cytoplasm and less condensed chromatin[7]. In the prostate, basal cells form a continuous layer that comprises the basement membrane. As opposed to secretory cells, basal cells are not androgen dependent, but do divide in response to androgens[3]. Because they respond to androgens, these cells may have a role in androgen dependent cancer.

Arteries supplying the prostate enter the periphery of the gland at various locations. Interlobular branches of the vessels follow the septa that divide the prostatic glands, and eventually break up into capillary networks that surround the acini[1]. Nerves of the prostate are primarily sympathetic fibers derived from the hypogastric plexus[1]. The nerves are distributed largely in the walls of the blood vessels and the smooth muscle. Additional nerve fibers are located in the glandular tissue outside the basement membrane of the acini[1].

Physiology of the Prostate Gland

Androgens are natural synthesized compounds that control the development and maintenance of male characteristics by binding to androgen receptors. Androgens are required for prostate development and normal prostate function. Testosterone, the primary circulating androgen, is produced from cholesterol in the testis. Testosterone can also be derived from androgens secreted by the adrenal gland and other tissues, including prostate tissue. Testosterone is transported to target tissues, and converted to 5α-dihydrotestosterone (DHT) by the enzyme 5α-reductase (Fig. 4). Dihydrotestosterone is more active than testosterone, and, has five times a higher affinity for the androgen receptors (AR) than testosterone[6]. Androgens engaging the AR regulate the proliferation of prostate cells through the cell cycle.

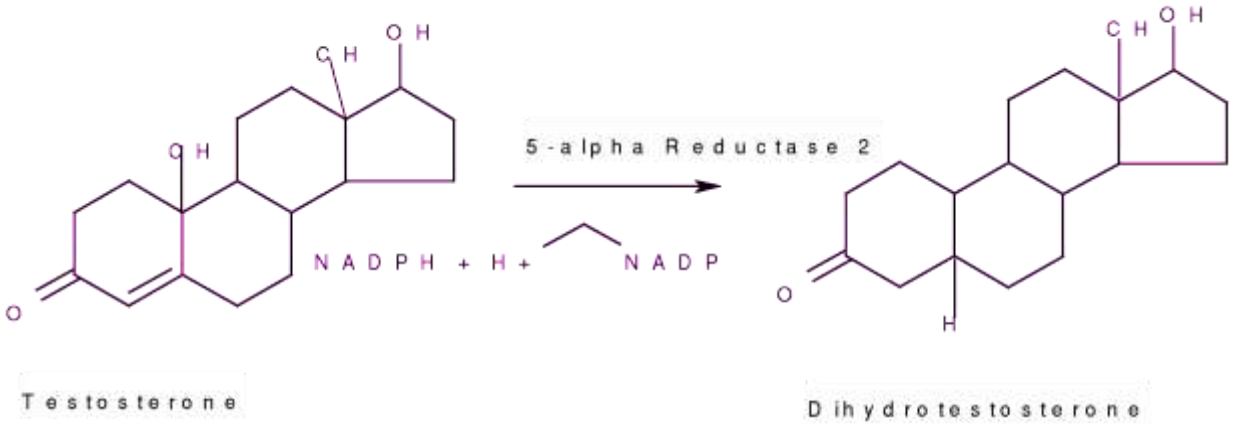

Figure 4. Conversion of testosterone to dihydrotestosterone by 5-alpha Reductase. Accessed from http://science.csustan.edu/stone/chem4400/sjbr/low2004.htm. Accessed 14 Nov 2012

The cell cycle is a series of events that takes place within a cell leading to the division of that cell. The cell cycle can be divided in to three phases: interphase, mitosis, and cytokinesis. Interphase is the period during which the cell grows and is prepared to divide. Mitosis is the process by which the nucleus and DNA are duplicated, and the cell splits in two through the process of cytokinesis. The end result of mitosis and cytokinesis is the production of two

daughter cells. In order for a cell to pass from interphase to mitosis it must pass specific "checkpoints". These checkpoints are in place to make sure that the cell is ready to proceed to the next phase of the cell cycle. Cancer is characterized by the uncontrolled division of cells. This occurs when the checkpoints in the cell cycle fail to work, and the cells continue to divide in the presence of damaged DNA.

Prostate cancer cell growth, through the process of cell division, depends on the ratio of cells proliferating to cells dying[6]. Androgens are the main regulator of this ratio by both stimulating proliferation and inhibiting apoptosis. This may be why prostate cancer depends on the crucial levels of stimulation by androgens for growth and survival. Androgen ablation causes cancer regression[4]. Without androgen, the rate of cell proliferation is lower and the rate of cell death increases, leading to extinction of these cancer cells[6].

Prostate Cancer

Prostate cancer is a hormone sensitive cancer influenced by testosterone, and is the second leading cause of cancer related death in men living in western countries[8]. Approximately 241,740 American men were diagnosed with prostate cancer in 2012, and of those, about 28,170 died from this cancer[2].

Two important characteristics of cancer are unregulated cell replication and angiogenesis. Angiogenesis is regulated by angiogenic inhibitors and inducers (Table. 1). The adult vasculature is typically maintained in a quiescent state by angiogenic inhibitors in the tissue matrix[10,11]. Angiogenesis, the formation of new blood vessels from pre-existing blood vessels, is necessary for the growth and metastasis of prostate tumors. Angiogenesis is regulated by the homeostasis of angiogenesis-inducing and angiogenesis-inhibiting factors[9] (Table. 1). In normal

tissues, the balance of these factors results in a quiescent vasculature. Pathological angiogenesis involves an increase in angiogenesis activators, such as platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and fibroblast growth factor (FGF), or a decrease in angiogenesis-inhibiting factors[12]. A wide variety of proteins are responsible for stimulating angiogenesis in prostate tissue. These proteins include VEGF and interleukin-8 (IL-8: Table 1)[11].

Table 1. Activators and Inhibitors of Angiogenesis in Cancerous Prostate Cells

Activators	Inhibitors
Vascular Endothelial Growth Factor A	Vascular Endothelial Growth Factor 1
Vascular Endothelial Growth Factor B	Angiotensin
Vascular Endothelial Growth Factor C	Plasminogen Activator 1
Vascular Endothelial Growth Factor D	Plasminogen Activator 2
Placenta Growth Factor Precursor (PLGF)	TIMP Metallopeptidase Inhibitor 1 (TIMP 1)
Angiopoietin 1 (AND 1)	Angiostatin
Platelet Derived Growth Factor (PGDF)	Endostatin
Transforming Growth Factor – Beta 1 (TGF B1)	Vasostatin
Fibroblast Growth Factor (FGH)	Tumstatin
Hepatocyte Growth Factor (HGF)	Arrestin
Monocyte Chemotactic Protein 1 (MCP-1)	Canstatin
Platelet Endothelial Cell Adhesion Molecule	Thrombospondin 1,2
Ephrins	Matrix Mettaloprotease Inhibitors (MMI)
Plasinogen Activators	Platelet Factor 4 (PF4)
Nitrous Oxide Synthase (NOS)	Prolactin
	Interleukin 4 (IL-4)
	Interleukin 12 (IL-12)
	Interleukin 8 (IL-8)

The progression of prostate cancer has been shown to be dependent upon angiogenesis[10,14]. In the adult rat, castration-induced prostate regression occurs in an orderly way in which decreased vascular endothelial growth factor (VEGF) levels, accelerated endothelial cell apoptosis, and decreased blood flow all precede epithelial apoptosis[14].

Conversely, testosterone stimulated prostate regrowth is preceded by endothelial cell proliferation and increased blood flow[14]. These are indicators that prostate growth is dependent on angiogenesis.

One angiogenesis activator is IL-8. Interleukin-8 is a multifunctional chemokine that influences angiogenesis. IL-8 belongs to the Glutamic acid-Leucine-Arginine Cysteine-X-Cysteine (ELR+CXC) chemokine subfamily[15] and is involved in inflammation mediated neutrophil infiltration, angiogenesis, and chemotaxis[16,17]. Normal prostate epithelial cells produce low amounts of IL-8[16]. However, IL-8 is overproduced in prostate cancer cells[16]. One way in which IL-8 promotes the progression of prostate cancer is by inducing the expression and activation of androgen receptors. Seaton et al.[18] showed that IL-8 signaling increases androgen receptor (AR) expression. The prostate cancer cell line, LNCaP, was stimulated with 3nM IL-8 *in vitro*. Initially, no response was seen, but after 16 hours, a 4.1-5.8 fold increase in AR expression was seen (Fig. 5a) [18].

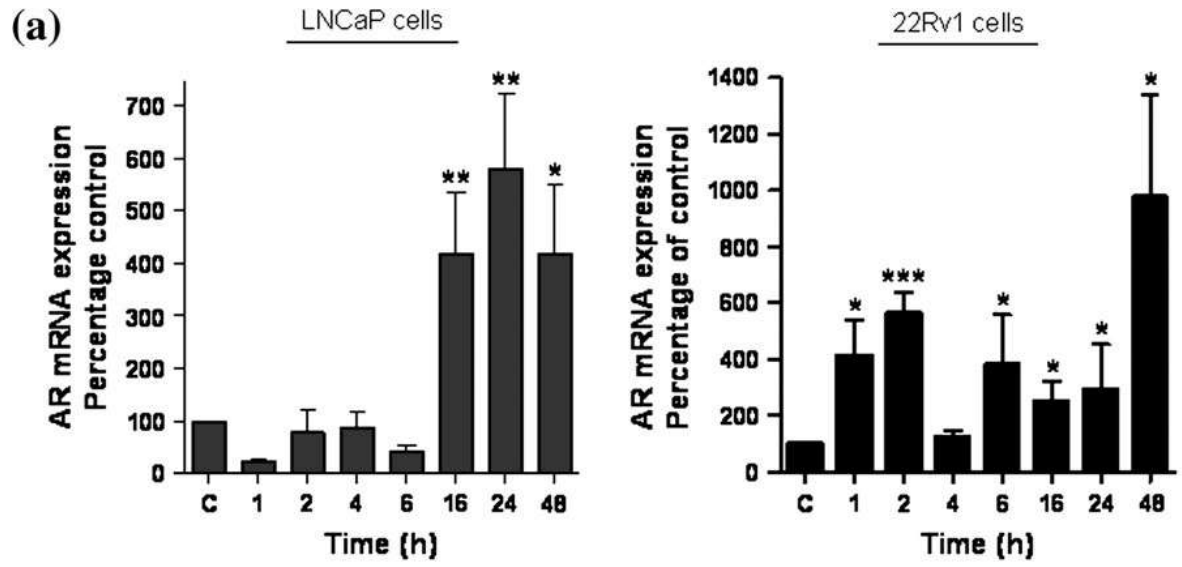

Figure 5. Bar graphs showing increase in AR expression with addition of IL8. Accessed from Carcinogenesis 2008.

Interleukin-8 has also been shown induce androgen independent growth in LNCaP and 22Rv1 cancer cell lines[18]. Treatment of LNCaP cells with IL-8 for 48 hours resulted in a dose- dependant increase in the growth of the cells, with a 17, 26, and 45% increase at 1, 3, and 10nM IL-8 respectively[18]. Flow cytometry experiments were also conducted to determine whether the increase in proliferation rate of the cells was accompanied by an increase in the percentage of cells entering the S phase (DNA synthesis) of the cell cycle[18]. Stimulation of LNCaP cells with 3nM IL-8 for 24 hours resulted in a 2.3 fold increase in the percentage of cells in the S phase. This data indicates that treatment with IL-8 results in an increase of cells that are duplicating their DNA in the S phase of the cell cycle.

Treatment: Castration

Historically, the standard first line treatment for prostate cancer usually involves androgen deprivation through castration[19]. Castration can involve a bilateral orchiectomy, removal of the testicles the primary site of androgen production, or can involve biochemical castration with luteinizing-hormone-releasing hormone (LHRH). Studies have shown that these treatments are 80% to 90% effective initially, but that after a median of 22 months, the cancer progresses to the castration refractory stage[13].

Prostate cancer that progresses after castration is called castration-refractory-prostate cancer, or metastatic-prostate cancer, and frequently metastasizes to the lymph nodes and bone[20]. Progression of the cancer to the metastatic phenotype requires alternative treatment methods which can include symptomatic care with narcotic analgesics, radiotherapy to alleviate bone pain. Treatment with bone-seeking isotopes such as strontium-89, and cytotoxic chemotherapy

are also used[21]. Metastatic-prostate cancer and its treatment with chemotherapy will be the focus of the remainder of the paper.

There are at least two causes for prostate cancer progression to the metastatic phenotype. The first cause is a change in the regulation of the androgen receptor (AR) due to increased sensitivity for androgens by over expression of nuclear coactivators[13]. A second cause leading to the progression of prostate cancer is the loss of phosphatase-and-tensin homolog gene (PTEN) with activation of the phosphatidylinositol 3-kinase-Akt pathway (Fig.6), leading to the release of B cell lymphoma 2 (BCL-2), a protein that regulates apoptosis, resulting in cell survival[13].

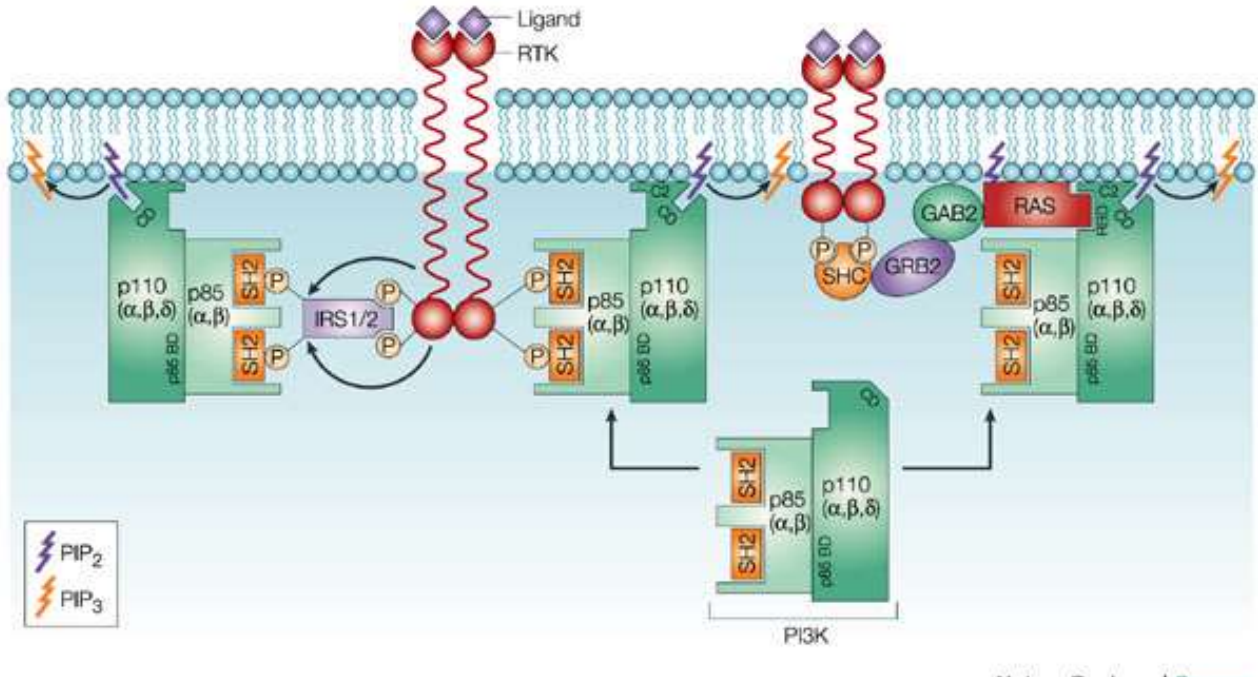

Figure 6. Phosphatidylinostiol 3-kinase-Atk pathway. Accessed from: http://www.nature.com/nrc/journal/v2/n7/fig_tab/nrc839_F2.html

Treatment: Chemotherapy

Prostate cancer was considered to be resistant to chemotherapy until the mid 1990's[10,20]. The first chemotherapy drug approved for the treatment of metastatic prostate cancer was Mitoxantrone which was given in combination with Prednisone, and was shown to have a role in the palliative treatment of metastatic prostate cancer[7,20]. Mitoxantrone is a synthetic antineoplastic chemotherapeutic agent and is a potent inhibitor of topoisomerase II, an enzyme responsible for repairing damaged DNA. Mitoxantrone works by causing damage to the DNA of T-cells, B-cells, and the prostate cancer cells. The damage to the DNA prevents these cells from proliferating, but suppresses the immune system. The suppression of the immune system may be one reason for the increased risk of infections. Prednisone is a glucocorticoid prodrug that is converted to 11-beta-hydroxysteroid dehydrogenase in the liver. Prednisone is used in combination with chemotherapeutic agents such as Mitoxantrone and Docetaxel to increase appetite, decrease nausea and vomiting, and to reduce inflammation, thus offsetting the energy depleting effects of the chemotherapy and making the treatment more tolerable for the patient. Studies showed that this treatment was successful in relieving pain and improving the quality of life in patients with prostate cancer, but did not improve overall survival rate[8].

Tannock et al[21] randomized 161 hormone refractory patients into two groups, one receiving Prednisone alone, and the other receiving Mitoxantrone plus Prednisone (10mg Mitoxantrone daily). Patients receiving the Mitoxantrone plus Prednisone achieved a statistically significant improvement of symptoms, including pain, compared with the patients who received Prednisone alone. Patients in the Mitoxantrone plus Prednisone group also reported secondary improvements in a six component quality-of-life-scale, with pain relief being the greatest improvement. The study showed that the treatment of Mitoxantrone plus Prednisone was

effective in improving the patient's quality of life, but showed no improvement in patient survival rate[11].

In 2004, the U.S. Food and Drug Administration approved the use of Docetaxel as the first line treatment method for patients with metastatic prostate cancer[11]. A combination of Docetaxel/Prednisone is the current chemotherapeutic standard of treatment for patients with metastatic prostate cancer[9]. Docetaxel is a semisynthetic taxane that delivers its effects primarily by binding to and stabilizing β-tubulin in the cell's microtubules during the G_2-M phases of the cell cycle; thus preventing the microtubules from disassembling. Disassembly of the microtubules is a precursor to cell division. Because the microtubules are stabilized, they cannot disassemble, and the cells cannot divide. This restricts the cell cycle of rapidly mitotic cells.

Docetaxel also functions through the phosphorylation of the apoptosis-regulator protein BCL-2[10]. This protein has been found to be over expressed in patients with prostate cancer, and leads to the decreased rate of apoptosis[12]. Docetaxel phosphorylates BCL-2, rendering it inactive, thus, allowing for apoptosis to occur[12]. When apoptosis occurs, tumors fail to grow uncontrollably. When compared to the treatment of Mitoxantrone plus Prednisone, Docetaxel was shown to give patients an average increased survival rate of three months[17].

Most patients have an initial response to the treatment with Docetaxel[12]. However, within 16-18 months, one half of the patients develop a resistance to this treatment[7]. Studies indicate an increased resistance is due to an over expression of P-glycoprotein (P-gp), a multidrug resistance associated membrane protein[8,23]. P-glycoprotein is a membrane transporter gene in human cells that mediates the efflux of drugs out of the cell. P-glycoprotein effluxes a variety of hydrophobic, neutral, and positively charged drugs from the cell[19]. When

over- expressed: P-gp leads to increased efflux of Docetaxel, and is correlated with decreased survival and poor prognosis (Fig.7)[7,22]. Because the Docetaxel is being transported out of the cell, it is unable to work inside of the cancer cells, allowing progression of the cancer.

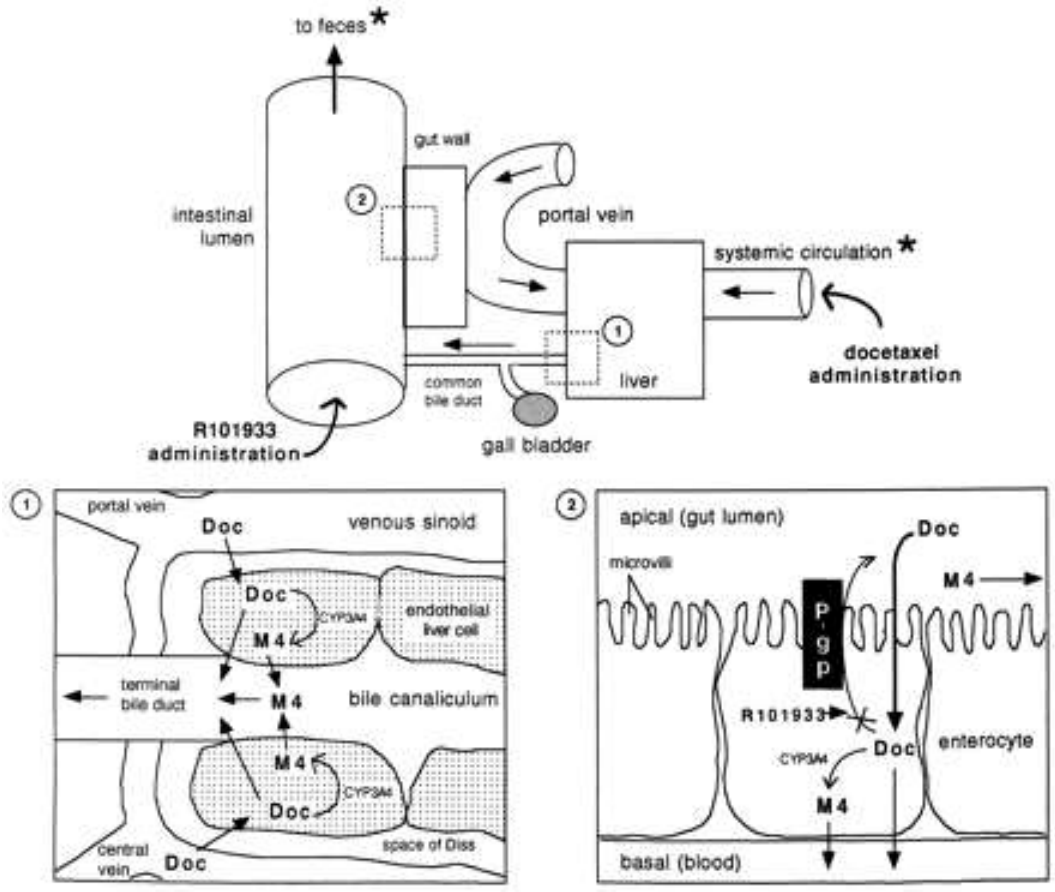

Figure 7. Schematic representation of the role of intestinal P-glycoprotein in docetaxel metabolism and elimination in humans. 1. Hepatic metabolism of Docetaxel 2. intestinal epithelium showing reputake of Docetaxel into lumen. Accessed from:cliniccancers.org 10 Dec. 2012

Resistance to Docetaxel results in this drug becoming an ineffective treatment. Resistance to Docetaxel develops due to mechanisms essential to the biology of prostate cancer or to more general mechanism of drug resistance intrinsic to different tumor types[9].

One general mechanism of drug resistance is impaired drug distribution[9]. Cancer cells form their own blood vessels through angiogenesis that alters the vasculature of the tissue. This altered vasculature leads to a poor distribution of the drugs within the cancerous tissue. This may be due to loss of cohesion in the tight junctions of the endothelium. Tight junctions create a barrier that separates not only the apical and basal compartments within an individual epithelial cell, but also to the external environment from internal body tissues. It is possible that the tight junction proteins are altered in prostate cancer cells, or that chemotherapeutic drugs weaken the tight junctions. It is possible that this could be related to the function of Pigment-Epithelial-Derived Factor (PEDF; discussed later).

One intrinsic drug mechanism is continued signaling by the androgen receptor even after castration has taken place[22]. Activation of the AR by adrenal androgens stimulated proliferation and inhibits apoptosis of prostate cancer cells, resulting in tumor growth and progression[20].

Another intrinsic mechanism is increased number and structural changes of the AR[7]. Increased expression, greater stability and nuclear localization of AR in metastatic prostate cancer are hallmarks of a hypersensitive AR which can be activated by lower concentrations of androgens. Over-expression of AR is necessary for growth of prostate cancer cells. Increased transcription of AR gene and persistence of AR were found in the nuclei of the metastases of men with metastatic prostate cancer. Over-expression of the AR may be due to amplification of the wild type AR gene, which occurs in approximately 30% of metastatic prostate cancer[7].

Vessels within cancerous tissue have variable structure and flow that leads to impaired delivery of nutrients, oxygen, and cancer drugs. Delivery of drugs is impaired because the drugs must be delivered through the vascular system and must penetrate tumor tissue through diffusion. Increased interstitial fluid pressure due to the leaky, disorganized vasculature coupled with poorly formed lymphatic drainage also impairs drug delivery in cancer tissues[19]. Due to the poor delivery of oxygen, cancer cells often develop hypoxic areas, which tend to be resistant to anticancer drugs. Multiple mechanisms may be involved in hypoxia induced resistance, including inhibition of cell proliferation, a hypoxia induced decrease cytotoxicity of some agents, and tissue acidosis[29].

Another mechanism of Docetaxel resistance can be linked to the production of IL-8. Interleukin-8 decreases the prostate cancer cells sensitivity to the cytotoxic effect of Docetaxel and is one of the reasons for drug resistance in metastatic-prostate cancer. The mechanism by which IL-8 decreases the cells sensitivity to Docetaxel is unclear[14]. Reactive oxygen species (ROS), generated either extracellularly or intracellularly through ligand receptor interactions, can function as signal transduction molecules to activate IL-8[30]. One way that may prevent the activation of IL-8 is by administration of antioxidants. Antioxidants work by breaking down ROS's, rendering them inactive. Because of the high rate of resistance to the current therapy, there is a current search for a more effective method of treatment for metastatic prostate cancer.

One area of research includes the study of Pigment-Epithelial-Derived-Factor (PEDF), a 50-kDa secreted glycoprotein present in many human tissues. Pigment-Epithelial-Derived Factor is a potent anti-angiogenic factor, was first discovered in 1991 as a factor secreted by the pigment epithelium of the human eye. It increases neuronal survival and differentiation, and blocks angiogenesis by inducing endothelial cell death[8, 23]. It is possible that endothelial cell

death is induced by interactions with the cell's tight junctions. Pigment-Epithelial-Derived Factor has been shown to be down regulated in tissues exhibiting prostate cancer where its loss has been linked to metastatic phenotype and poor prognosis[6].

Pigment-Epithelial-Derived Factor operates on cancer cells through the initiation of apoptosis, blocking angiogenesis and initiating differentiation of cancer cells[6]. Although the mechanism by which PEDF induces apoptosis is still unknown, it is thought that apoptosis is initiated through up-regulation of the Fas Ligand (FasL),which engages inducer-generated Fas receptor causing apoptosis[10,26]. Pigment-Epithelial-Derived-Factor is a selective inhibitor of angiogenesis in that it targets only new vessel growth while sparing the preexisting vasculature[24, 25]. Pigment-Epithelial-Derived Factor may work by strengthening the tight junctions in preexisting vasculature surrounding the cancerous prostate cells, thereby improving the vasculature in the prostate and improving the delivery of drugs to the tissue.

It was shown that re-expressing PEDF in rats delayed the growth and invasion of lung carcinoma, hepatocellular carcinoma, melanoma, and glioblastoma[7]. Moreover, decreased PEDF levels in the metastatic prostate adenocarcinoma in rats and humans compared with the non-metastatic disease imply that the loss of PEDF contributes to the progression of a metastatic phenotype[7]. It was shown that PEDF over expression in melanoma, prostate, and ovarian carcinoma, pancreatic cancer, Wilm's tumor, and neuroblastoma cell lines led to drastic reductions in tumor burden and metastasis, suggesting PEDF may be a promising therapeutic agent[26].

Pigment-Epithelial-Derived Factor works is by limiting the production of IL-8 in prostate cancer tissues. It is known that in one line of human prostate cancer cells, PC3 cells, expression

of PEDF reduced the production of IL-8 by 50%[8]. Because IL-8 leads to prostate tumor proliferation, it was hypothesized that by reducing the production of IL-8, PEDF would inhibit the proliferation of prostate tumors. After prostate cancer cells were treated with PEDF, IL-8 production was decreased and consequently, tumor size was reduced by 15% in the PC3 cell line. A negative correlation has been found between concentrations of PEDF and IL8 (Fig. 8)[19]. As the concentration of PEDF in the cell increases, the concentration of IL-8 decreases.

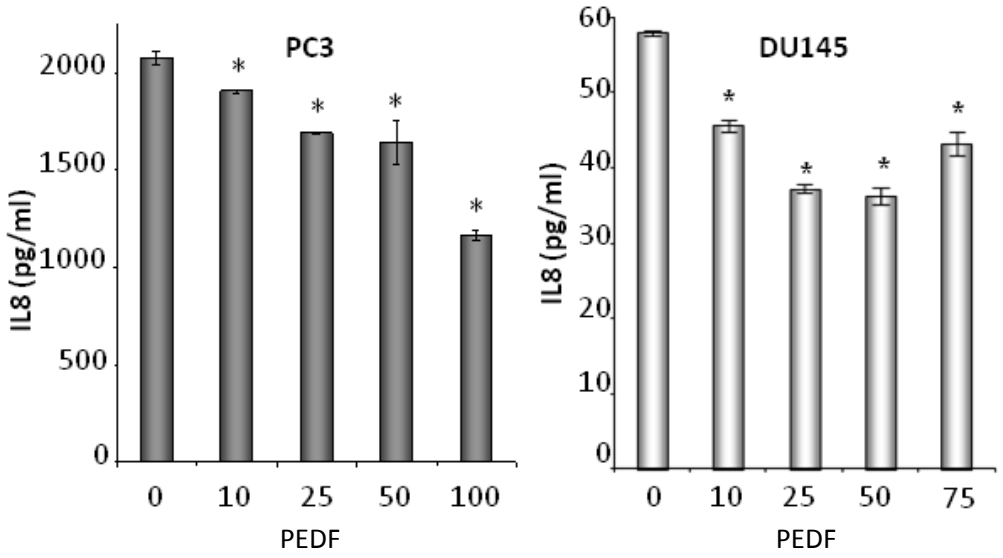

Figure 8. Relationship between concentrations of PEDF and IL8. Accessed from: J. Hirsch Cytokine[17]. Accessed 1 Dec 2012.

PEDF may delay the emergence of the development of Docetaxel resistance by inhibiting IL-8 production. As previously mentioned, the main reason for Docetaxel resistance is reduced cell sensitivity to the drug. With the addition of PEDF, cells would have increased sensitivity to the chemotherapy and the treatment would continue to be successful.

Current research on the effectiveness of PEDF/Docetaxel has been conducted using epithelial cell lines. I was involved in this research at Texas Tech University Graduate School of Biomedical Sciences using PC3 E13, PC3 P19, CL1 E10, and CL1 P15 cancer cell lines (unpublished data). Cells were counted using hemacytometer and were seeded 7,800 cells per well in 24 well plates. Two plates per cell line were seeded and incubated for 48 hours in incubator. After 48 hours, media was removed from the wells and 1 mL of serum free RPMI 1640 media was added to each well; the cells were then incubated for 5 hours. Cells were treated with varying concentrations of Docetaxel (0nM, 1nM, 5nM, 10nM, 25nM, 50nM, 100nM, 250nM, 500nM, 750nM, 1,000nM) and put plates in incubator for two days. After two days media was removed and 1mL of 2% Glutaraldehyde was added to each well and incubated for 10 minutes at room temperature. Glutaraldehyde was removed and 1mL of PBS 10X was added to wash cells. After removing PBS 1mL of .1% crystal violet was added to each well and the plates sat for 40 minutes. The cells were de-stained and photographs were taken of the cells in order for a growth curve to be conducted. It was shown that the cells that had PEDF grew at a slower rate (unpublished data). These results support previously published data showing that PEDF improves sensitivity to treatment with Docetaxel.

Conclusion

Prostate cancer, the second leading cause of death in men living in western countries, is a disease that continues to plague men daily. Research is currently being conducted in attempts to increase survival rates via treatment. One branch of research shows that PEDF can enhance treatment with Docetaxel leading to better chemotherapeutic agents for the treatment of prostate cancer and increased survival rates. Determining the underlying mechanisms for drug resistance is integral in the development of new treatment methods.

Research has been recently published that suggests some alternative causes for increase in testosterone levels leading to the increase of prostate cancer[2]. It was shown that men in modernized western countries have higher testosterone levels than those in developing countries[2]. One of the proposed reasons is due to a variation in nutritional status. Men in developed countries have more access to food, which eliminates energy constraints[2]. The diminished energy constraints allow these men to support higher levels of testosterone in the systems.

In a study using birds, it was shown that birds experience an increase in testosterone during breeding season[2]. In monogamously mating bird species, mating effort decreases after a mate is found and the male birds move in to a parenting role; at this time the testosterone level falls back down to normal levels[2]. Polygynous birds never experience the drop in testosterone levels because these birds continue to compete for mates throughout the breeding season. This suggested that the rise in testosterone was elicited by the aggressive competition between the birds[2]. These results are also applicable to primates[2].

Men in westernized areas often experience high levels of male-male competition, and the nature of this aggression leads to the support of higher testosterone levels. Aggressive social environments affect prostate cancer incidence through the responsiveness of male androgen physiology to competition[2]. The increased testosterone may lead to a higher incidence of prostate cancer, thus making PEDF relevant for treatment.

Pigment-Epithelium -Derived Factor may work to limit the production of IL-8, thereby improving the patient's reaction to the Docetaxel. Future research could be conducted to determine the role of PEDF/Docetaxel on angiogenesis in endothelial cells. This can be done *in*

vitro by treating the endothelial cells with PEDF/Docetaxel, and *in vivo* by using immunofluorescent staining to quantify blood vessels in tumors.

One of the benefits of doing the proposed research *in vivo* is being able to see the effects of PEDF on endothelial cells. The cell lines that were studied at Texas Tech were epithelial cell lines. Because PEDF works to inhibit angiogenesis in the body, this effect would most likely be seen on endothelial cells. There could be several drawbacks to doing this *in vivo* research. One drawback could be the ethical issues involved in using test rats. Another issue could be the increased cost that would come from using living specimens.

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