

a) Description of Research Training: Studying at UCLA in the laboratory of my prospective, post-doctoral supervisor Dr. [redacted] will provide me with excellent training in preparation for a future faculty position as a prostate cancer biologist. I will be exposed to a wide range cell and molecular biology techniques along with intense training involving generation of transgenic and knock-out animals. As part of my intellectual training I will work closely with Dr. [redacted] collaborators at UCLA who are interested in prostate cancer biology, including Drs. [redacted] and [redacted]. These investigators can all be considered leaders in their respective fields and are highly productive as bourn out in their publication record. Dr. [redacted] laboratory also has a monthly scientific meetings as well as a journal club with the other prostate cancer research groups at UCLA. I plan to be an active member of this joint team and will strive to form collaborations with other investigators such as Dr. [redacted] who, like Dr. [redacted], has made seminal contributions in the area of prostate cancer research.

I believe that the research concept at the UCLA prostate groups is one of creating prostate cancer models that are suitable for translational and therapeutic based research. This is apparent upon examining the recent research and publication record of the UCLA prostate group which within the previous year published three papers in journal *Cancer Cell* concerning c-myc (Dr. [redacted]), PTEN (Dr. [redacted]) and p53/PTEN prostate cancer murine models. Dr. [redacted] received her MD at the Beijing Medical University and subsequently received a Ph.D. from Harvard University. She received further training during a post-doctoral fellowship at The Whitehead Institute and Harvard University where she gained expertise in development of knock-out and transgenic animals. [redacted] interest in cancer research is apparent from some of her recent prestigious recent awards. Of particular note is her appointment as an Assistant Howard Hughes Investigator (Chevy Chase, MD) from which she has been provided with salary and operating funds. Dr. Wu has also received awards including the *CapCure Research Award* and the *Prostate Cancer Foundation Award*.

Dr. [redacted] has made seminal research contributions in studying the role of PTEN, the second most commonly mutated tumour suppressor gene and one that is lost in expression in up to 50% of advanced prostate cancer cases [1]. Consequently, Dr. [redacted] has is particularly focussed on the molecular mechanisms of PTEN controlled tumorigenesis. For this, she has undertaken a combination of molecular genetics, cell biology, and biochemical approaches. By analyzing cells and animals lacking the PTEN tumour suppressor, she has demonstrated that PTEN negatively regulates stem cell self-renewal, proliferation, and survival (Groszer et al. 2001 *Science* 294:2186-2189. The significance and impact of this study was discussed by Josef Penninger and James Woodgett in *Science Perspectives*, and by Sean Morrison in "News and Views" in *Nature Medicine*). Dr. [redacted]'s studies have provided a strong link between stem cell biology and cancer biology suggesting that tumours may originate through the transformation of stem cells. She has also established various animal models for human cancers, including mammary (Li et al., 2002 *Development* 129:4159-4170) and prostate cancer models (Wang et al., 2003 *Cancer Cell* 4:209-221). These murine cancer models offer unique tools for both exploring the molecular mechanism underlying human cancers and for the development of new therapies. One of her recent studies demonstrated that PTEN controls p53 protein level and transcription activity, providing a novel mechanism by which the loss of PTEN can functionally control 'two' hits in the course of tumour development by concurrently modulating p53 activity (Freeman et al., 2003 *Cancer Cell* 3:117-129. The significance of this study was discussed by Trotman and Pandolfi in "Previews" in *Cancer Cell*). Dr. [redacted] has also had considerable experience as a research supervisor and currently supervises seven postdoctoral fellows, two medical fellows, and three graduate students. To date, Dr. [redacted] has successfully trained 14 post-doctoral fellows, 12 graduate students, 3 undergraduate students and served on examination committees for 17 students. Many of her trainees have gone on to pursue university

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faculty positions. Dr. is also extremely involved in the graduate training program at UCLA and serves on several committees for graduate training (MMMP) and for the UCLA ACCESS curriculum.

Dr. also serves as Director for the UCLA Cancer Center ES cell Core Facility and also Director of the UCLA Technology Center for Molecular Genetics. These positions clearly demonstrate the degree to which Dr. is involved in the production of animal models for use in scientific research.

b) Background: Prostate cancer (PrCa) is the second leading cause of cancer related death in North American men [2, 3]. Advanced PrCa is treated by ablating the androgen receptor (AR) of its cognate ligand, dihydrotestosterone (DHT). While resulting in initial regression of the cancer, this treatment ultimately fails as cells harbouring AR evolve to a state of androgen independence (AI). The ability of PrCa cells to evade the requirement for androgens implies that other mechanisms are allowing prostate epithelium to grow, proliferate and differentiate [3]. Some of these mechanisms may include transcriptional co-activation of AR and growth factor activation of cell signalling pathways. Numerous reports have shown that transcriptional co-regulators of AR, such as co-activator Beta-catenin (β -cat) [4] or nuclear co-repressor NCoR [5], can strongly influence the ability of AR to promote growth of cancer cells in an androgen deprived environment [6].

Beta-catenin was originally defined as a component of the epithelial adherens junction, linking E-cadherin to the intracellular actin skeleton via α -catenin [7]. However, it has also gained particular attention as an oncoprotein, most notably in gastrointestinal pathologies [7]. More recently, β -cat has been implicated in affecting both the activity of AR [4] and in the progression of PrCa [8]. With the finding that β -cat acts as a potent co-activator of AR either in the presence of androgen (R1881 or DHT), Estradiol or OH-flutamide ranks β -cat as one of the more promiscuous AR co-activators [4]. We have shown that there is dynamic complexing between AR and β -cat such that upon exposure to agonist, these molecules physically complex and translocate to the nucleus [9]. These observations provided a novel means by which β -cat, a molecule that lacks conventional nuclear localization signal (NLS), can enter the nucleus. The AR can also repress, androgen-dependently, transcription of β -cat/Tcf (T-Cell Factor), an event associated with Wnt mediated oncogenesis [10] [11]. Despite the flurry of studies implicating β -cat with AI PrCa activity *in vitro*, considerably less *in vivo* data is available. Using transgenic animals it has been shown that expression of activated forms of β -cat, that is removal of Glycogen Synthase phosphorylation (GSK3 β) sites in exon 3 (Ser 33, 37, 45 and Thr 41) can induce Prostatic Intraepithelial Neoplasia (PIN) [8]. Other studies have indicated that β -cat does not produce PIN but, rather, transdifferentiation of epithelium [12]. Analysis of high grade PrCa patient samples has indicated a 5% rate of activating β -cat mutations at exon 3 [13]. Interestingly, this rate is relatively high in comparison to that found in colon cancer (where most activating Wnt mutations found are in the tumour suppressor Adenomatous Polyposis Coli, or APC). It is of further interest that in high grade PrCa the rate of nuclear β -cat (~20 %) superceeds the rate of activating mutations [14] [13]. The significance of these observations lies in the fact that nuclear β -cat is a hallmark of a cell with activated and, potentially, oncogenic β -cat/Tcf signalling. Given that the rate of nuclear β -cat is higher than would be predicated by the rate of activating mutations during high grade (AI) PrCas, other signalling pathways could be responsible for regulating the distribution of β -cat.

The serine/threonine kinase Akt has been described a central mediator of Phosphatidylinositol-3-Kinase (PI3K) mediated signalling with the capacity to direct regulation of Wnt signalling, cell cycle, cell death, protein synthesis, cell growth and cell survival [15-18]. Cellular control of PI3K signalling involves conversion of phosphatidylinositol 4,5-biphosphate (PIP2) to phosphatidylinositol 3,4,5-triphosphate (PIP3). PIP3 accumulation serves as a major substrate for downstream kinases including Akt, Phosphodependent kinase 1 (PDK1) and PDK2/Integrin Linked Kinase (ILK), all of which serve as

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positive regulators of the PI3K prosurvival pathway. Alteration of PIP3 supplies abrogates downstream activation of PI3K signalling [17]. Several lines of evidence suggest that PI3K signalling is the prominent means by which PrCa cells maintain growth in an AI manner [16, 19]. For example, using the following tissue culture conditions for the PSA secreting Lymph Node Metastatic Prostate cancer cells (LNCaP): serum free media, androgen depleted media, anti-androgens (bicalutamide) or addition of the PI3K inhibitor, LY294002, only inhibition of phosphorylated Akt (via PI3K) will result in programmed cell death [20]. Depletion of androgens can promote cell quiescence but will not induce apoptosis [20]. Despite the apparent role that Akt plays in AI PrCa maintenance, Akt fails to promote tumour formation in androgen depleted (castrated) environments [15] suggesting that Akt is necessary but not sufficient for PrCa progression. Akt has also been suggested to directly phosphorylate AR thereby regulating AR activation [21], though, other reports have indicated that mutations of phosphorylation sites on AR do not affect AR transcription [22]. Another role for Akt is to inhibit GSK3, a molecule that promotes apoptosis and is responsible for degradation of β -cat. Activated Akt maintains repressed levels of GSK, therefore, resulting in high levels of β -cat. Given these dynamics, alteration of Akt activity may allow a means by which to modulate β -cat levels and the activity of AR (directly via phosphorylation and indirectly via β -cat).

A signature event that occurs during prostate pathogenesis is chromosomal loss of the tumour suppressor PTEN (phosphatase and tensin homologue), an event that occurs in up to 50% of advanced PrCa and most PrCa cell lines (LNCaP, PC3) [1]. PTEN negatively regulates PIP2 \rightarrow PIP3 conversion by dephosphorylating PIP3 and, therefore, can be considered a potent inhibitor of PI3K signalling [17]. The fact that most PTEN mutations occur within the phosphatase domain underscores the importance of the PTEN phosphatase activity for its tumour suppressor function. Loss of PTEN expression results in pro-survival cell signalling and resistance to apoptotic stimuli. The lipid phosphatase activity of PTEN has also been implicated in stabilizing of intercellular junctions and in reverting cell invasiveness [23].

Importantly, we have previously observed constitutively high levels of nuclear β -cat in PTEN $-/-$ PrCa cells including PC3 and LNCaP cells [24]. When we introduced PTEN into these cells we observed activation of GSK-3 and reduced levels of β -cat and activity of its target gene, Tcf (Figure 1). PTEN mediates this action by down regulating Integrin-linked kinase (ILK) kinase activity, thus, leading to decreased GSK3 phosphorylation. In addition, ILK has been shown to directly phosphorylate and activate Akt, thereby inactivating GSK [25] (Figure 2). As GSK3 is critical in the regulation of β -cat cellular levels and localization, upstream PI3K molecules are likely also capable of altering β -cat signalling. In addition to being the dominant growth factor-activated cell survival pathway in LNCaP cells [26] PI3K signalling has been shown to be vital for the progression of LNCaP prostate cancer cells to AI [20] and regulation of the AR [27]. Consequently, several reports demonstrated that PTEN can serve as a negative regulator of AR transcriptional activity as well as androgen-induced cell proliferation and production of prostate specific antigen (PSA) [28] [29]. As AR has been shown to be functionally involved in progression of AI PrCa [30], it is possibly that loss of PTEN expression during advanced PrCa is a major contributor to aberrant AR activity. Importantly, it has been demonstrated that PI3K signalling stimulates the AR pathway through GSK3 β and nuclear β -cat accumulation. This study indicated that the repressive effects of PTEN are mediated by GSK3 β regulated degradation of β -cat [29]. β -cat mutants that are resistant to phosphorylation mediated degradation by GSK3 β are not altered by PTEN and, therefore, do not manifest a change in AR activity. While the functional role of β -cat in progression of PrCa is complex its role as an AR co-activator have been well-defined [31]. Thus, molecules, such as PTEN, with that capacity to alter levels of Akt and GSK3 β may have the ability to regulate oncogenic potential of β -cat. Cyclin D1 is a critical prometotic target gene of β -cat [32]. PTEN has also been shown to induce cell cycle arrest by decreasing the level and nuclear localization of cyclin D1 in PTEN deficient glioblastoma cell lines [33]. Given these compelling observations, it is

conceivable that loss of PTEN expression during PrCa progression may contribute towards increased nuclear expression of β -cat *in vivo* thus contributing to AI PrCa progression. While PTEN appears to regulate β -cat function *in vitro* [34], these data have not been adequately explored *in vivo*, either in transgenic or in xenograft PrCa mouse models.

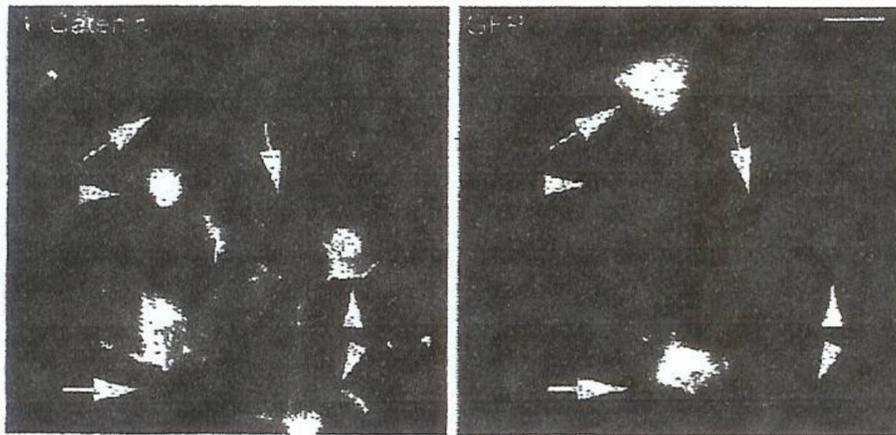


Figure 1. Levels of nuclear β -cat is reduced upon introduction of PTEN into PTEN^{-/-} PC3 PrCa cells. Immunofluorescence of β -cat illustrates that cells transfected with WT PTEN-GFP (arrows) do not show nuclear β -cat while non-PTEN transfected cells (arrowheads) show nuclear β -cat. Bar = 5 μ m. (Adapted from Persad et al., J. Cell Biol. 2000)

In addition to PTEN, the more recently described Carboxy Terminal Modulator Protein (CTMP) has been shown to bind Akt and dramatically reduce its phosphorylation, both at Thr 308 and most notably at Ser 473 sites [35]. CTMP can also revert the phenotype of v-Akt transformed cells [35]. Importantly, CTMP can inhibit Akt activity in the presence of physiological stimuli of the PI3K pathway such as insulin or Insulin-like Growth Factor-1 (IGF-1) and can inhibit Akt mediated phosphorylation (Ser9) of GSK3 β . The rationale for examining CTMP as a potential regulator of β -cat is three fold: (1) Molecules that specifically inhibit phosphorylated Akt or PI3K signalling (such as PTEN) are few in number and as Akt is hyperactivated in PrCa cells, identification of such targets is crucial in developing therapeutic understanding of PI3K inhibition; (2) Chromosome location 1q21 is not only the sight of frequent gene alterations in PrCa but is also the location of CTMP [36]. (3) Furthermore, while it is possible that CTMP mediated inhibition of PI3K may be similar to that of PTEN, it is also feasible that effects of depletion of PIP3 pools are not equal to inhibition of activated Akt in terms of β -cat regulation. For example, PIP3 can also serve as a substrate for PDK and ILK [25].

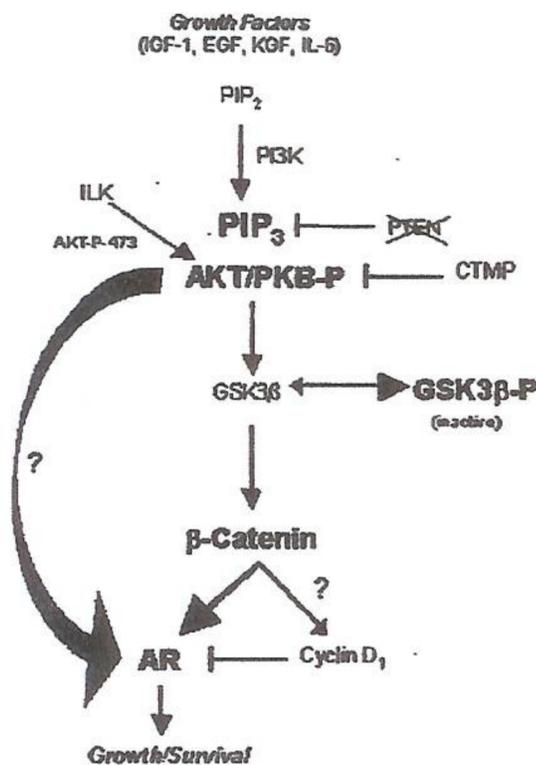


Figure 2. Intersection of Canonic Wnt Signalling with the PI3K pathway in PTEN^{-/-} PrCa cells. PTEN loss occurs frequently in advanced PrCa resulting in elevation of phosphorylated AKT and repression of the β -cat inhibitor, GSK3 β . This occurs by 5' dephosphorylation of PIP3 and the inhibition of PIP₂→PIP₃ conversion. Differently is CTMP which modulates PI3K signalling by directly inhibiting phosphorylation of Akt. PTEN^{-/-} cells contain high levels of available β -cat for ligand sensitive binding and transcriptional co-activation of AR. Cyclin D1, a downstream target of the β -cat/Tcf signalling, has been shown to bind and repress the AR, thus simultaneously generating promotive and antimitotic stimuli. (adapted from Mulholland et al., Endocrine Reviews, In Press, 2004)

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Hypothesis: If abrogation of PI3K signalling can reduce oncogenic β -cat/Tcf signalling in prostate cancer, then *in vivo* gain or loss of PTEN and/or CTMP could alter progression to Androgen Independence.

Rationale: In order to further understand the contributions that β -cat/Tcf signalling has to PrCa progression, we will exploit the documented relationship between PI3K, PTEN, β -cat and AR (Figure 2). By comparing the effects that PTEN and CTMP have on PIP3 and direct Akt inhibition, respectively, we will learn more about the functional contributions that each have on PI3K inactivation and modulation of β -cat/Tcf signalling. Besides molecules such as PP2A [37] and [38] ft1, relatively little is known about molecules with capacity to inhibit activity of Akt. CTMP negatively regulates Akt by direct binding at the plasma membrane and may prove to be important in preventing inappropriate PI3K activation. It may also prove to be crucial for preventing cell growth and proliferation during PrCa AI. While PTEN inhibits PI3K signalling indirectly by reducing the available pools of PIP3, CTMP inhibits Akt activity directly. Events that can alter the distribution of β -cat to allow modulation both of AR transcription (e.g., PSA production) as well as activity of β -cat/Tcf4 may be relevant to progression to AI PrCa. To date, the effects of *in vivo* PTEN gain-of-function have not been described in PrCa cells. If loss of PTEN instigates progression of advanced PrCa, we hypothesize that introduction of PTEN expression could abrogate *in vivo* PI3K signalling and, therefore, inhibit AI growth. Deciphering what genes are associated with PTEN signalling and how these related to potential oncogenic signalling of β -cat may be critical to determination of future therapeutic targets. Concurrent with assessing LNCaP tumour growth we will evaluate how induced PTEN expression can alter localization of β -cat. To date no reports have evaluated the effects that CTMP may have in PrCa cells. Given that Akt is a central mediator of pro-survival signalling and that molecules capable of inhibiting Akt are few, CTMP appears to be an ideal candidate for assessment in PrCa.

Research Objectives & Methods:

- 1a) Does *in vivo*, bi-allelic loss of PTEN lead to higher frequency of nuclear β -catenin?
- 1b) Does this pool of β -catenin contain, activating (GSK3 β resistant) mutations?

Frequently, patients from which PrCa biopsies are obtained have received neoadjuvant hormone therapy, potentially confounding the assessment of androgen regulated pathways. We will, therefore, use a gene-targeted approach and take advantage of a recently developed murine PrCa model (Figure 3). Using a PTEN $-/-$ prostate conditional mouse [39] we will assess alterations of E-cadherin and the Wnt/ β -cat signalling components including APC, Axin, GSK3 β and Tcf4. More specifically, we will harvest prostate tissue that is hyperplastic, neoplastic, invasive adenocarcinoma, metastatic and AI in progression. We will also compare prostate pathologies from PTEN $+/+$, PTEN $+/-$ and PTEN $-/-$ mice using immunohistochemistry to qualitatively evaluate the distribution of key Wnt/ β -cat components. Importantly, we will determine whether early loss of PTEN results in increased levels of β -cat and/or increased nuclear localization of β -cat. We will also qualitatively evaluate β -cat signalling by Western Blotting and co-immunoprecipitations of known interactions such a that between β -cat and Tcf, which is associated with oncogenic signalling. Decreased expression of Wnt degradative components would be suggestive of an active Wnt signalling cascade.

6. Main Body: Deciphering Beta-Catenin Contributions in Androgen Independent Prostate Cancer by Modulation of PI3K Signalling.

The relevance of using a PTEN^{-/-} mouse is further validated when comparing levels of phospho-Akt (Ser473) in animals harbouring prostates with WT PTEN (Figure 3b). Phospho-Akt levels are substantially elevated in cells lacking PTEN suggesting PI3K signalling is activated, likely contributing to the observed development of metastatic PrCa by 12 weeks.

Previous reports have shown that GSK3 β mutations (Ser 33, 37, 45 and Thr 41) in PrCa occur in 5% of advanced cancers while nuclear β -cat occurs at a frequency of ~20% [14, 40]. Using the PTEN prostate conditional knock-out model developed in laboratory of Dr. Wu we will determine: (1) whether β -cat activating mutations are present in PTEN null prostate cancers (as found in human samples); and (2) whether the rate of β -cat mutations is increased in PTEN null prostate cancers, as compared to PTEN^{+/-} or PTEN^{+/+} mice. Using laser capture micro dissection capabilities present at UCLA we will stain tissue sections with β -cat antibodies, use digital imaging software to encircle cells deemed as having nuclear β -cat, followed by dissection of epithelial nuclei. Collected tissue will be followed by DNA extraction and sequencing using β -cat/exon 3 primers. The presence of nuclear β -cat without GSK3 resistant mutations would be suggestive that an alternative means of nuclear accumulation could have functional importance.

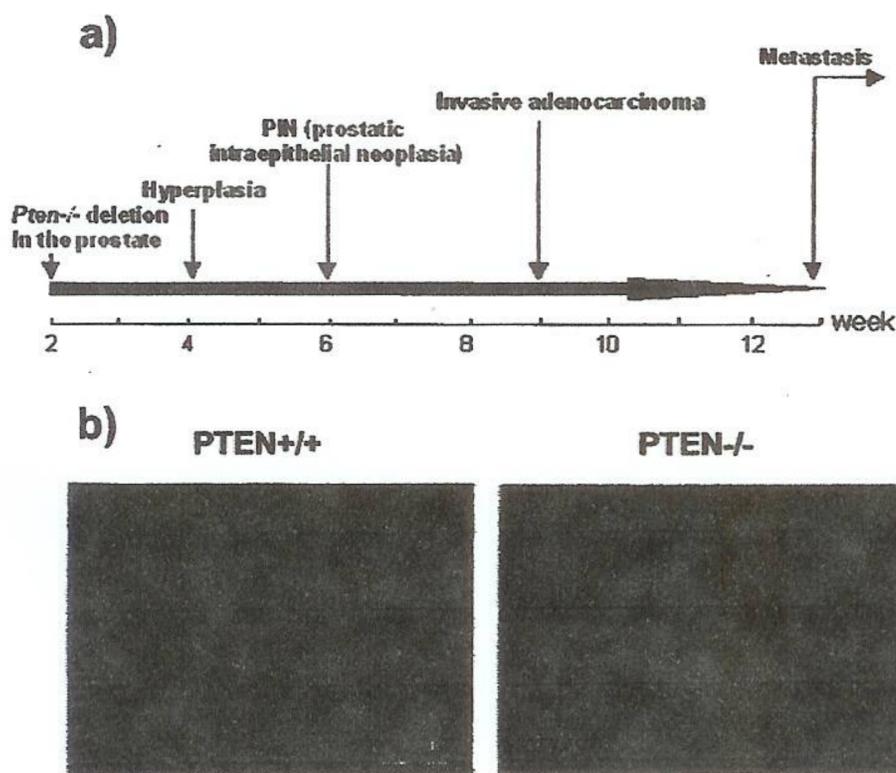


Figure 3. a) Formation of Androgen Independent PrCa in PTEN^{-/-} mice. Over the course of 12 weeks prostate specific PTEN^{-/-} mice show similar stages of pathology as observed in human PrCa. By 12 weeks mice display metastatic prostate cancer and display AI PrCa growth. b) Activation of PI3K in PTEN^{-/-} mice. Distribution of PTEN (red) and phospho-Akt (S473) (green) in PTEN^{+/+} and PTEN^{-/-} prostatic epithelium (anterior prostate). Activated Akt levels are low in PTEN containing cells but are elevated considerably in epithelium lacking PTEN (adapted from Wang et al., Cancer Cell, 2003).

To further understand the behaviour of epithelium in PTEN null prostate cancers, Dr. Wu has is also developing explant cell lines from PTEN^{-/-} mouse prostates using SV40 T antigen transformation. By doing so, we will be able expand these cells for more extensive biochemical and transfection studies but also relate these observations to well-established PrCa cell lines (LNCaP, PC3). We will also develop cell lines from PTEN^{+/-} and PTEN^{-/-} epithelium. Even further, Dr. Hong Wu is currently mating PTEN^{-/-} mice with mice harbouring activating gene mutations for β -cat (β -cat Δ Ser33, 37, 45, Thr 41). By comparing PTEN^{-/-}/ β -cat(Δ Ser33, 37, 45, Thr 41) mice with PTEN^{-/-} mice we will be in an excellent position to evaluate potential synergistic interactions between loss of PTEN and β -cat towards in contributions towards PrCa progression.

2) Does PTEN gain of function modulate β -cat/Tcf signalling *in vivo*?

Previous *in vitro* analysis has shown that transient expression or viral infection of PTEN results in reduced growth and proliferation of PrCa cells [41]. The expression of PTEN in PSA secreting PrCa cells under inducible, stable conditions has not been well characterized. While technically difficult, we have made use of a stable transfected, Tet repressor to ensure that basal levels of non-induced PTEN are at a minimum to ensure the generation of "non-leaky" PTEN expression. We have isolated and characterized several clones both for WT and MT (C124s) LNCaP-PTEN that show excellent PTEN induction and maintain their expression through passaging. Figure 4 shows that LNCaP-WT PTEN cells can efficiently reduce Phospho-Akt (Ser 473) levels under several serum conditions. Given the ability of PTEN to function as a tumour suppressor, I am also evaluating the ability of PTEN to induce apoptosis of cells under a variety of tissue culture media conditions (full serum, androgen depleted media and serum free media). These experiments are important in validating the ability of PTEN to induce apoptosis in tumours both in the presence or absence of androgens and validates assaying *in vivo*, xenograft tumour growth.

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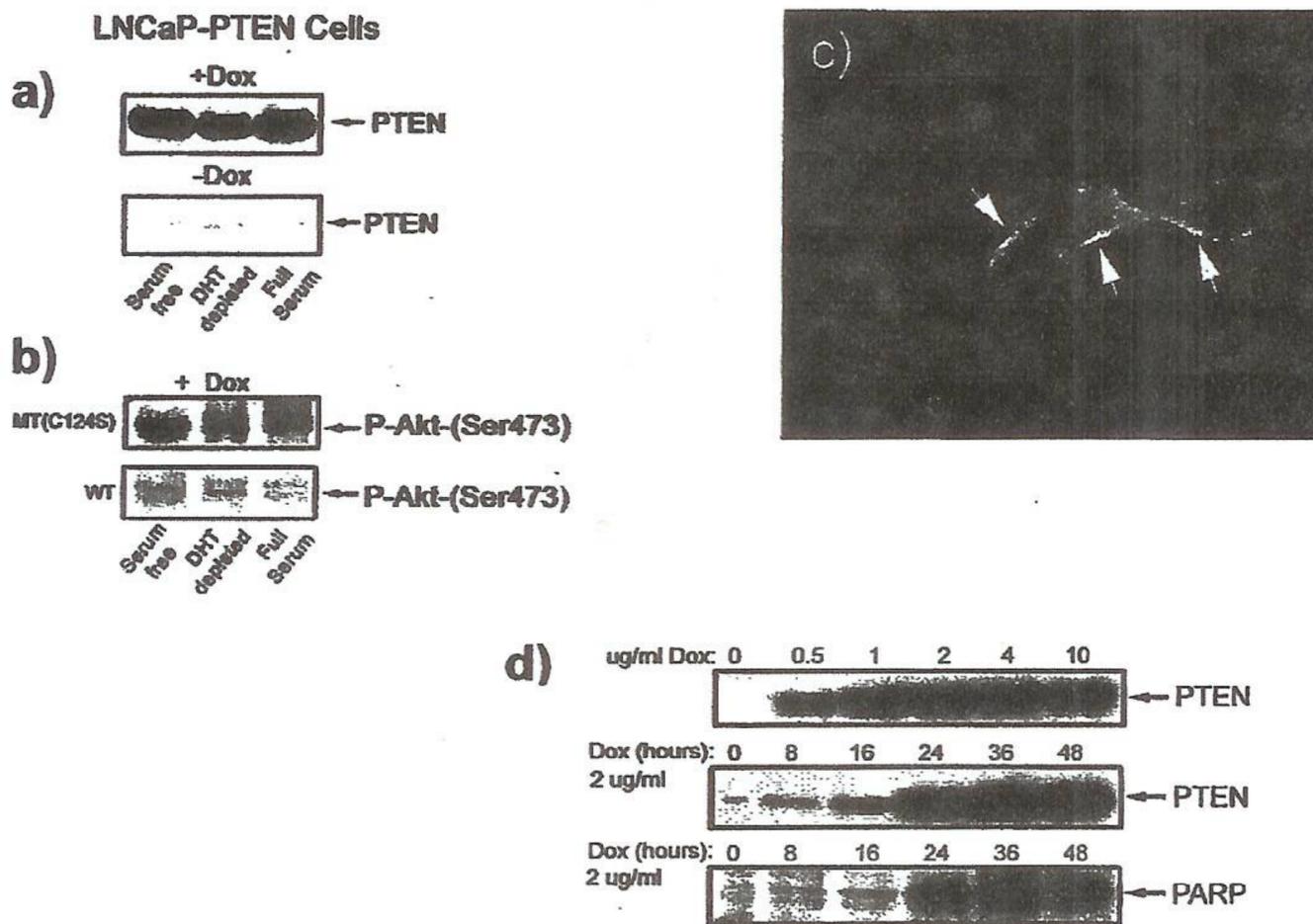


Figure 4. Validation of functional induction of PTEN expression in LNCaP cells grown *in vitro*. a) Induced PTEN expression in PSA secreting PrCa cells (LNCaP) in different media conditions upon the addition of 2 ug/ml doxycyclin (+Dox) for 24 hours. b) Detection of phospho-Akt (Ser473) levels upon *in vitro* induction of functional (WT) and catalytic mutant (C124S) PTEN in LNCaP-PTEN stable cells. Induction of WT PTEN shows inhibition of phosphorylated (Ser 473) Akt while the PTEN catalytic mutant does not. c) Validation that LNCaP-PTEN stable cells show a submembrane cellular distribution upon induction (arrows). d) Dose-response induction of PTEN in LNCaP-PTEN cells cultured in serum free media with increasing concentrations of doxycyclin and time course exposure to 2 ug/ml doxycyclin. PARP expression is readily apparent after 16 hours of PTEN induction validating that PTEN can induce apoptosis (Mulholland et al., Unpublished Results, 2004).

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In vivo, gain-of-function, analysis of induced PTEN will entail the expansion of LNCaP-PTEN clones and the subcutaneous injection of a LNCaP cell/Matrigel composite into the dorsal flank of nude mice (Figure 5). Tumours will be allowed to form over 4-5 weeks followed by androgen depletion (castration) in order to mimic androgen withdrawal therapy as used in clinical treatment of human PrCa. We will assay serum PSA levels (secreted by LNCaP cells) using commercial PSA ELISA kits and also measure dimensions (LxWxH x 0.5236) [42] of progressing tumours. PTEN induction will occur by addition of doxycycline (administered *via* drinking water *ad libitum*). LNCaP-parental tumour growth curves and PSA secretion have been shown to follow reproducible patterns before and after surgical castration. We will be able to assess levels of the PIP3 substrate, as compared to PIP2, using specific PH (Pleckstrin Homology domain) tagged fluorescent probes (Echelon Biosciences). Quantitation of these signals will validate whether PTEN is depleting PIP3 pools in our xenograft system upon PTEN induction. Generation of xenograft mouse models has been well established by investigators both at the UCLA prostate group by both Dr. Hong Wu and Dr. Charles Sawyers. It is anticipated that if PTEN maintains its tumour suppressor function in PrCa xenografts, reduced growth and PSA production will be readily detectable upon Dox-mediated induction of PTEN expression (Figure 5). We will relate growth profiles and PSA levels with β -cat signalling, both on a quantitative (message and protein) and qualitative level (cellular distribution of β -cat)

3) Is CTMP a negative regulator of PI3K and β -cat/Tcf in PrCa?

Using both transient and stable (inducible) expression systems we will evaluate the effects of CTMP on PI3K and β -cat signalling in PrCa (LNCaP and PC3) cells. Specifically, we will determine whether CTMP can:

- abrogate levels of phosphorylated (Thr 308/Ser 473)-Akt?
- diminish the pro-survival capacity of PI3K signalling (ie. does CTMP decrease *in vitro* growth factor mediated cell growth and proliferation)?
- alter LNCaP tumour growth and PSA levels?
- mediate a change in β -cat/Tcf signalling similar to PTEN?

To validate that CTMP is acting to repress Akt we will use phospho-specific antibodies that differentiate between total Akt, Akt-Thr308 and Akt-Ser473 activation. To determine the anti-PI3K effects of CTMP we will evaluate PI3K mediators of growth and translation including relative levels of mTOR [43] and p70 S6K phosphorylation [44]. Relative growth rates will be carried out by MTT assay while mitotic activity with BrdU incorporation and cell cycle (FACS) analysis. These studies will serve as pilot studies for *in vivo* xenograft experiments to be carried out with stable, doxycycline inducible LNCaP-CTMP cells which we are currently developing. As with inducible expression of PTEN, we will evaluate the ability of CTMP to alter both tumour volume (mm³) and serum PSA levels (ng/ml) with attention to localization and expression of β -cat/Wnt expression at select points of xenograft progression (intact, pre-castrated, post-castrated and AI) +/- induced CTMP expression (Figure 5).

6. Main Body: Deciphering Beta-Catenin Contributions in Androgen Independent Prostate Cancer by Modulation of PI3K Signalling.

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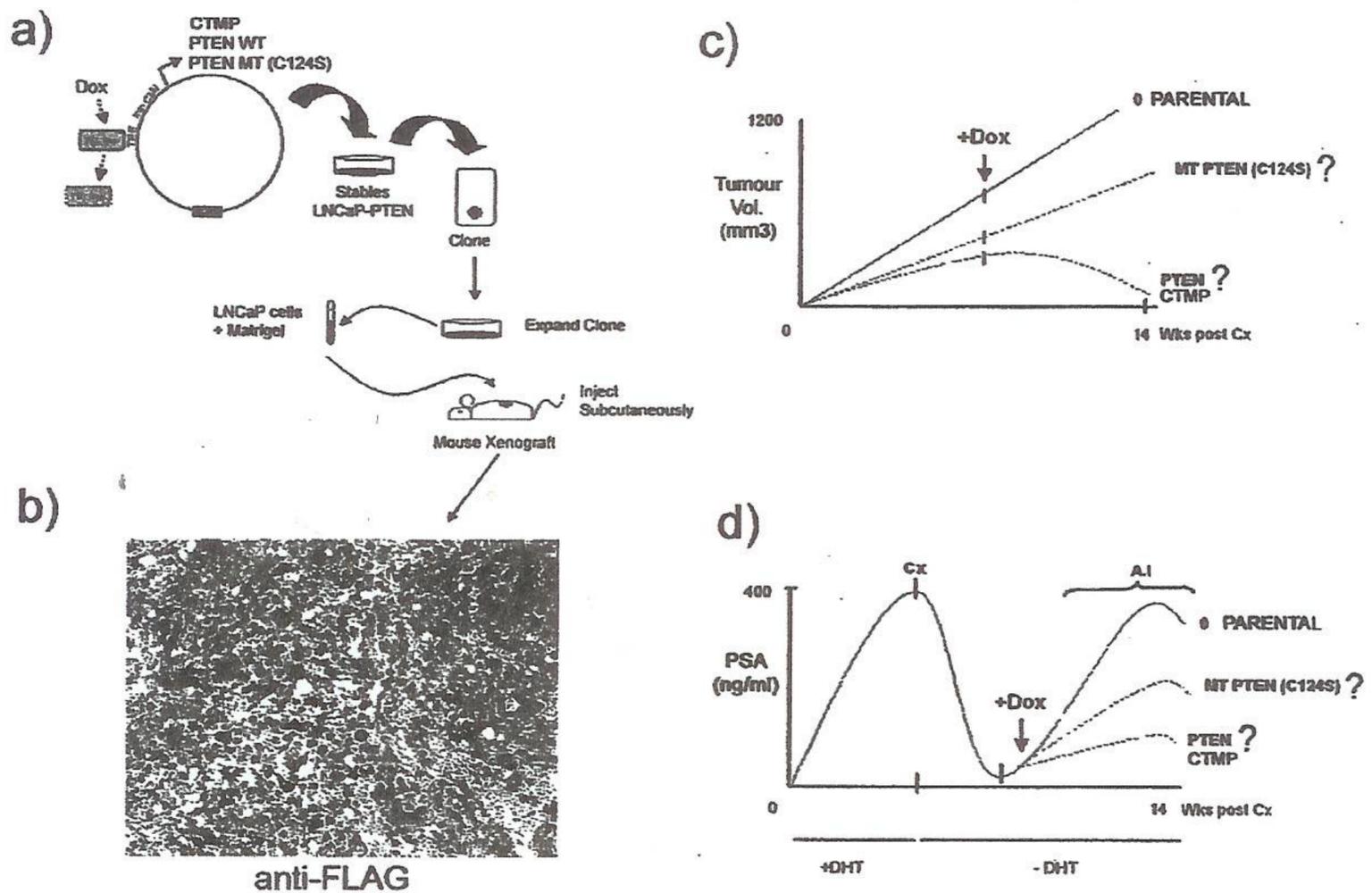


Fig 5. Generation of an inducible LNCaP-PTEN cell line and Xenograft.

a) Stable LNCaP-PTEN and LNCaP-CTMP clones are under the control of a Tet responsive element and minimal CMV promoter. PTEN or CTMP was transfected into LNCaP cells and clones isolated by hygromycin selection. Select clones were expanded and mixed with matrigel for injection in the dorsal flank of nude mice ($\sim 2.5 \times 10^6$ cells per injection). b) Validation of stable, in vivo protein expression in an LNCaP xenograft tumour (consisting of a 30% inducible population of LNCaPs) upon doxycyclin treatment, tissue sectioning and staining (anti-FLAG) for a previously studied protein. Tumours were grown for 4 weeks, administered 10 mg/ml doxycyclin in drinking water, harvested and stained for the presence of FLAG (FLAG-protein X). Positively staining cells indicates that robust protein induction can be maintained in vivo over the duration of weeks. c) Prospective AI LNCaP tumour growth profile as a function of induced PTEN (WT vs. MT) or CTMP expression in the absence of androgens (castrated). d) PSA levels will be measured in PTEN and CTMP induced tumour and compared to parental and mutant controls.

For both *in vitro* *in vivo* analysis of CTMP and PTEN we will pay particular attention to localization and expression of β -cat signalling components. While β -cat and Wnt pathway mutations are not common in PrCa xenografts there have been reports of infrequent APC and CTMP mutations [14, 40]. Therefore, if there is an observed change in β -cat distribution, as a function of PTEN or CTMP induction, we will isolate representative cells for β -cat exon 3 sequence analysis.

4) **How do PTEN and CTMP compare in their repressive PI3K effects?** Defining which molecules are necessary for proliferation and growth in PrCa cells with hyperactive PI3K signalling is crucial for the development of targeted inhibition of androgen independent PI3K signalling. To determine whether inhibition of PIP3 pools and PIP2→PIP3 conversion is functionally comparable to abrogation of phosphorylated Akt mediated control of β -cat/Tcf signalling, we will employ both focussed, commercial PI3K/prostate cancer cDNA arrays consisting of 100 genes (Superarray/GEArray) and large scale, cDNA arrays (>10,000 genes) available at UCLA. These studies will allow us both to confirm the functionality of our stable cell lines and to determine differences between the effects of PTEN and CTMP mediated alteration of β -cat signalling components. To carry this out we will extract RNA samples both from cell lines and xenograft tumours using LNCaP-parental, LNCaP-PTEN WT, LNCaP-PTEN (C124S) and LNCaP-CTMP induced tumours harvested during the AI stage of xenograft tumour progression.

Summary: While it has been widely hypothesized that PI3K signalling is necessary for AI growth, it is likely that PI3K signalling regulates downstream effector molecules manifesting the capacity for PrCa epithelia to evade androgen ablation therapy. Using recently generated knock-out mice and inducible PrCa (PTEN & CTMP) expression systems, we are in an excellent position to evaluate how modulation of PI3K signalling can alter the oncogenic potential of Beta-catenin/Tcf signalling in PrCa progression. *In vivo* analysis of Beta-catenin/Tcf signalling to PrCa progression in conjunction with tumour analysis with cDNA microarray and protein screening techniques will be crucial to the future development of targeted inhibitors and may generate clinically relevant data for assessing response to novel therapies.

c) Career Development Plan: My proposed studies with the UCLA will provide me with excellent preparation for a career in prostate cancer biology. The UCLA prostate cancer group is one of the best in the United States and will provide me with the opportunity to continue the high level of training that I have received during my Doctoral studies at *The Prostate Centre* (Vancouver, BC). UCLA will also provide me with new and exciting training in exploring the development of novel prostate cancer models. In terms of intellectual training, I will work closely with UCLA collaborators of Dr. [redacted] who are interested in prostate cancer biology, including Drs. [redacted] and [redacted]. These investigators can all be considered leaders in their respective fields and are highly productive as bourn out by in publication record. Dr. [redacted] s laboratory is a member of the UCLA Cancer Center, which offers weekly seminars and a yearly scientific retreat. I will present my research work at least once a year in this campus-wide research seminar series in addition to our weekly lab meetings. I will also attend at least one National or International Scientific Conference per year. UCLA has obtained funding from the National Cancer Institute as a part of Mouse Models for Human Cancer Consortium and Dr. Wu is the Co-PI for the UCLA center grant. Dr. [redacted] is also a co-investigator of the UCLA SPORE grant. Dr. [redacted] laboratory has a monthly scientific meetings as well as a journal club with rest of the prostate cancer research groups at UCLA. I will be an active member of this joint team. Currently, the field of prostate cancer lacks biologically relevant cell lines (PSA secreting) and animal models that show prostate cancer progression in a similar manner to humans. Dr. [redacted] has recently developed a novel animal model (a PTEN -/- conditional mouse) which develops metastatic and androgen independent prostate cancer. By learning technology in the production of transgenic and knock-out animals in the context of sound biological hypothesis I hope to gain training that will allow me to conduct research that provides greater clinical relevancy. By completing my Doctoral studies at The Prostate Centre, Vancouver and my Post-doctoral training with the prostate group at UCLA I will be in an excellent position to complete for an excellent faculty position in prostate cancer research.