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MURINE PROSTATE CANCER DEVELOPMENT AND $\alpha_V\beta_6$ INTEGRIN EXPRESSION IN THE PTEN-NULL MOUSE MODEL OF PROSTATE CANCER

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ABSTRACT

Prostate cancer is projected to account for 27,000 deaths in the U.S. in 2007 (Jemal et al, 2007). Although much research has been conducted, the exact mechanisms of prostate cancer initiation and development are unknown. This MQP assessed the development of prostatic intraepithelial neoplasia (PIN) and adenocarcinoma, versus the expression of $\alpha_V\beta_6$ integrin which has previously been implicated in other types of cancer signaling, at different time points in the specific background of PTEN-null mice, a model for prostate cancer. It was determined that integrin $\alpha_V\beta_6$ was expressed in some stages of both PIN and adenocarcinoma.

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BACKGROUND

Prostate Cancer

Prostate cancer, the leading cause of new cancer cases in men (excluding skin cancer), is projected to account for 218,890 new cases in 2007 (Jemal et al., 2007). Many treatment options exist for prostate cancer and newer targeted treatments are being developed based on current research (Pomerantz and Kantoff, 2007). However, prostate cancer is still projected to account for over 27,000 deaths in 2007.

Risk Factors

Different factors can affect susceptibility to prostate cancer including age, ethnicity, hormonal issues, diet and genetic factors (Gronberg, 2003; Crawford, 2003 Schaid, 2004; Hsing and Chokkalingam, 2006). Age is a major risk factor for prostate cancer; the majority of cases are diagnosed in men 65 and older (Gronberg, 2003; Crawford, 2003; Hsing and Chokkalingam, 2006). Ethnicity is another important factor; Asians have the lowest incidence, and African Americans have the highest (Crawford, 2003; Gronberg, 2003). Residents of China have the lowest reported rates of prostate cancer (Hsing and Chokkalingam, 2006; Gronberg, 2003). This disease is not common in Asia and South America; however, it is common in Europe and North America (American Cancer Society, 2007). Many scientists hypothesize a combination of environmental factors, genetic differences and life style differences among ethnicities account for the different rates of prostate cancer in these populations (Gronberg, 2003; Hsing and Chokkalingam, 2006).

Much research has been conducted regarding prostate cancer and dietary factors; unfortunately, many of these studies show conflicting results. Some studies suggest that an intake of animal and red meat increases the risk of prostate cancer (Hsing and Chokkalingam, 2006). Some studies have found selenium and vitamin E to have anti-cancer effects (Hsing and Chokkalingam, 2006). High calcium intake has been epidemiologically linked to prostate cancer (Sonn et al, 2005). Some studies have shown anti-cancer effects of green tea and soy protein (Sonn et al, 2005). Further research is needed to confirm these and other claims (For review see: Crawford, 2003; Sonn et al, 2005; Hsing and Chokkalingam, 2006).

Genetic factors and family history has been heavily investigated as possible causes of prostate cancer. Case control studies have shown men with a first degree relative with prostate cancer are at higher risk, however, this data may be affected by referral bias (Schaid, 2004). The high values obtained from these studies may have also been affected by the high prevalence of prostate cancer. Twin studies indicate that genetic factors may influence prostate cancer susceptibility (Hsing and Chokkalingam, 2006) and suggest that this susceptibility is more complex than rare autosomal dominant mutations (Schaid, 2004). However, some control studies suggest the susceptibility may be autosomal dominant in nature (Schaid, 2004; Hsing and Chokkalingam, 2006). Linkage studies have implicated different chromosomal regions including PCAP, HPCX, CAPB, HPC20, HPC2, ELAC2 and a region on chromosome 8 (8p22-23) (Schaid, 2004; Hsing and Chokkalingam, 2006). Despite all of the genetic research related to prostate cancer, the disease has not been linked to one single gene. However, some studies suggest that different mutations may affect the aggressiveness of prostate cancer and that these

mutations may not be the same as those that confer susceptibility to the malignancy (Schaid, 2004). However, further research is needed to confirm these results.

Androgens and Prostate Cancer

Androgen hormones are essential to prostate development and may play a role in prostate cancer (Hsing and Chokkalingam, 2006). Androgen target gene expression is regulated by the Androgen receptor (AR) which, upon binding to testosterone in the cytoplasm will translocate to the nucleus and will recognize DNA sequences in the promoter or enhancer regions of androgen genes to either up or downregulate their expression (Jenster, 1999). AR can also be activated by growth factors in an androgen independent manner (Jenster, 1999). AR and androgens are involved in normal development as well as cancer growth. Linkage studies suggest a link between AR activity and age of prostate cancer onset (Jenster, 1999). Many studies report AR mutations in prostate cancer. These mutations can alter specificity of the ligand, inhibit AR, and some mutations do not affect the function of AR *in vitro* (for a Review see: Jenster, 1999). The inhibition of the AR pathway may prevent differentiation helping to lead towards oncogenesis. Decreasing the ligand specificity will allow AR to be more easily activated allowing for growth and providing a growth advantage under low androgen conditions such as patients undergoing androgen ablation therapy (Jenster, 1999). Either of these methods may contribute to prostate cancer, though the exact mechanism or mechanisms are unknown. AR can be activated in both a ligand-dependant and ligand-independent manner (see Figure 1); activation of AR can promote growth and survival in the prostate and AR activation may be involved in prostate cancer (Scher et al, 2004). Androgen and AR play an important role in prostate cancer development; therefore,

different levels of expression between individuals may affect susceptibility to prostate cancer (Hsing and Chokkalingam, 2006).

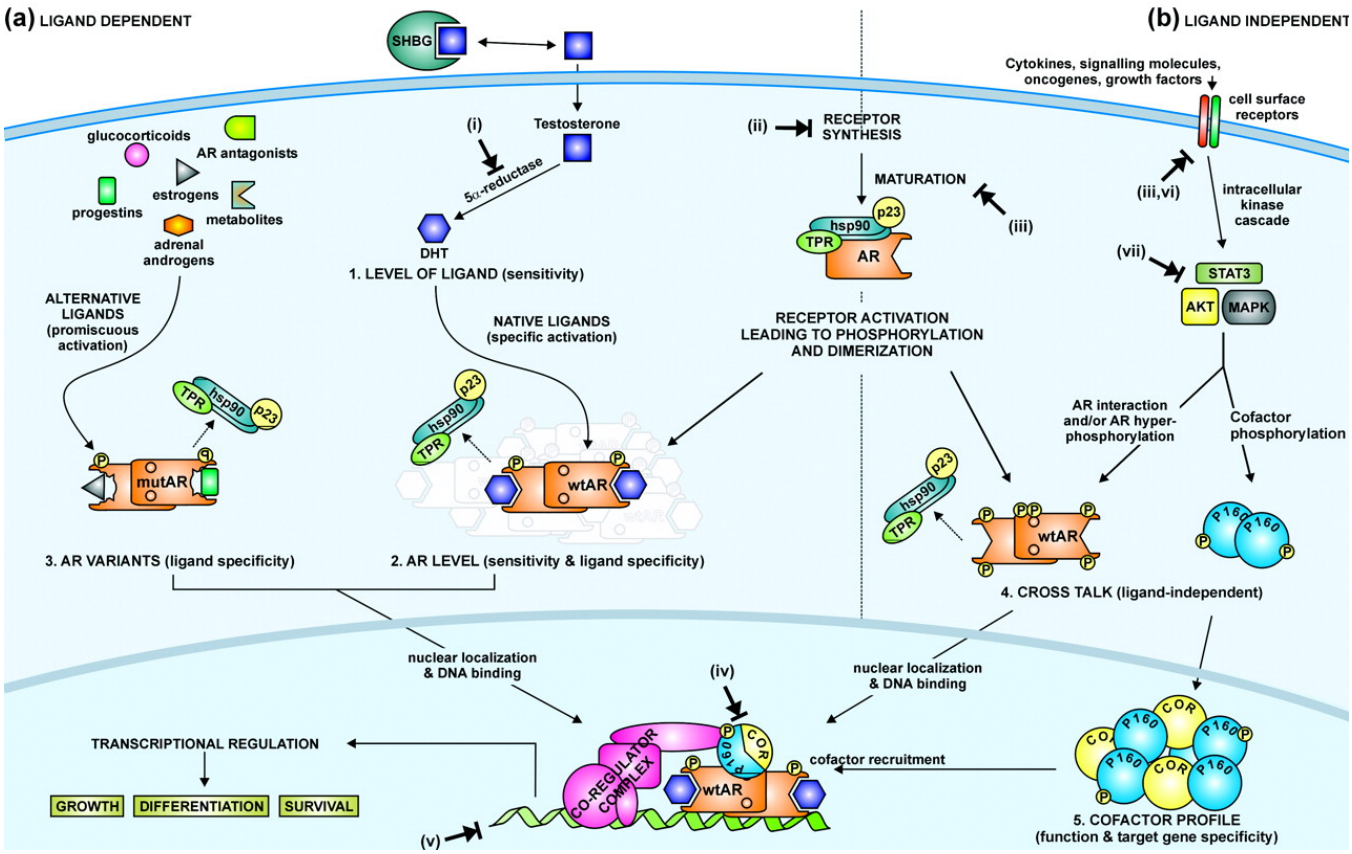

Figure 1. AR Signaling in Prostate Cancer. AR can be activated by different mechanisms; androgens such as testosterone can activate AR in a ligand-dependent manner. Different mutations can allow other molecules to also activate AR. AR can also be activated in a ligand-independent manner through cross-talk with other signaling molecules. The activation of AR promotes survival, growth, and differentiation (Scher et al, 2004).

AR is also known to interact with PTEN, a tumor suppressor often lost in prostate cancer. *In vitro* PTEN directly combines with AR preventing nuclear translocation (Lin et al, 2004). The AR levels are also decreased through PTEN-mediated degradation (Lin et al, 2004). Another group found PTEN inhibition of AR to be AKT dependent in LnCaP cells (Nan et al, 2003). These findings have implications for hormone-refractory prostate cancer. Jiao et al (2007)

presented data suggesting that the tumor growth in PTEN-null cell lines is due to AR activity. However, further research is needed to fully understand the role of this PTEN and AR interaction in an animal model.

Detection and Treatment

This disease usually develops later in life. Health professionals use a rating system of four stages to rate the progression of prostate cancer (NCI, 2006). Stage 1 prostate cancer is local and not detectable by imaging or direct exam. Stage 2 denotes more advanced cancer progression than stage 1 but the cancer growth is still limited to the prostate. Stage 3 prostate cancer has spread to other areas near the prostate. Stage 4 prostate cancer is metastatic and has spread to areas outside the prostate such as the lymph nodes or bladder. On a molecular level, lesions known as prostatic intraepithelial neoplasia (PIN) develop prior to adenocarcinoma; PIN lesions usually develop prior to invasive cancer (Abate-Shen and Shen, 2000). Prostate inflammatory atrophy (PIA) may be a precursor to PIN and prostate cancer. In some cases PIA can progress to PIN and cancer, however, not all cases of cancers are preceded by PIA (De Marzo et al, 2004). Some mutations found in PIN and adenocarcinoma are also present in PIA (De Marzo et al, 2004).

Prostate cancer detection is commonly accomplished through digital rectal examination and detection of serum prostate specific antigen (PSA) (Stanford et al, 1999). Despite its name, expression of this protein is not specific to the prostate (Reviewed in Gao et al, 1997). These methods combined provide better results than only performing either test (Gao et al, 1997). The PSA test is not specific to prostate cancer; it is detectable in patients with Benign Prostate

Hyperplasia (Gao et al, 1997). The PSA test may also lead to overdiagnosis, diagnosis of cancers that are not clinically significant, or to not diagnosing some instances of prostate cancer (Hernandez and Thompson, 2004; Shah et al, 2005; Harris and Lohr, 2002). Using the ratio of free PSA/total PSA may increase the accuracy of this test (Basso et al, 2000).

Many groups are currently investigating the causes of and possible alternative treatments for prostate cancer. The current treatment options vary based on the stage of the cancer and patient age when detected (Stanford et al, 1999; NCI, 2006). Widely used treatment options include monitoring, surgery, radiation, and hormone therapy. Clinical trials are currently testing new methods including cryosurgery, chemotherapy, biologic therapy, and high-intensity ultrasound, among others. Radical prostatectomy is the most common treatment used for localized prostate cancer (Stanford et al, 1999). Radiation and no treatment (monitoring) are common in older men (Stanford et al, 1999).

In Vivo Prostate Cancer Models

One important aspect of all medical research is the availability of a reliable animal model. Many mouse models currently exist for prostate cancer.

TRAMP Model

The Transgenic Adenocarcinoma of the Mouse Prostate (TRAMP) developed in 1995 by Greenberg and co-workers used a rat probasin promoter to express the semian virus 40 (SV40) oncogene. This construct promoted the expression of both the large and small T antigen in the

prostate of TRAMP mice. TRAMP mice develop fast-growing tumors, and adenocarcinoma can be detected as early as 20 weeks of age (Greenburg et al, 1995).

LADY Model

Another mouse model (LADY) used a SV40 construct which only expressed the large T antigen (Kasper et al, 1998). The different lines of this transgenic mouse have different rates of cancer development ranging from 10-30 weeks (Powell et al, 2003). Powell and coworkers (2003) reviewed the different animal models available for prostate cancer; they noted the LADY and TRAMP models promote faster cancer development than is common in the slow developing human disease.

PTEN-null Model

The PTEN-null model used in this MQP study utilized a method of prostate specific Cre-recombination developed by Wu and co-workers (2001). This method uses the control of the prostate specific ARB2PB promoter to delete specific genes in the prostate using the Cre-Lox system. In the Cre-Lox system, the Cre recombinase protein recognizes specific LoxP sites and DNA recombination occurs (Abremski and Hoess, 1984; Sauer and Henderson, 1988). A conditional knockout of the PTEN tumor suppressor in the prostate was created using this technique by flanking the PTEN gene with LoxP sites (Wang et al, 2003). In this case the Cre protein is placed under the control of the ARB2PB promoter making the knockout specific to the prostate. This tumor suppressor is often inactivated in prostate cancer cell lines and tumors (Cairns et al, 1997; Vliesta et al, 1998). This tumor suppressor is required for embryonic development (Cristofano et al, 1998); therefore, a conventional knockout of PTEN is not

possible. The prostate cancer that presents in these mice is antrogen-independent and does not respond to castration (Wang et al, 2003). The authors reported metastasis to the lymph node and lung. Wang et al (2003) claimed adenocarcinoma formation occurred in as little as 9 weeks, and that the cancer progression modeled the human disease better than previous models.

Previous Work with the PTEN-null Model

Some previous research involving the expression of specific proteins and markers has been published in this model, see Table 1. Note the expression of known PIN and adenocarcinoma markers such as Ki76 and P63. Also note that anti-apoptotic markers have also been shown to be over-expressed in this model. The expression and localization of androgen receptor was also documented. Many other aspects of this model have yet to be evaluated and future research in this area is needed. Future research may reveal other molecules that play a role in prostate cancer signaling which could parallel the human disease.

Parameters	Molecule	method(s)
Stem cell marker	Sca-1 (3)	FACS
Cell cycle regulator	AR(1,2)	IHC
Cell cycle regulator	AR upon castration: Nuclear in normal and primary tumor; but diffuse cytoplasmic in recurrent (1,2)	IHC
Anti-apoptotic	Bcl-2 (3)	IF
Neuroendocrine	synaptophysin(1)*	IHC, IF
Neuroendocrine	synaptophysin(2)*	IHC
Neuroendocrine	Synaptophysin upon castration: Few in normal; more in primary tumor; many more in recurrent (1)	IHC
Cytoskeleton	CK5 (1,3)	IHC(1), IF(3)
Cytoskeleton	CK8 (1,3)	IHC(1), IF(1,3)
Cell proliferation marker	Ki67 (1,2,3,4)	IF(1,3),IHC(2)
Basal cell marker	P63 (3)	IF
Cytoskeleton	CK5 and CK8 upon castration: expressed in both primary and recurrent tumor (1)	IHC
homeobox gene	NKX3.1 (2)	IF
heterodimeric glycoprotein	Clusterin (2)	IHC
Bone extracellular structural Protein	Osteopontin (2)	Microarray
Signaling molecule	p-Akt (2,4)	IF(2), IHC(4)
Cyclin-dependent kinase inhibitor	p27Kip1 (4)	IHC

KEY: Present, upregulated, downregulated, * conflicting

IF= immunofluorescent IHC = immunohistochemistry

1: Liao,et al cancer res 67(15),2007 2:Wang,et al cancer cell 4, 2003 3:Wang,et al PNAS 103(5), 2006 4:Trotman et al Plos bio 3(1), 2003

Table 1. Previous Work in the PTEN-null Model of Prostate Cancer. The previously published work performed with this mouse model is shown. Molecules marked in red note upregulation compared to wild type, green denotes down regulation compared to wild type, and black indicates detection of expression in the model without comparison to wild type. Also note that some molecules have been analyzed after castration.

Integrins

Integrin Signaling

Integrins are transmembrane heterodimers consisting of an α and β subunit (Humphries and Newham, 1998; Hynes, 1999). Because of the multiple types of α and β integrin subunits, these subunits can combine in at least 24 different combinations (Hynes, 1999). Figure 2 shows a diagram of integrin structure. Integrins form a link between the ECM and the surface of cells (Humphries and Newham, 1998; Hynes, 1999). This integrin dependent adhesion has many effects on the cell.

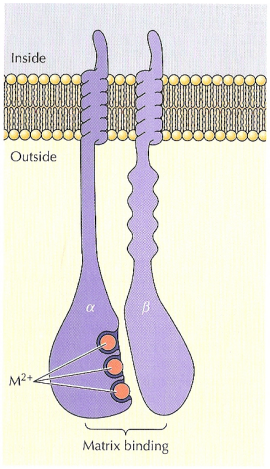

Figure 2. Diagram of Integrin Structure. (Cooper & Hausman, 2004)

Integrins have been shown to affect many signaling pathways. One method by which integrins affect signaling is by interactions with growth factors; this interaction is essential to growth factor signaling (Alam et al, 2007). MAP kinase activation also can occur as a result of integrin dependent cell adhesion (Chen et al, 1994). MAP kinase signaling is involved in cell proliferation and survival (Chang and Karin, 2001). Activation of cdk's, which are regulated by

integrins and growth factors, are required for cells to complete the G1 phase and enter S phase of the cell cycle (Giancotti and Ruoslahti, 1999; Assoian and Schwatz, 2001). Assoian and Schwartz (2001) proposed a model in which growth factors bound to integrins activate Rac and ERK leading to activation of cyclin D1 (see Figure 3). Cyclin D1 associates with cdk4/6 causing pRb-E2F to dissociate which activates cyclin A allowing the cell cycle to enter S phase during which DNA synthesis occurs (Cooper & Hausman, 2004). The mouse Rb protein has been sequenced (Bernards et al, 1989), and its expression occurs in the brain, kidney, spleen thalamus, and lungs of adult mice (Bernards et al, 1989).

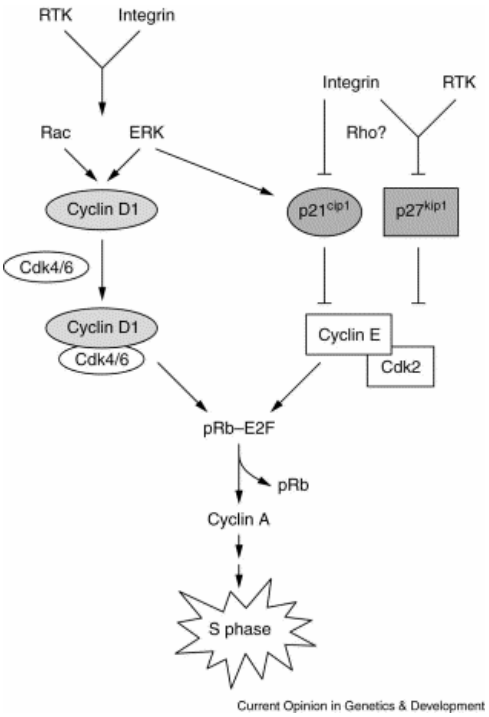

Figure 3. Integrin Signaling Pathways. Assoian and Schwartz, 2001.

Due to the role integrins play in cell cycle signaling, they have also been implicated in cancer signaling. Guo and Giancotti (2004) proposed a model in which FAK activation by

integrins promotes cell survival, proliferation, and migration through a signaling cascade. For example, Fridrichs and co workers (1995) linked the over-expression of integrin α_6 in human breast cancer to poor survival. β_1 and $\alpha_v\beta_3$ integrins are expressed in ovarian cancer cell lines and primary tumors (Cannistra et al, 1994). Integrins $\alpha_2\beta_1$, $\alpha_3\beta_1$, $\alpha_5\beta_1$ and $\alpha_6\beta_4$ are over-expressed in skin carcinoma (Reviewed in Mizejewski, 1999). Integrins are also under-expressed in some cancers such as integrins $\alpha_1\beta_1$, $\alpha_2\beta_1$ and $\alpha_5\beta_1$ in primary colon cancer (Reviewed in Mizejewski, 1999). Some of the β_1 integrin heterodimers are also over-expressed in some metastatic cancers such as melanoma, skin, and breast cancer (Reviewed in Mizejewski, 1999). In prostate cancer, integrins β_1 and β_3 are up-regulated and α integrins α_3, α_4, and α_5 are downregulated in prostate cancer (For Review see: Fornaro et al, 2001). This information has been discovered through immunohistochemical techniques. These expression patterns suggest that integrins play an important role in the growth and development of cancer.

Integrin $\alpha_V\beta_6$

The focus of this MQP study is the integrin $\alpha_V\beta_6$. Sheppard and co workers (1990) sequenced this integrin in both guinea pigs and a human pancreatic carcinoma cell line, while Arend and coworkers (2000) sequenced the mouse $\alpha_V\beta_6$ integrin. The domains of the human sequence are shown in Figure 4. Shepard and co workers (1990) found many similarities to previously sequenced beta integrins. Of particular interest was the cytoplasmic domain which contains the same sequence as β_1 and β_3 as well as 11 additional amino acids at the carboxyl end of the domain.

Figure 4. $\alpha_V\beta_6$ Domains. (Arend et al, 2000)

Agrez et al. (1994) showed that integrin β_6 can enhance proliferation of a cell line. They further showed that the 11 amino acid extension of the cytoplasmic domain is required for this effect. Other functions of the cytoplasmic domain such as cell adhesion and focal contact localization can function without this 11 amino acid portion (Agrez et al, 1994) but do not occur in the absence of the entire cytoplasmic domain (Cone et al 1994; Agrez et al, 1994). These functions are also performed by β_1 and β_3. This data shows the importance of the cytoplasmic domain in the functions of integrin $\alpha_V\beta_6$.

Integrin β_6 is known to bind to only one alpha subunit (α_V) (Goel and Languino, 2004). This integrin has been shown to bind to FN (Busk et al, 1992), tenascin (Goel and Languino, 2004), vitronectin (Goel and Languino, 2004), and osteopontin (Goel and Languino, 2004). This integrin is only expressed in epithelial cells and is not detectable in most resting epithelium, including lungs and skin (Bruess et al, 1993).

This integrin is expressed during wound healing in human skin grafts (Breuss et al, 1995), in the basal level of healing of human oral mucosa (Haapasalmi et al, 1996), and in full thickness skin wounds (Haapasalmi et al, 1996). However, *in vivo* over-expression of $\alpha_V\beta_6$ integrin can lead to skin lesions (Hakkinen et al, 2004). Another group showed that the over-

expression of $\alpha_V\beta_6$ in animals compromised by stress lead to faster healing times compared to stressed wild type animals (AlDahlawi et al, 2006). *In vivo* models and human pathological samples show upregulated $\alpha_V\beta_6$ expression during lung inflammation compared to normal lung tissue (Breuss et al, 1995). These results suggest $\alpha_V\beta_6$ plays a role in wound healing and inflammation.

Transforming Growth Factor Beta 1 (TGFβ1) is also known to interact with and become activated by integrin $\alpha_V\beta_6$ (Munger et al, 1999). Lack of this activation had been shown to confer disease susceptibility and lead to age dependent emphysema *in vivo* (Morris et al, 2003). It has been suggested that expression of $\alpha_V\beta_6$ integrin plays a role in TGFβ1 regulation (Sheppard, 2005). This growth factor can induce apoptosis, regulate cell growth, as well as function as a tumor suppressor (Akhurst and Derynck, 2001). However, TGFβ1's role of controlling cell growth is often lost in tumors even though TGFβ1 is present (Akhurst and Derynck, 2001). Other studies have also linked TGFβ1 over-expression to poor clinical prognosis (Akhurst and Derynck, 2001). TGFβ1 can actually support metastasis and tumor invasion (Akhurst and Derynck, 2001).

$\alpha_V\beta_6$ activating TGFβ1 reveals a possible role for $\alpha_V\beta_6$ in cancer progression. Integrin $\alpha_V\beta_6$ is known to have altered expression in different cancers and cancer models. Bates and co-workers (2005) showed that high expression of $\alpha_V\beta_6$ is a marker for cancer progression and poor clinical outcome in colon cancer. Their data indicated that $\alpha_V\beta_6$ is not expressed in normal colon cells but is highly expressed in invasive cancer. Another group found that Stat3 is commonly activated in prostate cancer and has been shown to mediate the activation of integrin $\alpha_V\beta_6$ *in*

vitro (Azare et al, 2007). Cell migration is affected by integrin $\alpha_V\beta_6$ in cells expressing stat3 *in vitro* (Azare et al, 2007). $\alpha_V\beta_6$ expression has been documented in carcinomas of the ovary, colon, pancreas, gastric region, breast and oral cavity (Reviewed in Thomas et al, 2006. With this evidence in mind this MQP will focus on the expression of the integrin $\alpha_V\beta_6$ and its role in prostate cancer development.

PROJECT PURPOSE

The purpose of this project was to analyze expression of integrin $\alpha_V\beta_6$ in the PTEN-null mouse model of prostate cancer. The initiation and progression of prostate cancer was also analyzed in this model by assaying the development of prostatic intraepithelial neoplasia (PIN) and adenocarcinoma. The main hypothesis tested is that $\alpha_V\beta_6$ will be highest at peak times of carcinogenesis in the model. This study will also assist in the development of studies testing the *in vivo* administration of different therapeutic compounds including those that block $\alpha_V\beta_6$.

MATERIALS AND METHODS

Primary Antibodies

The following antibodies were used in this study: anti-mouse integrin $\alpha_V\beta_6$ CH2A1 (Biogen Inc, Cambridge, MA) and anti-human IgG control.

Mice

To generate *Pten*$^{LoxP/LoxP}$/ *PB-Cre4* mice: ARR2Probasin-Cre transgenic mice PB-Cre4 on Balb/cB6 background (Wu et al, 2001) were crossed to Pten$^{LoxP/LoxP}$ mice on a 129/Balb/c backgound (Wang et al, 2003). The male offsprings with *Pten*$^{LoxP/+}$/ *PB-Cre4* genotype were then crossed to Pten$^{LoxP/LoxP}$ females to get male Pten$^{LoxP/LoxP/}$ *PB-Cre4* (target) and Pten$^{LoxP/LoxP}$ (littermate control) mice. These *Pten*$^{LoxP/LoxP/}$ *PB-Cre4* mice were designated as *Pten*$^{pc-/-}$. We used only F2 generation male offspring for this study. All mice were maintained under specific pathogen-free conditions, and experimental protocols were approved by the IACUC at University of Massachusetts Medical School in Worcester, MA. Prostate cancer was induced Cre-recombinase-induced excision of PTEN in prostate tissue. Mice were sacrificed at different time points to evaluate cancer progression and integrin expression. These mice were perfused with 4% PFA, and then fixed in formalin for twenty-four hours prior to dissection and paraffin embedding.

Mouse Tissue

PTEN-null murine prostate tissues were obtained by dissection. All sections were cut from paraffin- embedded blocks and placed on charged slides. All slides contained prostate tissue from mice with a prostate-specific Cre-recombinase-induced PTEN deletion at different ages.

Hematoxylin and Eosin Staining

Hematoxylin and Eosin (H & E) staining was performed following a protocol modified from one published in *Short Protocols in Molecular Biology, second edition* (1992). The slides were deparaffinized in xylene and then rehydrated using ethanol and Milli-Q filtered water. The slides were then stained in hematoxylin and eosin. Slides were dehydrated in ethanol and equilibrated in xylene. Cover slips were applied using permount.

Immunohistochemistry

Immunohistochemistry was performed following protocols provided by the Languino Lab at University of Massachusetts Medical School. Integrin $\alpha_V\beta_6$ staining used the pepsin protocol which follows: Slides were deparaffinized in xylene and then rehydrated in ethanol and Milli-Q filtered water. Endogenous peroxidase activity was blocked by incubation in 3% hydrogen peroxide for 15 minutes. Slides were washed twice in PBS. Sections were unmasked using pepsin in an incubator at 37 degrees for 5 minutes. Slides were washed twice in PBS. Advin and biotin blocking solutions (Vector) were applied to slides for 10 minutes each with two washes in PBS between applications. After 2 washes in PBS, slides were blocked in 0.25% casein (Vector) in PBS for 15 minutes. Slides were drained and primary antibody was applied to

all slides (200 µl / slide). The slides were left overnight at 4 degrees. The following day slides were washed twice in PBS. Biotinylated secondary anti-human IgG antibody was applied to the slides for 30 minutes at room temperature. Slides were washed twice and treated with strepto-advin peroxidase (1:200 in PBS) (Vector) for 30 minutes at room temperature. After 2 washes in PBS, slides were stained using di-aminobenzidine (DAB) for approximately 5 minutes or until staining developed. Slides were washed in running tap water and counter stained with hematoxylin prior to dehydration in ethanol and equilibration in xylene. Coverslips were applied using permount.

Review of Slides

All slides were reviewed by microscopy using an Olympus BX41TF optical microscope equipped with an EvolutionsMP 5.0 RTV digital camera attached to a computer. Digital images were captured using QCapture-pro software. H & E stained sections of prostate gland from each mouse were examined microscopically by a veterinary pathologist to include anterior, dorsal, lateral, and ventral lobes. The prostatic lobes were individually scored for the approximate percentage of each pathological stage (normal, prostatic intraepithelial neoplasia {PIN}, or invasive adenocarcinoma) present in that lobe. Immunohistochemically stained slides were reviewed in a similar manner with the percent of cells staining, and the intensity was scored by lobe. The intensity of the staining was rating using a system of +, ++ and +++ as previously described by Armes et al (1999). In this system + indicates weak pale brown weak staining, ++ indicates moderate brown staining, and +++ indicates strong dark brown staining.

RESULTS

Prostate Cancer Development in the PTEN-Null Mice

The rate of prostate cancer progression varied between animals. Prostatic intraepithelial neoplasia (PIN) and adenocarcinoma were detectable in all animals at the 11 week and later time points (Table 2). The earliest invasive cancer was seen in the 11 week animal. The variation between animals will be important to consider in future studies. More animals were not available because being a conditional knockout mice are breed at low yield making limiting availability. Most animals older than 20 weeks developed more than 25% cancer in at least one lobe. The 30 week animal presented cancer in almost all glands in the VP, DP, and LP.

Tag#	Age (weeks)	VP	DP	LP	AP	% of total AdCa	% of total PIN
2243	9.000	PIN	PIN	PIN	PIN	0	75
2245	12.143	normal	Normal	normal	Normal	0	0
2256	11.286	PIN	PIN	PIN	AdCa	10	80
2261	16.714	PIN	AdCa	AdCa	PIN	25	75
2246	17.000	PIN	AdCa	PIN	AdCa	<5	~100
2267	20.000	25% AdCa	30% AdCa	90% AdCa	10% AdCa	50	50
2296	21.429	25% Adca	20% AdCa	50% AdCa	10% AdCa	75	25
2269	24.286	10% AdCa	25% AdCa		100% PIN	10	90
2271	24.286	100% PIN	90% AdCa	75% AdCa	25% AdCa	50	50
2252	30.5714	90% AdCa	100% AdCa	100% AdCa		95	5

Table 2: PIN and Cancer Development in PTEN-Null Mice. In this table the percentage of total gland tissue that contain PIN and adenocarcinoma cancer is reported for each animal included in the study. These numbers were determined through review of slides which were stained with H & E. Note, blank fields denote that the lobe was not present in the section.

Figure 5 shows selected images of H & E stained tissue at various stages of prostate cancer. PIN is visible as an increased number of cells within a gland, while adenocarcioma is apparent with an increased amount of cells and stromal invasion.

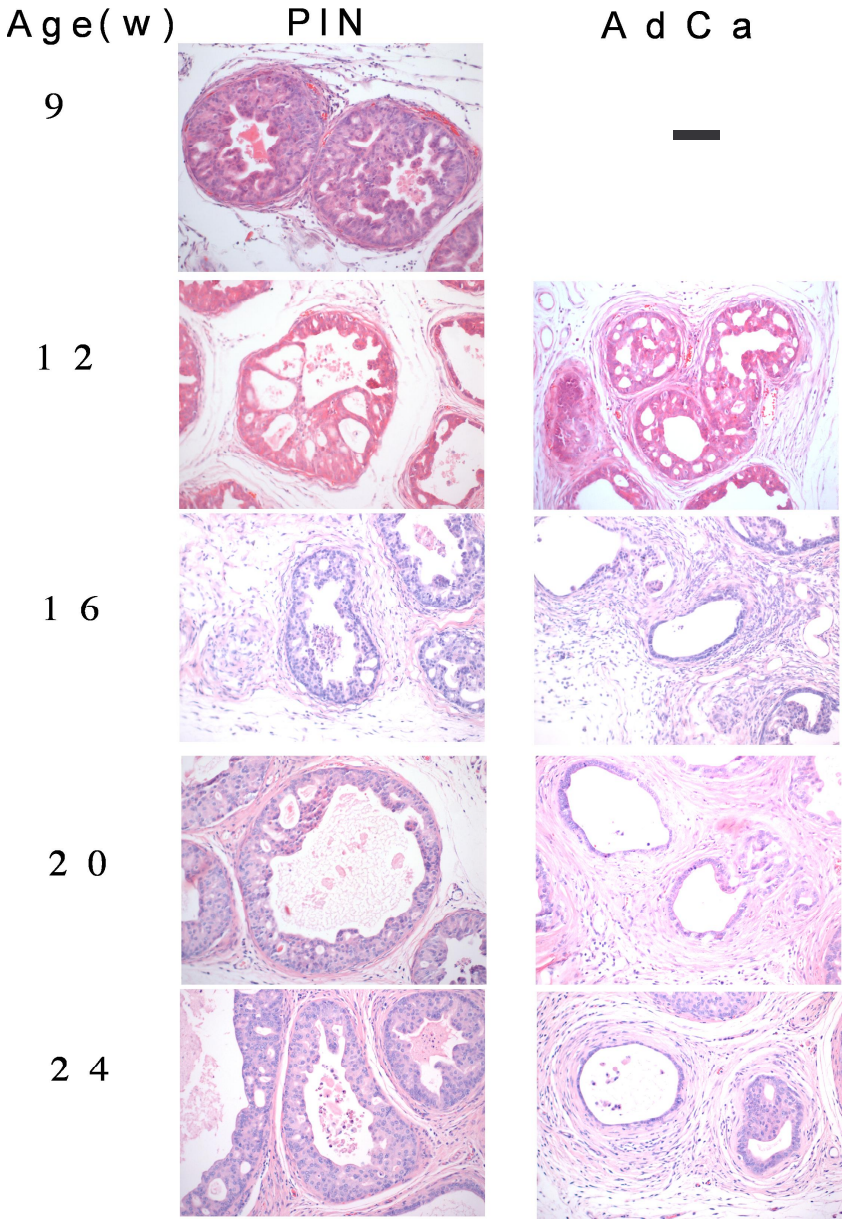

Figure 5. Selected H & E Images from PTEN-Null Mice at Different Ages. These images show examples of PIN (left column) and adenocarcinoma (right column) from animals at different ages. See Table 2 for the percentages of PIN and adenocarcinoma on each slide.

$\alpha_V\beta_6$ Expression

With respect to $\alpha_v\beta_6$ expression, the α_v subunit most frequently combines with β_6 in mouse prostate tissue, so monitoring the abundance of β_6 protein by immuno-histochemistry (IHC) is sometimes used as a surrogate for quantitating $\alpha_v\beta_6$. In most animals, a higher percent of cells stained positive for $\alpha_v\beta_6$ and the staining was more intense in adenocarcinoma than in PIN (Table 3, Compare Figures 6 and 7). The most intense and highest percent of $\alpha_v\beta_6$ staining of PIN glands occurred in the anterior prostate (AP). $\alpha_v\beta_6$ staining was also seen in PIN lesions and adenocarcinoma at earlier time points (data not shown). Note the limited $\alpha_v\beta_6$ staining in the PIN at 9 weeks versus PIN staining at later time points (Figure 6). Also note the increase in staining intensity between 16 and 30 week animals (Figure 7).

Animal		% of AdCa Cells Expressing $\alpha_V\beta_6$				% of PIN Cells Expressing $\alpha_V\beta_6$			
Tag#	**Age (w)**	**VP**	**DP**	**LP**	**AP**	**VP**	**DP**	**LP**	**AP**
2261	16.7143	No AdCa	50% ++	33% ++	33% ++	0	0	0	0
2246	17	50% ++	25% ++	10% +	10% +	0	>5%, +	>5%, +	5% +
2267	20	25% ++	90% ++	75% ++	90% ++	25% +	75% ++	75% +	75% ++
2296	21.4286	50% ++	50% ++	50% ++	50% ++	10% +	25% +	10% +	90% ++
2269	24.2857	10% +	50% ++		No AdCa	10% +	10% +		25% +
2271	24.2857	No AdCa	75% ++	75% ++	75% ++	10% +	50% +	No PIN	25% ++
2252	30.5714	90% ++	90% ++	90% ++		90% ++	No PIN	No PIN	

Table 3: $\alpha_V\beta_6$ Stain in PIN and Adenocarcinoma. This table shows the percent of PIN lesions and the percent of adenocarcinoma lesions staining positive for integrin β_6. AP, anterior prostate; DP, dorsal prostate; LP, lateral prostate; VP, ventral prostate. The intensity of the staining was rated using a system of +, ++ and +++ as previously described by Armes et al, 1999.

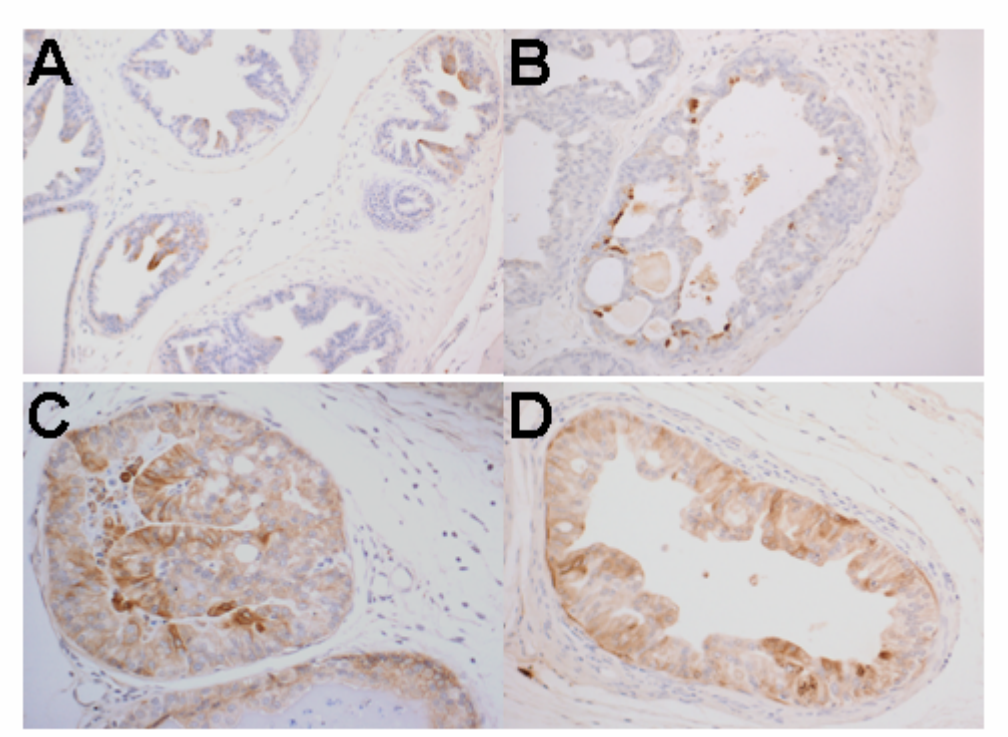

Figure 6. Selected IHC Images (β_6 staining) of PIN from 9, 20 and 24 Week Animals. Images of PIN in PTEN-null mice at 9 weeks (A), 16 weeks (B), 20 weeks (C) and 30 weeks (D). All images at 200X.

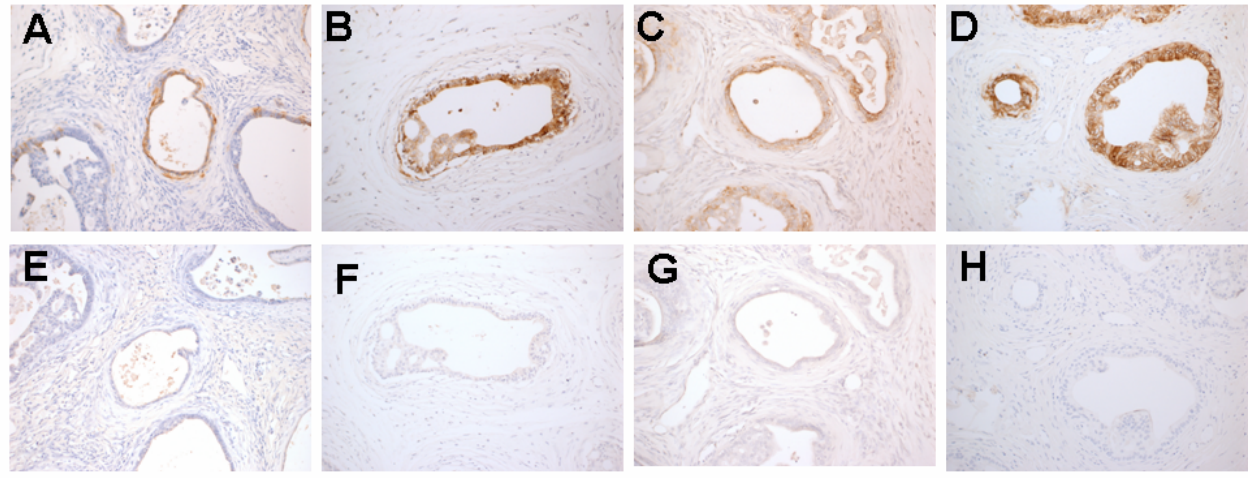

Figure 7. Selected IHC Images (β_6 staining) of Adenocarcinoma from 16-30 week Animals. These images are examples of adenocarcinoma staining for integrin β_6. The animals are 16 weeks (A and E), 20 weeks (B and F), 24 weeks (C and G) and 30 weeks (D and H) weeks old. All images are at 200X. Negative controls stained with an antibody to human IgG (E-H). For details on staining intensity see Table 3.

DISCUSSION

Therapeutic and Pharmaceutical Targets in Prostate Cancer

The data from this MQP demonstrate that β_6 integrin staining (and by deduction $\alpha_v\beta_6$ staining) increases in prostatic intraepithelial neoplasia (PIN) and adenocarcinoma tissues in a mouse model for prostate cancer. Because $\alpha_V\beta_6$ is expressed during prostate cancer but not in resting epithelium, this integrin may serve as a therapeutic or diagnostic target for this type of cancer; a test for integrin $\alpha_V\beta_6$ may assist in diagnosis of prostate cancer, while a drug that blocks the function of this integrin may slow cancer progression and prevent metastasis. If the effect of this integrin is limited to the prostate, it would have to be detected before the cancer has metastasized. However, this treatment might be helpful after radiation or surgery for preventing cancer from returning. Side effects of lowering $\alpha_v\beta_6$ expression would need to be considered, as this integrin plays an important role in wound healing. This concept could be tested in the PTEN-null model, or a similar *in vivo* model for prostate cancer.

Differences Between Mouse and Human Prostate Tissue

Another important aspect of this animal model that must be considered when interpreting the data from this project, is the morphological differences between human and mouse prostates. In mice, the prostate is composed of four distinct lobes: the anterior prostate (AP), the ventral prostate (VP), the dorsal prostate (DP) and the lateral prostate (LP); histological and biochemical differences exist between these lobes (Shappell et al, 2004). In some cases the DP and LP will be analyzed together as the dorsolateral lobe (DLP). The human prostate contains different zones that are histologically different, however, after development they are not discrete lobes

(Shappell et al, 2004). These zones include the anterior fibromuscular stroma, the periurethral transitional zone (TZ), peripheral zone (PZ) and the central zone (CZ) (Schappell et al, 2004). Cancer is often found in the PZ which contains about 75% of the prostate tissue (Schappell et al, 2004). Price (1963) proposed that the DLP is the mouse equivalent of the human PZ. Work by Berquin and co-workers (2005) found similarities in gene expression at the mRNA level between these areas through laser dissection followed by micro array analysis. When considering these differences, it is important to notice that the majority of cancer in this murine model was found in the LP and DP. The results and morphology in the AP were not the same as the other lobes. A higher level of $\alpha_V\beta6$ expression in PIN was observed than in the other glands, and less cancer developed in the AP than in other lobes (Table 2, data not shown). In the other lobes, more intense expression of αvβ6 integrin was correlated with more advanced adenocarcinoma. This difference may be due to differences in Cre expression, gene expression, and morphology between the AP and the other lobes of the murine prostate.

Variation Between Animals

In this study we also saw a variation in cancer development and PIN between animals of the same age. These animals shared the same genetic background and genotype, and were often littermates. It is important when considering data from human and animal studies that not all animals or humans are exactly the same. For this reason variation between animals could not be avoided in this study. Due to the limited number of animals available for this pilot study, only two mice were perfused at each of the later time points. Only one animal was available at the 30 week time point due to animals being used for other studies. When analyzing the data presented here it is important to note the differences among animals at the same time point (see Table 2).

Also note that one animal was perfused at 20 weeks and another at 21 weeks. We perfused the 21 week animal because the tissue from one of the animals that we perfused at 20 weeks was of poor quality, most likely due to poor fixation. Usable data was not obtainable from this tissue.

Cre Expression Variance

The Cre expression, and therefore removal of the PTEN tumor suppressor, is not equal in all lobes under this system. Wu et al (2001) showed the greatest expression to be in the lateral lobe. The lowest levels of Cre expression were seen in the dorsal and anterior lobes. Cre expression was not analyzed in the mice in this MQP due to the lack of availability of a reliable Cre antibody, however the data in this MQP showed the lowest amount of cancer in the VP and AP. The expression of Cre in the mice in this MQP may not be the same as the mice used in previous report due to the differences in genetic backgrounds.

In Vivo Perfusion as an Improvement

For this MQP, anesthetized animals, as by IACUC-approved protocols, were euthanized via perfused using an *in vivo* perfusion apparatus, and then dissected tissues were fixed in formalin for 24 hours. Perfusion was used because it has been shown to improve preservation of the ultrastructure and improve IHC results (Grabenbauer et al, 2001). Using perfusion-fixation followed by immersion in formalin rather than just immersion in formalin may have improved our results relative to previous studies.

Role of $\alpha_V\beta_6$ Integrin Expression in Adenocarcinoma and Transformation

The data from this MQP show that integrin $\alpha_V\beta_6$ is expressed in both PIN and adenocarcinoma. This integrin may play a role in transformation from PIN to cancer due to its ability to increase cell proliferation. This integrin has been previously reported to increase the growth of colon carcinoma cells due to an 11 amino acid sequence specific to this integrin (Agrez et al, 1994). The expression of this integrin during wound healing may also indicate a role in prevention of apoptosis and may promote cancer growth. The effects of integrins on the cell cycle may also be involved. The activation of TGFβ_1 by $\alpha_V\beta_6$ previously shown by Munger et al (1999), may also help promote tumor growth. All of the downstream effects of this integrin's expression may greatly affect the progression from PIN to adenocarcinoma and metastasis.

Based on the data presented here, a model of integrin $\alpha_V\beta_6$ expression has been proposed (See Figure 8). In this model, those animals with the PTEN tumor suppressor floxed, and the PBCre gene predominately expressed in the prostate, will develop PIN and adenocarcinoma as previously reported (Wang et al, 2003). However in the proposed model, note that not all animals express cre-recombinase (lower right side of figure). In the model also note that $\alpha_V\beta_6$ integrin is not expressed in normal prostate, and the level of $\alpha_V\beta_6$ integrin expression in the prostate increases as PIN and adenocarcinoma develop.

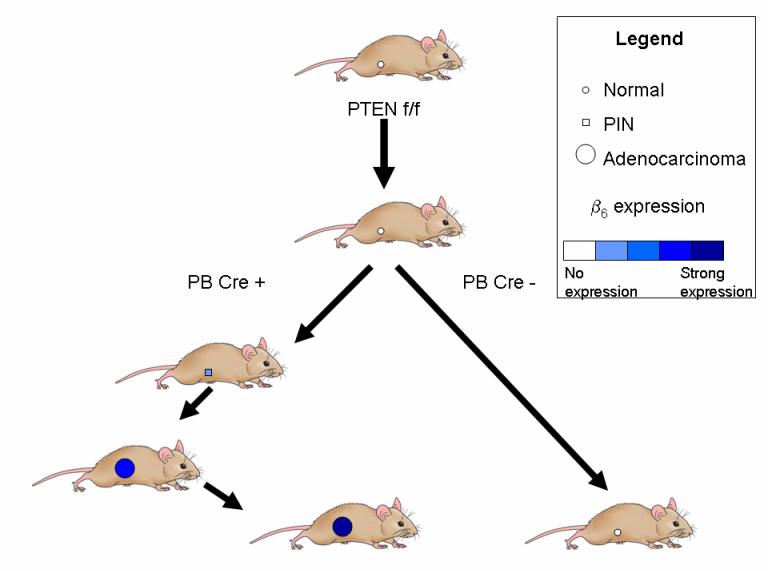

Figure 8. Proposed Model of Integrin $\alpha_v\beta_6$ Expression in PTEN-null Mice. In this model, PTEN-null animals first develop PIN (square box), which progresses to invasive adenocarcinoma (circles). As the PIN and adenocarcinoma progress, the level of integrin $\alpha_V\beta_6$ expression (blue) increases. In this model, $\alpha_v\beta_6$ expression is first detected with the advent of PIN, but becomes maximal during PIN conversion to an adenocarcinoma.

Based on our observations with $\alpha_V\beta_6$ integrin, a model is proposed (Figure 9) for the interaction of TGFβ1 with $\alpha_V\beta_6$ in prostate cancer. TGFβ1 was previously shown to interact with, and become activated by, integrin $\alpha_V\beta_6$ (Munger et al, 1999), so it is proposed here that $\alpha_V\beta_6$ upregulated in prostate cancer (as found in this project) interacts with inactive TGFβ1 causing its activation. The activated TGFβ1 then binds TFGβ1-receptor to induce cancer causing signal transduction events. Although previous studies have shown that TGFβ1 can induce apoptosis, regulate cell growth, and function as a tumor suppressor, other studies have shown that TGFβ1's role of controlling cell growth is often lost in tumors even though TGFβ1 is still present, and instead have linked TGFβ1 over-expression to poor clinical prognosis, metastasis, and tumor invasion (Akhurst and Derynck, 2001). Overproduction of TGFβ1 in

prostate cancer has been shown to promote tumor growth and has been linked to a higher rate of metastasis (Steiner and Barrack, 1992).

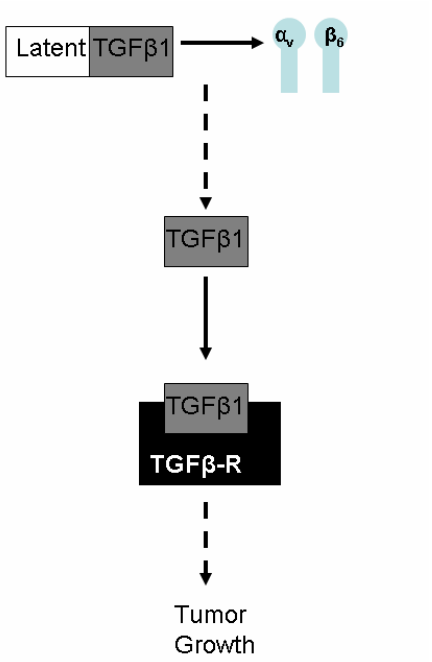

Figure 9. Proposed Model for the Interaction of TGFβ1 and Integrin $\alpha_V\beta_6$ Signaling in Prostate Cancer. Inactive TGFβ1 (upper gray box) directly interacts with (solid arrow) αvβ6 (light blue) to activate TGFβ1 (diagram center), as previously shown by Munger et al, 1999. It is proposed here that αvβ6 is upregulated in prostate cancer, which binds inactive TGFβ1 to activate it. The activated TGFβ1 then binds to cell surface TGFβ1-receptors (black box) which leads to (dashed arrow) increased tumor growth.

FUTURE STUDIES

These PTEN-null mice can be used for numerous *in vivo* studies relating to prostate cancer. Although previous research in this model has been conducted as to the types of proteins upregulated and downregulated during cancer formation (highlighted in Table 1), similar research of other molecules may prove beneficial. Different treatment and prevention options can be tested on these animals, and the cancer progression can be compared the data presented here. This data will be useful in designing new experiments. Based on the recent prostate cancer models of inflammation progressing to cancer, it may be beneficial to assess the effects of T-cells and macrophages on the role $\alpha_V\beta_6$ in cancer progression. AR mutations have also been implicated in prostate cancer signaling, therefore it may prove beneficial to further study the role that AR plays in cancer progression and how it is related to $\alpha_V\beta_6$. The integrin $\alpha_V\beta_6$ may also be a possible pharmaceutical or diagnostic target. This project supports the ongoing research into the causes and possible cures of prostate cancer through *in vivo* experimentation.

REFERENCES

Abate-Shen C and Shen MM (2000). Molecular Genetics of Prostate Cancer. *Genetics and Development.* **14(19):** 2410-2434.

Abremski K and Hoess R (1984). Bacteriophage P1 Site-Specific Recombination. *The Journal of Biological Chemistry.* **259(3):** 1509-1514.

Agrez M, Chen A, Cone RI, Pytela R and Sheppard D (1994). The $\alpha_V\beta_6$ Integrin Promotes Proliferation of Colon Carcinoma Cells through a Unique Region of the β_6 Cytoplasmic Domain. *The Journal of Cell Biology.* **127(2):** 547-556.

Akhurst RJ and Derynck R (2001). TGF-β Signaling in Cancer – a Double-Edged Sword. *TRENDS in Cell Biology* **11(11):** S44-S51.

Alam N, Goel HL, Zarif MJ, Butterfield JE, Perkins HM, Sansoucy BG, Sawyer TK and Languino LR (2007). The Integrin Growth Factor Duet. *Journal of Cellular Physiology.* **213:** 649-653.

AlDahlawi S, Eslami A, Hakkinen L and Larjava HS (2006). The $\alpha_V\beta_6$ Integrin Plays a Role in Compromised Epidermal Wound Healing. *Wound Repair and Regeneration.* **14:** 289-297.

American Cancer Society (2007). Cancer Facts and Figures 2007. Atlanta: American Cancer Society.

Arend L, Smart A and Briggs J (2000). Mouse β_6 Integrin Sequence, Pattern of Expression, and Role in Kidney Expression. *J AM Nephrol.* **11:** 2297-2305.

Armes JE, Trute L, White D, Southey MC, Hammet F, Tesoriero A, Hutchins A-M, Dite GS, McCredie MRE, Giles GG, Hopper JL, and Venter DJ (1999). Distinct Molecular Pathogeneses of Early-Onset Breast Cancers in BRCA1 and BRCA2 Mutation Carriers: A Population-based Study. *Cancer Res*earch. **59**: 2011-2017.

Assoian RK and Schwatz MA (2001). Coordinate Signaling by Integrins and Receptor Tyrosince Kinases in the Regulation of G_1 phase Cell-Cycle Progression. *Curr Opin Genet Dev.* **11(1):** 48-53.

Azare J, Leslie K, Hikmat A, Gerald W, Weinreb P, Violette SM and Bromberg J (2007). Constitutively Activated Stat3 Induces Tumorigenesis and Enhances Cell Motility of Prostate Epithelial Cells through Integrin β_6. *Molecular and Cellular Biology.* **27(12):** 4444-4453.

Basso D, Fogar P, Piva MG, Navaglia F, Mazza S, Prayer-Galetti T, Castellucci E and Plebani M (2000). Total PSA, Free PSA/Total PSA Ratio, and Molecular PSA Detection in Prostate Cancer: Which is Clinically Effective and When?. *Adult Urology.* **55(5):** 710-715.

Bates R, Bellovin DI, Brown C, Maynard E, Wu B, Kawakatsu H, Sheppard D, Oettgen P and Mercurio AM (2005). Transcriptional Activation of Integrin β_6 during the Epithelial-Mesenchymal Transition Defines a Novel Prognistic Indicator of Aggressive Colon Cancer. *The Journal of Clinical Investigations.* **115(2):** 339-347.

Bernards R, Shackleford GM, Gerber MR, Horowitz JM, Friend SH, Schartl M, Bogenmann E, Rapaport JM, McGee T, Dryja TP and Weinberg RA (1989). Stucture and expression of the murine retinoblastoma gene and characteristics or its encoded protein. *Proc. Natl. Acad. Sci. USA.* **86:** 6474-6478.

Berquin IM, Min Y, Wu R, Wu H and Chen YQ (2005). Expression Signature of the Mouse Prostate. *Journal of Biological Chemistry.* **280(43):** 36442-36451.

Bruess JM, Gillett N, Lu L, Sheppard and Pytela R (1993). Restricted Distribution of Integrin β_6 mRNA in Primate Epithelial Tisues. *Journal of Histochemistry and Cytochemistry.* **41(10):** 1521-1527.

Breuss JM, Gallo J, DeLisser HM, Klimanskaya IV, Folesson HG, Pittet JF, Nishmura SL, Aldape K, Landers DV, Carpenter W, Gillet N, Sheppard D, Matthey MA, Albelda SM, Kramer RH and Pyteka R (1995). Expression of the β_6 Integrin Subunit in Development, Neoplasia and Tissue Repair Suggest a Role in Epithelial Remodeling. *Journal of Cell Science.* **108:** 2241-2251.

Busk M, Patela R and Sheppard D (1992). Characterization of the Integrin $\alpha_V\beta_6$ as a Fibronectin-Binding Protein. *The Journal of Biological Chemistry.* **267(9):** 5790-5796.

Cairns P, Okami K, Halachmi S, Halachmi N, Esteller M, Herman JG, Jen J, Isaacs WB, Bova S and Sidransky D (1997). Frequent Inactivation of *PTEN/MMAC1* in Primary Prostate Cancer. *Cancer Research* **57:** 4997-5000.

Cannistra S, Ottensmeier C, Niloff J, Orta B and DiCalo J (1994). Expression and Function of β_1 and $\alpha_V\beta_3$ Integrins in Ovarian Cancer. *Gynecologic Oncology* **58:** 216-225.

Chang L and Karin M (2001). Mammalian MAP Kinase Signalling Cascades. *Nature* **401:** 37-40.

Chen Q, Kinch MS, Lin TH, Burridge K and Juliano RL (1994). Integrin-Mediated Cell Adhesion Activates Mitogen-Activated Protein Kinases. *The Journal of Biological Chemistry.* **269(3):** 26602-26605.

Cone RI, Weinacker A, Chen A and Sheppard D (1994). Effects of Beta Subunit Cytoplasmic Domain Deletions on the Recruitment of the Integrin Alpha v Beta 6 to Focal Contacts. *Cell Adhes Commun.* **2(2):** 101-113.

Cooper & Hausman (2004) The Cell Cycle. In: *The Cell a Molecular Approach, Third Edition.* Washington, DC: ASM Press. 591-629.

Crawford D (2003). Epidemiology of Prostate Cancer. *Journal of Urology.* **62(S6A):** 3-12.

Cristofano AD, Pesce B, Cordon-Cardo C and Pandlfi PP (1998). Pten is Essential for Embryonic Development and Tumor Suppression. *Nature Genetics* **19:** 348-355.

De Marzo AM, DeWeese TL, Platz E, Meeker AK, Nakayama M, Epstein JI, Isaacs WB and Nelson WG (2004). Pathological and Molecular Mechanisms of Prostate Carcinogenesis: Implications for Diagnosis, Detection, Prevention and Treatment. *Journal of Cellular Biochemistry.* **91:** 459-477.

Fridrichs K, Ruiz P, Franke F, Gille I, Terpe HJ amd Imhof B (1995). High Expression Level of α_6 Integrin in Human Breast Carcinoma is Correlated with Reduced Survival. *Cancer Research.* **55:** 901–906.

Gao X, Porter AT, Grignon DJ, Pontes JE and Honn KV (1997). Diagnostic and Prognostic Markers for Human Prostate Cancer. *The Prostate.* **31:** 264-281.

Giancotti F and Ruoslahti E (1999). Integrin Signaling. *Science.* **285(5430):** 1028-1033.

Goel HL and Languino LR (2004). Integrin signaling in cancer. In: Kumar R, editor. *Molecular targeting and signal transduction*. Norwell: Kluwer Academic Publishers. 15-31.

Greenburg NM, DeMayo F, Finegold MJ, Medina D, Tilley WD, Aspinall JO, Cunha GR, Donjacour AA, Matusik RJ and Rosen JM (1995). Prostate Caner in a Transgenic Mouse. *Proc. Natl. Acad. Sci. USA.* **92:** 3439-3443.

Gronberg (2003). Prostate Cancer Epidemiology. *The Lancet.* **361:** 859-864.

Guo W and Giancotti FG (2004). Integrin Signalling During Tumor Progression. *Nature Reviews Molecular Cell Biology.* **5:** 816-826.

Fornaro M, Manes T and Languino LR (2001). Integrins and Prostate Cancer Metastases. *Cancer and Metastasis Reviews.* **20:** 321-331.

Haapasalmi K, Zhang K, Tonnesen M, Olerud J, Sheppard D, Salo T, Kramer R, Clark RAF, Uitto VJ and Larjava H (1996). Keratinocytes in Human Wounds Express $\alpha_V\beta_6$ Integrin. *The Journal of Investigative Dermatology.* **106(1):** 42-48.

Hakkinen L, Koivisto L, Gardner H, Saarialho-Kere U, Carroll JM, Lasko M, Rauvala H, Laato M, Heino J and Larjava H (2004). Increases Expression of β6 Integrin in Skin Leads to Spontaneous Development of Chronic Wounds. *American Journal of Pathology.* **164(1):** 229-242.

Harris R and Lohr K (2002). Screening for Prostate Cancer: An Update of the Evidence for the U.S. Preventive Service Task Force. *Annals of Internal Medicine*. **137(11):** 917-929.

Hernandez J and Thompson I (2004). Prostate-Specific Antigen: A Review of the Validation of the Most Commonly Used Cancer Biomarker. *Cancer* **101(5):** 894-904.

Hsing A and Chokkalingam A (2006). Prostate Cancer Epidemiology. *Frontiers in Bioscience.* **11:** 1338-1413.

Humphries MJ and Newham P (1998). The Structure of Cell-Adhesion Molecules. *Trends in Cell Biology.* **8:** 78-83.

Hynes R (1999). Cell Adhesion: Old and New Questions. *Membrane Trafficking.* **9(12):** M33-M37.

Jemal A, Siegel R, Ward E, Murray T, Xu J and Thun MJ (2007). Cancer Statistics 2007. *CA Cancer J Clin.* **57:** 43-66.

Jenster G (1999). The Role of Androgen Receptor in the Development and Progression of Prostate Cancer. *Seminars in Oncology.* **26(4):** 407-421.

Jiao J, Wang S, Qiao R, Vivanco I, Watson PA, Sawyers CL and Wu H (2007). Murine Cell Lines Derived from *Pten* Null Prostate Cancer Show the Critical Role of PTEN in Hormone Refractory Prostate Cancer Development. *Cancer Research.* **67(13):** 6083-6091.

Kasper S, Sheppard PC, Yan Y, Pettigrew N, Borowsky AD, Prins GS, Dodd JG, Duckworth ML and Matusik RJ (1998). Development, progression, and androgen-dependence of prostate tumors in probasin-large T antigen transgenic mice: a model for prostate cancer. *Lab Invest.* **78(3):** 319-33.

Liao CP, Zhong C, Saribekyan G, Bading J, Park R, Conti PS, Moats R, Berns A, Shi W, Zhou Z, Nikitin AY and Roy-Burman P (2007). Mouse models of prostate adenocarcinoma with the capacity to monitor spontaneous carcinogenesis by bioluminescence or fluorescence. *Cancer Research.* **67(15):** 7525-7533.

Lin H, Hu Y, Lee DK and Chang C (2004). Regulation of Androgen Receptor Signaling by PTEN (Phosphate and Tensin Homolog Deleted on Chromosome 10) Tumor Suppressor through Distinct Mechanisms in Prostate Cancer Cells. *Molecular Endocinology* **18(10):** 2409-2423.

Mizejewski GJ (1999). Role of Integrins in Cancer: Survey of Expression Patterns. *Proc Soc Exp Biol Med.* **222:** 124-138.

Morris D, Huang X, Kaminski N, Wang Y, Shapiro S, Dolganov G, Glick A and Sheppard D (2003). Loss of Integrin $\alpha_V\beta_6$ Mediated TGFβ Activation Causes Mmp12-Dependent Emphysema. *Nature.* **422(13):** 169-173.

Munger JS, Huang X, Kawakatsu H, Griffiths MJD, Dalton SL, Wu J, Pittet JF, Kaminski N, Garat S, Matthay MA, Rifkin DB and Sheppard D (1999). The Integrin $\alpha_V\beta_6$ Binds and Activates

Latent TGFβ1: A Mechanism for Regulating Pulmonary Inflammation and Fibrosis. *Cell.* **96:** 319-328.

Nan B, Snabbon T, Unni E, Yuan XJ, Wang YE and Marcelli M (2003). The PTEN Tumor Suppressor is a Negative Modulator of Androgen Receptor Transcriptional Activity. *Journal of Molecular Endocrinology.* **31:** 169-183.

National Cancer Institute (2006). Physician Data Query. Cancer.gov. http://www.cancer.gov/cancertopics/pdq/treatment/prostate/patient/allpages#Section_AboutPDQ_14 Last updated: 12/12/2006 Accessed: 9/27/2007.

Pomerantz M and Kantoff P (2007). Advances in the Treatment of Prostate Cancer. *Annual Review of Medicine.* **58:** 205-220.

Powell WC, Cardiff RD, Cohen MB, Miller GJ and Roy-Burman P (2003). Mouse Strains for Prostate Tumorigenesis Based on Genes Altered in Human Prostate Cancer. *Current Drug Target.* **4:** 263-279.

Price D (1963). Comparative Aspects Of Development And Structure In The Prostate. *Natl Cancer Inst Monogr.* **12:** 1-27.

Sauer B and Henderson N (1988). Site-Specific DNA Recombination in Mammalian Cells by the Cre Recombinase of Bacteriophage P1. *Proc. Natl. Acad. Sci. USA.* **85:** 5166-5170.

Schaid (2004). The Complex Genetic Epidemiology of Prostate Cancer. *Human Molecular Genetics* **13(R1):** R103-R121.

Shah JB, Reese AC, McKiernan JM and Benson MC (2005). PSA Updated: Still Relevant in the New Mellennium. *European Urology.* **47:** 427-432.

Shappell SB, Thomas GV, Roberts RL, Herbert R, Ittmann MM, Rubin MA, Humphrey PA, Sundberg JP, Rozengurt N, Barrios R, Ward JM and Cardiff RD (2004). Prostate Pathology of Genetically Engineered Mice: Definitions and Classification. The Consensus Report from the Bar Harbor Meeting of the Mouse Models of Human Cancer Consortium Prostate Pathology Committee. *Cancer Research.* **64:** 2270-2305.

Shepard D, Rozzo C, Starr L, Quarantall V, Erle DJ and Pytela R (1990). Complete Amino Acid Sequence of a Novel Integrin β6 Subunit Identified in Epithelial Cells Using the Polymerase Chain Reaction. *The Journal of Biological Chemistry.* **265(20):** 11502–11507.

Sheppard (2005). Integrin-Mediated Activation of Latent Transforming Growth Factor β. *Cancer and Metastasis Reviews.* **24:** 395-402.

Sonn GA, Aronson W and Litwin MS (2005). Impact of Diet on Prostate Cancer: a Review. *Prostate Cancer and Prostatic Diseases.* **8:** 304-310.

Stanford JL, Stephenson RA, Coyle LM, Cerhan J, Correa R, Eley JW, Gilliland F, Hankey B, Kolonel LN, Kosary C, Ross R, Severson R, West D. *Prostate Cancer Trends 1973-1995*, SEER Program, National Cancer Institute. NIH Pub. No. 99-4543. Bethesda, MD, 1999.

Steiner MS and Barrack ER (1992). Transforming Growth Factor-β1 Overproduction in Prostate Cancer: Effects on Growth *in Vivo* and *in Vitro. Molecular Endocrinology.* **6:** 15-25.

Thomas GL, Nystrom ML and Marshall JF (2006). $\alpha_V\beta_6$ Integrin in Wound Healing and Cancer of the Oral Cavity. *J Oral Pathol Med*. **35:** 1-10.

Trotman LC, Niki M, Dotan ZA, Koutcher JA, Di Cristofano A, Xiao A, Khoo AS, Roy-Burman P, Greenberg NM, Van Dyke T, Cordon-Cardo C and Pandolfi PP (2003). Pten dose dictates cancer progression in the prostate. *PLoS Biology.* **3(1):** 385-396.

Vliesta RJ, Alewijk DCJG, Hermans KGL, Steenbrugge GJ and Trapmen J (1998). Frequent Inactivation of PTEN in Prostate Cancer Cell Lines and Xenografts. *Cancer Research.* **58:** 2720-2733.

Wang S, Gao J, Qunying L, Rozengurt N, Pritchard C, Jiao J, Thomas G, Li g, Roy-Burman R, Nelson PS, Liu X and Wu H (2003). Prostate-Specific Deleation of the Murine Pten Suppressor Gene Leads to Metastatic Prostate Cancer. *Cancer Cell.* **4:** 209-221.

Wang S, Garcia AJ, Wu M, Lawson DA, Witte ON and Wu H (2006). Pten deletion leads to the expansion of a prostatic stem/progenitor cell subpopulation and tumor initiation. *Proceedings of the National Academy of Science.* **103(5):** 1480-1485.

Wu X, Wu J, Huang J, Powell WC, Zhang J, Matusik RJ, Sangiorgi FO, Maxson RE, Sucov HM and Roy-Burman P (2001). Generation of a Prostate Epithelial Cell-Specific Cre Transgenic Mouse Model for Tissue-Specific Gene Ablation. *Mechanics of Development.* **101:** 61-69.