Olga Miakicheva
UCSD School of Medicine, Class of 2018
Independent Study Project
March 7, 2018

Bone Metastatic Prostate Cancer

An Overview of Cell Signaling, Novel Cell Culture Environment, and a Push for Cross-Investigator Collaboration.

Introduction

Prostate cancer (CaP) continues to be the second leading causes of male cancer mortality in the United States despite our efforts to mitigate the cancer via better screening techniques and cancer management[1]. Due to clinical advancements more men are diagnosed at an earlier, organ-confined, stage of prostate cancer decreasing the rate of metastatic disease to 4%[2]. Nonetheless, metastatic disease is still a significant risk factor for those men who are not cured of the organ-confined disease. The treatment of recurrent prostate cancer usually consists of Androgen Deprivation Therapy (ADT), followed by chemotherapy and radiotherapy if the cancer becomes resistive and non-responding [3], [4]. Unfortunately, many bone metastasized prostate cancer cells become resistant to ADT and eventually leave no viable treatment options[3], [4]. Even more shocking is the fact that 100% of men who died of prostate cancer had bone metastases[4].

In patients these bone metastases are linked to both osteoblastic and osteolytic lesions leading to pain, discomfort and bone fractures. Medical and research communities worked to develop various models to study the metastatic disease due to the lack of curative treatments and unknown reasons for prostate cancers affinity to bone. Currently, there are several cancer bone metastasis-derived orthotopic bone models, using prostate carcinoma cell lines which include: PC3, LAPC9, VCaP and finally PCSD1 [3], [4]. Each one of the models produces slightly different bone-remodeling outcomes: PC3 forms purely osteolytic lesions in intra-tibial xenografts, VCaP produces mixed osteoblastic/osteolytic lesions, while LAPC9 forms purely osteoblastic lesions but also shifts to differentiate from the original patient-derived cell lines based on genomic sequencing data. Finally, PCSD1 can form both osteoblastic and osteolytic lesions in the murine model [3], [4].

PCSD1 is a novel, primary prostate cancer bone metastasis-derived xenograft model developed in Dr. Christina Jamieson's UCSD laboratory aimed for use as a therapeutic testing tool for developing novel CaP therapies [3]. A recent study showed that PCSD1 tumors were castration resistant, meaning they grew without the activation of the androgen receptor, in the bone-niche as compared to soft tissue environments[4]. Upon investigation, it was discovered that the WNT5A signaling pathway activates a WNT5A/BMP-6 loop, and this enables the proliferation, differentiation, migration, polarity and apoptosis via beta-catenin-dependent canonical and beta-catenin-independent non-canonical pathways[5], [6].

Earlier studies revealed that osteoblastic differentiation mediated by BMP-2 is associated with increased expression of WNT5A and Ror2 [5]. Another study confirmed that WNT5A has a profound effect on CaP cell migration using PC3 cell line on a bone marrow stromal cell (BMSC) conditioned media [7]. Another finding about WNT5A is that it is expressed at higher levels in specific regions of bone, such as the growth plates [8]. The discovery of association

between WNT5A and bone-niche CaP allows room for potential novel therapeutic agents aimed at bone-metastatic prostate cancer. Here, we aim to delineate existing WNT5A/CaP research as well as demonstrate the involvement of WNT5A in the PCSD1 model.

As previously stated, various cell lines are created and studied in order to understand pathophysiology of cancer cell interactions as well as their responses to possible treatments. However, standard in-vitro cultured do not accurately mimic in-vivo organ systems. In order to mimic the structural relationships between cell-of-interest and their microenvironment various 3D in vitro model systems have been developed [9]. The novel 3D organotypic systems, or organoids, can be formed from various tissue types including primary tissue and stem cells and are capable of mimicking in-vivo characteristics of real tissues[10]. Here, we aim to further evaluate the progress of organoid research as it relates to prostate cancer as well as outline organoid quantification techniques.

Despite a plethora of new CaP research, even the most interactive investigators struggle to comprehend the full scope of the field. In order to continue studying bone-metastatic prostate cancer efforts have to be made in order to provide a collaborative environment between prostate cancer investigators, clinicians and ultimately patients. With the help of the Albert Foundation our lab discovered just how hard it is for patients to find out if any research is being done in regard to their specific cancer. It was determined that despite online searching bone metastatic prostate cancer there is a lack of free primary literature that is easily available to the public. During my time at CAMJ lab I designed and built a website that provides information about the bone-metastatic prostate cancer research that is being performed at UCSD by Dr. Christina Jamieson, as well as an online repository of other bone-metastatic prostate cancer animal models investigated at other institutions.

Each section of this document will discuss the various projects that I was able to spearhead while at the CAMJ lab and hope that this description will ultimately prove beneficial to other investigators in the field of bone-metastatic prostate cancer research.

WNT5A And Bone-Niche Prostate Cancer Literature Review

The discovery of association between WNT5A and bone-niche CaP allows room for potential novel therapeutic agents aimed at bone-metastasized prostate cancer. My aim here is to further collect information about the involvement and importance of the WNT5A signaling pathway in prostate cancer, prostate development and its link to other cancers. A systematic electronic literature search was conducted to identify any publications relating to the Wnt signaling pathway as it relates to prostate cancer using PubMed (http://www.pubmed.gov/) and Cochrane Library (http://www.cochranelibrary.com/) from January 1991 to November 2016. Several combinations of the following search terms were used to identify pertinent publications: “WNT5A”, “Wnt signaling pathway”, “prostate cancer”, “malignancy”, “cancer”, and “beta catenin pathway”. Only peer-reviewed published articles were included in the analysis of current state of research of WNT5A in prostate cancer. The research was categorized into groups based on final conclusions including how it relates to prostate cancer, how the Wnt pathways impacts bone lesions and metastases, and the importance of Wnt in prostate development.

Wnt Pathway and Prostate Development

Many cancers appropriate signaling pathways normally used during organogenesis and development, leading to abhorrent cell multiplication. WNT5A has shown to be a key player in several steps of embryogenesis. Several studies further demonstrate the involvement of the Wnt

pathway in prostatic development. WNT5A is essential for normal prostate development where it regulates bud outgrowth, ductal elongation, branching, cell polarity and lumenization. WNT5A participates in prostatic bud patterning by restricting mouse ventral prostate development to prevent prostatic fistulation to the hindgut.

Author and Title	Journal and Date	Summary and Conclusion
Huang L, Pu Y, Hu WY, et al. The role of WNT5A in prostate gland development. [11]	Dev Biol. 2009	In rat prostate, Wnt5A mRNA is expressed by distal mesenchyme during the budding stage and localizes to periductal mesenchymal cells with an increasing proximal-to-distal gradient during branching morphogenesis Steroids modulate prostatic Wnt5A expression during early development with testosterone suppressing WntA and neonatal estrogen increasing expression WntA is not essential for functional differentiation Wnt5A suppresses prostae Shh expression while Shh stimulates Wnt5A expression in a lobe-specific manner during early development indicating that Wnt5A participates in cross-talk with other members of the gene regulatory network that control prostate development. Wnt5A does not influence prostatic expression of other Wnt morphogens, it suppresses Wif-1 expression and can thus indirectly modulate Wnt signaling. Wnt5A is essential for normal prostate development where it regulates bud outgrowth, ductal elongation, branching, cell polarity and lumenization.
Allgeier SHI et al. Wnt5A selectively inhibits mouse ventral prostate development. [12]	Dev Biol. 2008	Wnt5A participates in prostatic bud patterning by restricting mouse ventral prostate development. In the 70% of WntA null male fetuses where the UGS was present, it was morphologically deformed, devoid of a pelvic urethra, and attached by a fistulous connection to the hindgut
Zhang TJ1, et al. SAGE reveals expression of Wnt signalling pathway members during mouse prostate development. [13]	Gene Expr Patterns. 2006	Antagonist of the Wnt pathway, secreted frizzled related protein 2 is highly expressed in the early prostate libraries and down regulated at later developmental stages. Only Wnt4 transcripts were detectable in the developing prostate Wnt pathway members are expressed in the developing prostate Wnt5A, the most abundantly expressed Wnt gene in the UGS
Yamaguchi TP, Bradley A, McMahon AP, Jones S. A Wnt5A pathway underlies outgrowth of multiple structures in the vertebrate embryo. [14]	Development. 1999	A loss-of-function mutation of Wnt5A leads to an inability to extend the A-P axis due to a progressive reduction in the size of caudal structures Due to outgrowth defects observed in the developing face, ears and genitals, our data indicates that WntA regulates a pathway common to many structures whose development requires extension from the primary body axis The reduced number of proliferating cells in both the progress zone and the primitive streak mesoderm suggests that one function of WNT5A is to regulate the proliferation of progenitor cells.

WNT5A and Prostate Cancer

Most of the research I've analyzed aims to find the crucial link between Wnt pathway and prostatic cancer development, progression, and aggression. Although WNT5A appears to be

upregulated in some of the most aggressive prostate cancers, there is data to demonstrate that some of the most aggressive CaP shows a downregulation of WNT5A. The combination of research is indicative of a very complicated crosstalk of WNT5A and other receptors and ligands in the B-catenin pathway requiring a much more careful dissection of the signals.

Author and Title	**Journal and Date**	**Summary and Conclusion**
Liang X et al. MicroRNA-1297 inhibits prostate cancer cell proliferation and invasion by targeting the AEG-1/Wnt signaling pathway. [15]	Biochem Biophys Res Commun. 2016	miR-1297 significantly downregulated in hCaP Overexpression of miR-1297 inhibited CaP cell proliferation and invasion miR-1297 suppression promoted CaP cell proliferation and invasion Astrocyte elevated gene-1 (AEG-1) is a predicted target of miR-1297 miR-1297 inhibits prostate cancer proliferation and invasion by targeting AEG-1, ultimately impacting the Wnt pathway
Shu X, et al. Genetic variants of the Wnt signaling pathway as predictors of aggressive disease and reclassification in men with early stage prostate cancer on active surveillance.[16]	Carcinogenesis. 2016	Expression of KLK3 is regulated by β-catenin allows Wnt/AR crosstalk Somatic genetic alterations in the top 6 mutated Wnt pathway genes were observed in 30% of prostate cancer cases: FZD3: 11%, DVL2: 8%, PPP2CB: 8%, FZD2: 7%, FZD6: 7%, APC: 5%; cBioportal rs752822 and rs2735839 may assist in risk-stratifying GS 7 patients and predict prostate cancer reclassification
Thiele S et al. WNT5A and Its Receptors in the Bone-Cancer Dialogue.[17]	J Bone Miner Res. 2016	Summary of the role of the noncanonical Wnt ligand WNT5A in the development and metastatic process of osteotropic cancer entities
Tseng JC et al. CAPE suppresses migration and invasion of prostate cancer cells via activation of non-canonical Wnt signaling. [18]	Oncotarget. 2016	Caffeic acid phenethyl ester (CAPE) is a main bioactive component of honeybee hive propolis CAPE treatment suppressed the migration and invasion of PC-3 and DU-145 CaP cells. CAPE treatment induced receptor ROR2 in non-canonical Wnt signaling pathway but suppressed abundance of β-catenin, NF-κB activity, PI3K-Akt signaling, and epithelial-mesenchymal transition (EMT). Overexpression or knockdown of ROR2 suppressed or enhanced cell migration of PC-3 cells, respectively. CAPE treatment reduced canonical Wnt signaling. Intraperitoneal injection of CAPE reduced the metastasis of PC-3 xenografts in tail vein injection nude mice model.
Miyamoto, et al. RNA-Seq of single prostate CTCs implicates noncanonical Wnt signaling in antiandrogen resistance.[19]	Science. 2015	Using CTC cells, RNA sequencing. Ectopic expression of WNT5A in prostate cancer cells attenuates the antiproliferative effect of AR inhibition WNT5A suppression in drug-resistant cells restores partial androgen sensitivity
Thiele S, et al. WNT5A has anti-prostate cancer effects in vitro and reduces tumor growth in the skeleton in vivo.[20]	J Bone Miner Res. 2015	In vitro, WNT5A overexpression induced CaP cell apoptosis and reduced proliferation and migration, WNT5A knock-down showed opposite effects.

		In vivo, local tumor growth and tumor growth in the bone microenvironment was considerably diminished after WNT5A overexpression in PC3 cells. WNT5A exhibits antitumor effects in CaP cells
Lee GT, et al. Prostate cancer bone metastases acquire resistance to androgen deprivation via WNT5A-mediated BMP-6 induction. [21]	Br J Cancer. 2014	Bone-CaP interaction leads to castration resistance via WNT5A/BMP-6 loop In vitro co-cultures show CaP cells proliferated under an androgen-depleted condition when incubated with bone stromal cells. qPCR shows induction of BMP-6 by CaP cell lines in the presence of bone stromal cells WNT5A derived from bone stromal cells ups BMP-6, upping CaP proliferation via a physical interaction between Smad5 and β-catenin. Intracellularly, WNT5A increased BMP-6 expression via protein kinase C/NF-κB pathway in CaP cell lines
Zhao S, et al. MiR-26a inhibits prostate cancer progression by repression of WNT5A.[22]	Tumor biology 2014	Significant decrease of miR-26a in CaP tissues versus their normal Stable miR-26a inhibited cell proliferation, metastasis, and epithelial mesenchymal transition and induced G1 phase arrest in prostate cancer miR-26a inhibited CaP progression via WNT5A
Jin F, Qu X, Fan Q, Wang L, Tang T, et al. Regulation of prostate cancer cell migration toward bone marrow stromal cell-conditioned medium by WNT5A signaling.[7]	Mol Med Rep. 2013	Expression of 22 genes associated with bone metastasis was measured in three PCa cell lines (LNCaP, PC3 and DU145) Significantly higher levels of WNT5A mRNA expression were identified in the PC3 cells, compared with those in LNCaP and DU145 cells. Silencing WNT5A expression with siRNA reduced the migration capacity of PC3 cells by 50%. The addition of rmWNT5A improved the migration capacity of PC3 cells in a concentration-dependent manner. WNT5A promotes PC3 cell migration toward BMSC-CM
Zheng D, et al. Role of WNT7B-induced noncanonical pathway in advanced prostate cancer.[23]	Mol Cancer Res. 2013	WNT7B is necessary for the growth of CaP cells, promotes androgen-independent growth of CRPC cells likely through the activation of protein kinase C isozymes, and induces osteoblast differentiation in vitro through a direct cell-cell interaction
Khaja ASS, et al. Emphasizing the role of WNT5A protein expression to predict favorable outcome after radical prostatectomy in patients with low-grade prostate cancer.[24]	Cancer Med. 2012	Preserved high protein expression of WNT5A in prostate cancer is associated with longer relapse-free time after radical prostatectomy Patients with preserved high-WNT5A protein levels in their tumor cells have a lower risk of recurrence after radical prostatectomy compared to patients with low-WNT5A protein expression
Kypta, R.M., et al. Wnt/beta-catenin signalling in prostate cancer. [25]	Nat. Rev. Urol, 2012	Activation of the Wnt/β-catenin pathway has effects on prostate cell proliferation, differentiation and the epithelial–mesenchymal transition
Takahashi, S et al. Noncanonical Wnt signaling mediates	Proc Natl Acad Sci U S A. 2011	CaP tumor growth was significantly potentiated by introduction of the AR T877A mutation Genetic screening of mice identified Wnt-5a as an activator.

androgen-dependent tumor growth in a mouse model of prostate cancer.[26]		Enhanced Wnt-5a expression in malignant prostate tumors but not in BPH Noncanonical Wnt signal stimulates development of prostatic tumors with AR hyperfunction
Syed Khaja AS, Helczynski L, Edsjo A, et al. Elevated level of WNT5A protein in localized prostate cancer tissue is associated with better outcome. [27]	PLoS One. 2011	Preserved overexpression of WNT5A protein in patients with localized prostate cancer predicts a favorable outcome after surgery WNT5A impairs the invasive properties of prostate cancer cells Patients with high expression of WNT5A protein had significantly better outcome in terms of time to biochemical recurrence compared to patients with low expression levels A combination of high WNT5A expression with low levels of Ki-67 or androgen receptor expression had even better outcome compared to all other groups. WNT5A expression significantly correlated with VEGF and with Ki-67 and androgen receptor expression
Yamamoto H, Oue N, Sato A, et al. WNT5A signaling is involved in the aggressiveness of prostate cancer and expression of metalloproteinase. [28]	Oncogene. 2010	Abnormal expression of WNT5A and beta-catenin was observed in 27 (28%) and 49 (50%) of 98 prostate cancer cases Simultaneous expression of WNT5A and beta-catenin was observed in only five cases, suggesting their exclusive expression. The positive detection of WNT5A was correlated with high Gleason scores and biochemical relapse of prostate cancer, but that of beta-catenin was not. Knockdown and overexpression of WNT5A in human prostate cancer cell lines reduced and stimulated, respectively, their invasion activities, and the invasion activity required Frizzled2 and Ror2 as Wnt receptors WNT5A activated Jun-N-terminal kinase through protein kinase D (PKD) and the inhibition of PKD suppressed WNT5A-dependent cell migration and invasion WNT5A induced the expression of metalloproteinase-1 through the recruitment of JunD to its promoter region
Wang Q, Symes AJ, Kane CA, et al. A novel role for Wnt/Ca2þ signaling in actin cytoskeleton remodeling and cell motility in prostate cancer. [29]	PLoS One. 2010	The activity of Ca2+/calmodulin dependent protein kinase (CaMKII), a transducer of the non-canonical Wnt/Ca2+ signaling, increased by 8 fold in cancer cells; no change was observed in β-catenin expression, known to activate the canonical Wnt/β-catenin pathway Expression of numerous genes (e.g., CCND1, CD44) under the control of β-catenin transcription is down-regulated. Incubation of normal prostate cells with recombinant WNT5A protein induced actin remodeling with a regular wound edge and increased wound healing capacity. non-canonical Wnt/Ca2+ signaling via CaMKII acts as a novel regulator of structural plasticity and cell motility in prostate cancer
MacDonald, B.T. et al. (2009) Wnt/beta-catenin signaling: components, mechanisms, and diseases. [30]	Dev Cell. 2009	Review article highlights some key aspects of Wnt/beta-catenin signaling in human diseases including congenital malformations, cancer, and osteoporosis, and discuss potential therapeutic implications.
Wang Q, Williamson M, Bott S, et al. Hypomethylation of	Oncogene. 2007	Hypomethylation of WNT5A, S100 calcium-binding protein P (S100P) and cysteine-rich protein 1(CRIP1) was confirmed in the cancer cells

WNT5A, CRIP1 and S100P in prostate cancer.[31]		Bisulfite sequencing of a section of the 5' untranslated region (UTR) of WNT5A revealed that three CpG sites (15, 24 and 35) were consistently methylated (93%) in the normal cell line and normal tissues, but not in the prostate cancer cell line and eight primary prostate cancers Likely that an epigenetic mechanism regulates WNT5A expression in prostate cancer.
Verras, M. et al. Roles and regulation of Wnt signaling and beta-catenin in prostate cancer. [32]	Cancer Lett 2006	Abnormal expression of Wnt ligands, receptors, inhibitors, and other co-regulators play important role in CaP
Giles, R.H. et al. (2003) Caught up in a Wnt storm: Wnt signaling in cancer. [33]	Biochim Biophys Acta. 2003	The Wnt signaling pathway, named for its most upstream ligands, the Wnts, is involved in various differentiation events during embryonic development and leads to tumor formation when aberrantly activated.

WNT5A in other bone metastatic cancers

The Wnt pathway is also implicated in several other cancers, specifically breast cancer and melanoma. Figure 1 from paper "WNT5A and Its Receptors in the Bone-Cancer Dialogue" by Thiele S, et al. briefly demonstrates the common involvement of WNT5A signaling across all three cancers, highlighting the ligand-receptor pair's ability to control cell migration, invasion, and progression.

Breast cancer

Cell surface receptors are a hot topic in breast cancer but remain elusive in patients with "triple negative" diseases. In these individuals the role of WNT5A seems to be protective. In fact, patients with lower WNT5A levels in tumor tissue, have an increased risk of death.

Author and Title	**Journal and Date**	**Summary and Conclusion**
Zhong Z, Shan M, Wang J, Liu T, Shi Q, Pang D. Decreased WNT5A expression is a poor prognostic factor in triple-negative breast cancer.[34]	Med Sci Monit. 2016	WNT5A expression was evaluated in 90 TNBC specimens Pts with negative WNT5A expression had significantly poorer recurrence-free survival (RFS) than patients with positive WNT5A expression (P=0.008). Negative WNT5A protein expression might contribute to the tumor progression and poor prognosis of TNBC and might be a new therapy target in TNBC.
Cai J, Guan H, Fang L, et al. MicroRNA-374a activates Wnt/b-catenin signaling to promote breast cancer metastasis. [35]	J Clin Invest. 2013	In early stages, BrCa cells undergo an Wnt/β-catenin signaling is known to drive epithelial-mesenchymal transition (EMT) and metastasis Wnt/β-catenin signaling is hyperactivated in metastatic breast cancer cells that express microRNA 374a. miR-374a directly targeted and suppressed multiple negative regulators of the Wnt/β-catenin signaling cascade, including WIF1, PTEN, and WNT5A. miR-374a maintains constitutively activated Wnt/β-catenin signaling
Jonsson M, Dejmek J, Bendahl P-O., Andersson T. Loss of Wnt-5a protein is associated with early	Cancer Res. 2002	Wnt-5a gene plays a role as a tumor suppressor. Loss of WNT5A protein expression was significantly associated with higher histological grade and absence of estrogen and progesterone receptors.

relapse in invasive ductal breast carcinomas. [36]		WNT5A expression was lost in tumors from 78% of the patients with recurrent disease (n = 32) compared with 35% of the recurrence-free Recurrence-free survival was significantly shorter in the Wnt-5a-negative group (P < 0.001)

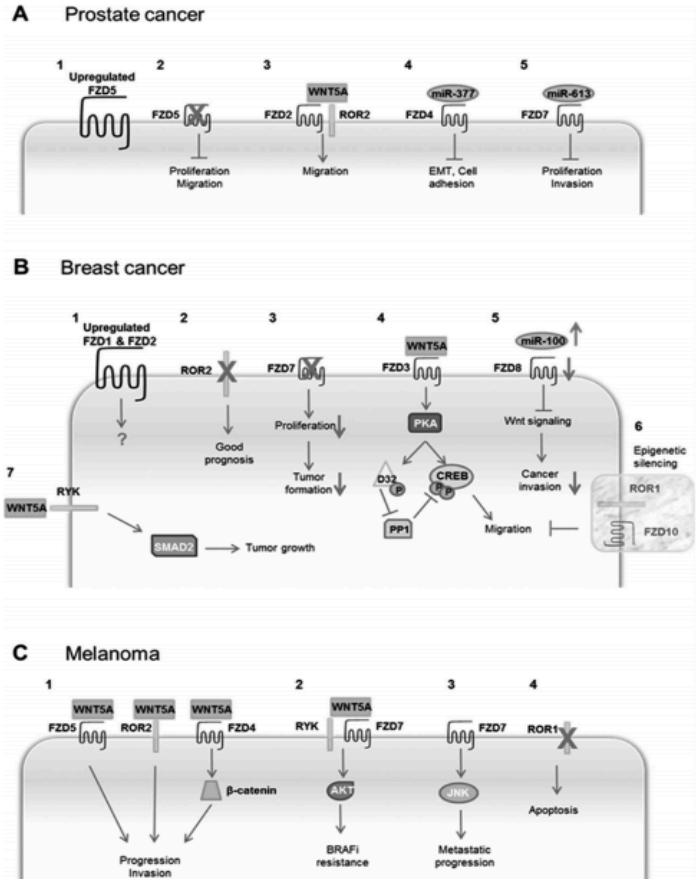


Figure 1: Summary of WNT5A signaling in CaP, Breast Cancer, and Melanoma.

Melanoma and beyond

In melanoma, WNT5A has been shown to increase motility and invasion of melanoma cells. However, the effects of WNT5A were also shown to depend on ROR2 and ROR1. ROR1, when inactivated, drove cancerous melanocytes to apoptosis.

Notably, WNT5A and ROR1 have been implicated and well studied in leukemias, specifically Chronic Lymphocytic Leukemia by Kipps group and Catriona Jamieson. The investigators developed an antibody against ROR1 that is currently in clinical trials for CLL and breast cancer demonstrating a real-time translation of cancer molecular signaling to therapeutics[37], [38].

Author and Title	**Journal and Date**	**Summary and Conclusion**
Choi M, Widhopf G. Pre-clinical Specificity and Safety of UC-961, a First-In-Class Monoclonal	Clin Lymphoma Myeloma Leuk 2015	Due to expression of ROR1 on the cell surface of leukemia cells in chronic lymphocytic leukemia (CLL), but not on normal B-cells or other post-partum tissues, ROR1 is a therapy candidate. UC-961 is a first-in-class humanized monoclonal antibody (mAb) that binds the extracellular domain of ROR1. This paper outlines some of the

Antibody Targeting ROR1[38].		preclinical studies leading to an investigation new drug (IND) designation, enabling clinical studies in patients with CLL.
O'Connell MP, Marchbank K, et al. Hypoxia induces phenotypic plasticity and therapy resistance in melanoma via the tyrosine kinase receptors ROR1 and ROR2. [39]	Cancer Discov. 2013	The Wnt signaling pathway, can effectively guide the phenotypic plasticity of tumor cells, when primed to do so by a hypoxic microenvironment. Increased WNT5A signaling can give rise to a subpopulation of highly invasive cells that are intrinsically less sensitive to novel therapies for melanoma, and targeting the WNT5A/ROR2 axis could improve the efficacy and duration of response for patients with melanoma on vemurafenib.
Grossmann AH, Yoo JH, Clancy J, et al. The small GTPase ARF6 stimulates b-catenin transcriptional activity during WNT5A-mediated melanoma invasion and metastasis. [40]	Sci Signal. 2013	In melanoma cells, WNT5A stimulated the disruption of N-cadherin and β-catenin complexes by activating the guanosine triphosphatase adenosine diphosphate ribosylation factor 6 (ARF6). Binding of WNT5A to the Frizzled 4-LRP6 (low-density lipoprotein receptor-related protein receptor complex activated ARF6, which liberated β-catenin from N-cadherin, thus increasing the pool of free β-catenin, enhancing β-catenin-mediated transcription, and stimulating invasion.
O'Connell MP, Fiori JL, Xu M, et al. The orphan tyrosine kinase receptor, ROR2, mediates WNT5A signaling in metastatic melanoma.[41]	Oncogene. 2010	ROR2 is expressed predominantly in metastatic melanoma, implicating WNT5A as a mediator of melanoma metastasis Increases in WNT5A cause increases in ROR2 expression, as well as the PKC-dependent, clathrin-mediated internalization of ROR2 Using in vitro and in vivo metastasis assays, we show that ROR2 is necessary for the WNT5A-mediated metastasis of melanoma cells

Conclusion

Overall, it is evident that interest in the Wnt/Catenin pathway in prostate cancer has become an increasingly studied field. We know WNT5A plays a role in establishing castration resistance and is a ripe target for androgen insensitive PCa. Nonetheless, the novel research has not yet been able to establish a specific Wnt pathway target that can used therapeutically for prostate cancer. I believe that the difficulty lies in seemingly contradictory information regarding the importance of WNT5A in both prostate cancer aggressiveness and its role as a protective factor for recurrence. Nonetheless, existing ROR1-specific antibody therapeutic UC-961 implies that molecular targeting can be useful in treatment of malignancies and should be pursued.

WNT5A And PCSD1

Initial Proposal

Bone metastatic prostate cancer continues to be a hard-to treat problem for men all across the world. A newly developed xenograft PCSD1 model is a pre-clinical therapeutic development and testing platform to understand the mechanism of resistance to androgen deprivation therapy of bone metastatic prostate cancer. However, the dual osteoblastic and osteolytic nature of prostatic cancer tumors leads to many hypotheses regarding the effect of microenvironment on the cancer cell. The hypothesis I tested is that the signaling molecule WNT5A, which can be found in various parts of the developing bone, allows CaP cells to grow in an androgen-independent fashion in the bone-niche.

Hypotheses and Experimental Setup

The two main hypothesis that I initially aimed to explore were as follows:
1) There is a specific distribution of WNT5A, Ror1, and Ro2 signaling molecules on the bony substrate that can effect the development of the prostate cancer bone tumors.
2) PCSD1 cells are influenced by presence of WNT5A in the bone-microenvironment.

Results

Immunohistochemical (IHC) staining of WNT5A proved to be extremely difficult to perform on existing xenograft model sections despite several rounds of condition optimizations and trials. Staining with a rabbit-anti-human WNT5A antibody showed good positive and negative control validity but did could not be detected on experimental sections [Fig 2-4]. Experimental conditions including greater concentration of primary antibody, newer sections of femurs as well as decreased time in overnight storage chamber were considered. Staining appeared to be improved with a 1:100 concentration of antibody [Fig. 5].

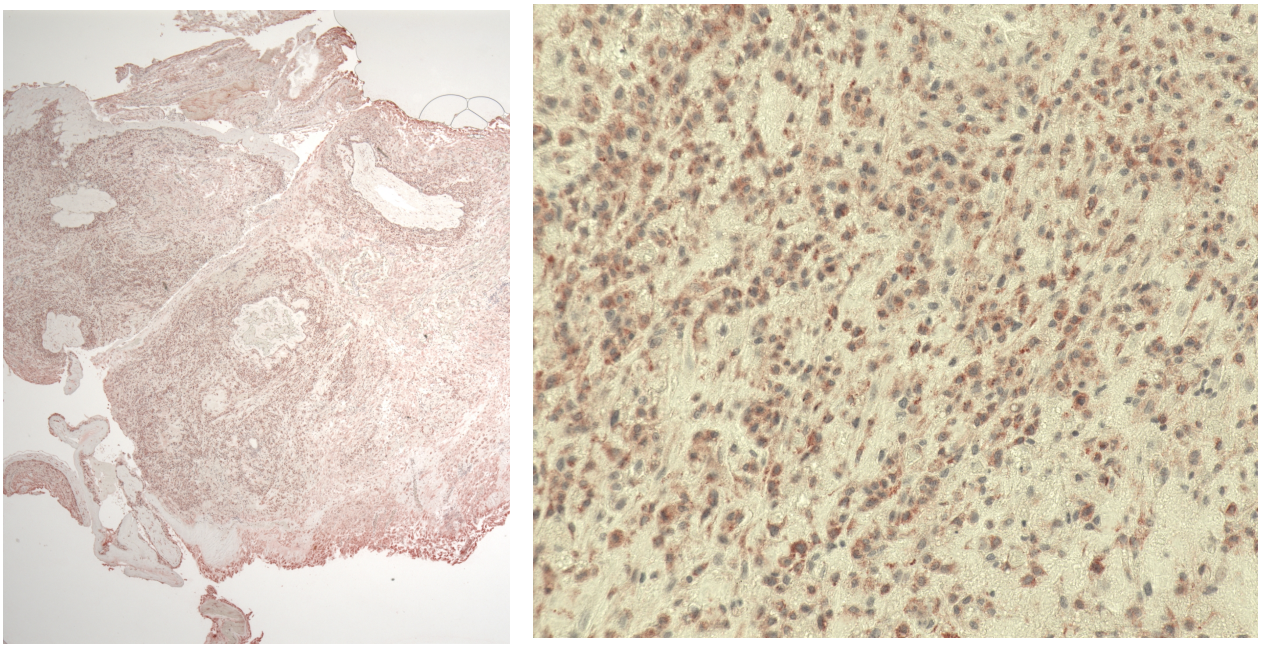


Figure 2. Left: Placenta, HRP stain of WNT5A, 2x. Right: Placenta, HRP stain (brown) of 1:200 WNT5A, 20x.

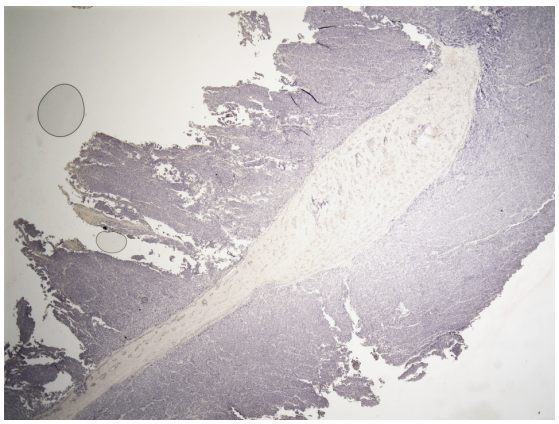


Figure 3. (Left) Xenograft prostate cancer bone tumor, HRP stain of 1:200 WNT5A, 2x.

Figure 4. (Right) Xenograft prostate cancer bone tumor, HRP stain of WNT5A, 20x.

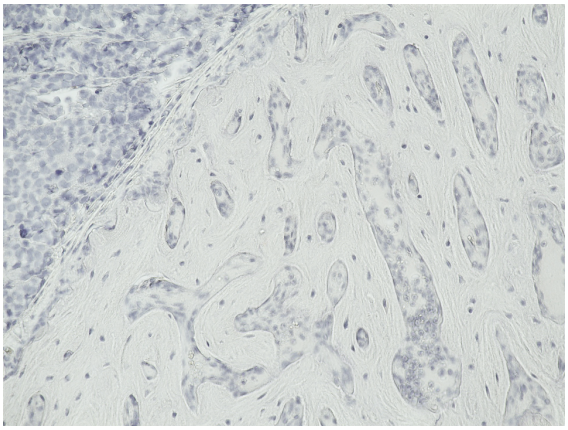
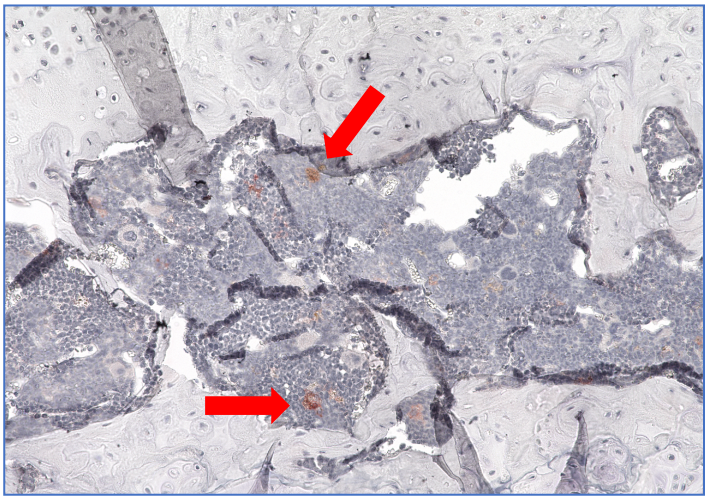
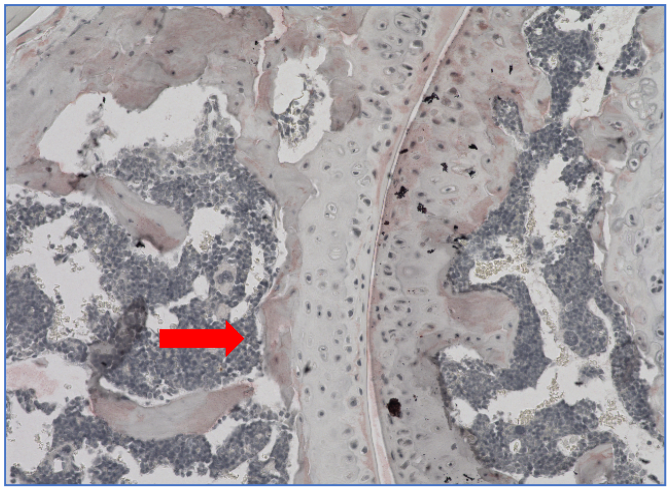
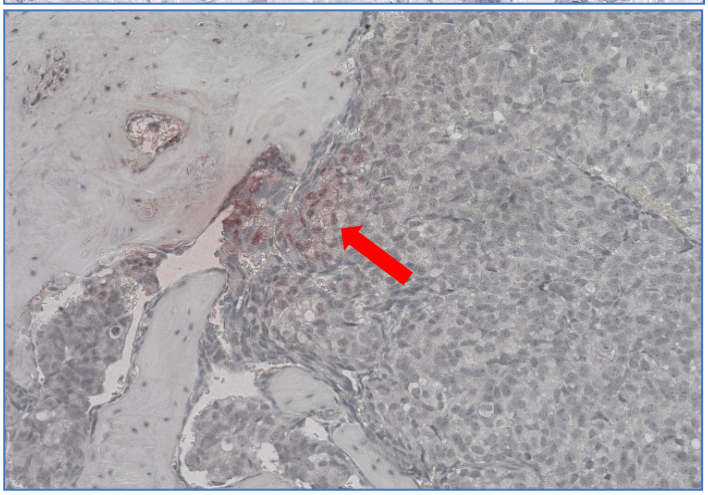


Figure 5.
Right: 1:100 WNT5A (brown) of a PCSD1+HS5 co-injected right leg. 10x.
Bottom : Different areas of 1:100 WNT5A(Brown) of PCSD1 only injected right leg. 20x.







Development of in-vitro cell cultures

In order to simulate more organ-like growth conditions for PCSD1 in-vitro model, we attempted growing PCSD1-GFP cells on HS5+HS27 stroma. The first phase of the experiment involved culturing irradiated human stromal cells, (HS5 and HS27) with PCSD1 on 7 and 14-day cycles. The cells continued to be GLF positive and were monitored with fluorescent microscopy [Fig 6, 7]. FACs cell sort revealed only a 33% return of cancer cells. Notably, non-irradiated stromal cells produced a better growth environment. In order to further quantify cells with fluorescent microscopy, the cells were grown on sterilized glass cover-slips but cell-adhesion to glass was very poor. Cover slips were consequently treated with Poly-d-lysine to encourage cell adhesion. Cytologic PSMA staining of PCSD1 cells grown in in-vitro co-cultures was performed [Fig. 8]. Cells appear to be washed off cover slips as well as impacted by profound edge effect. We did discover that the GFP signal was only moderately quenched in this protocol. Given the poor results of co-culture experiment we chose to not continue the experiment.

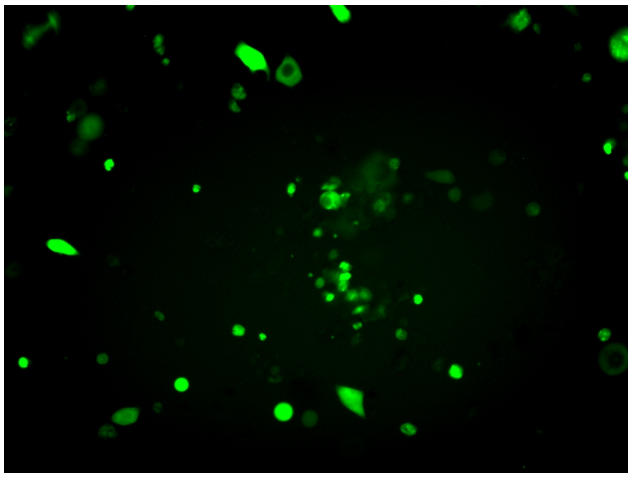


Figure 6: 20x view of PCSD1-GFP–Luciferase cells grown on HS5/HS27 mix. Transitional, elongated, cancer cells noted.

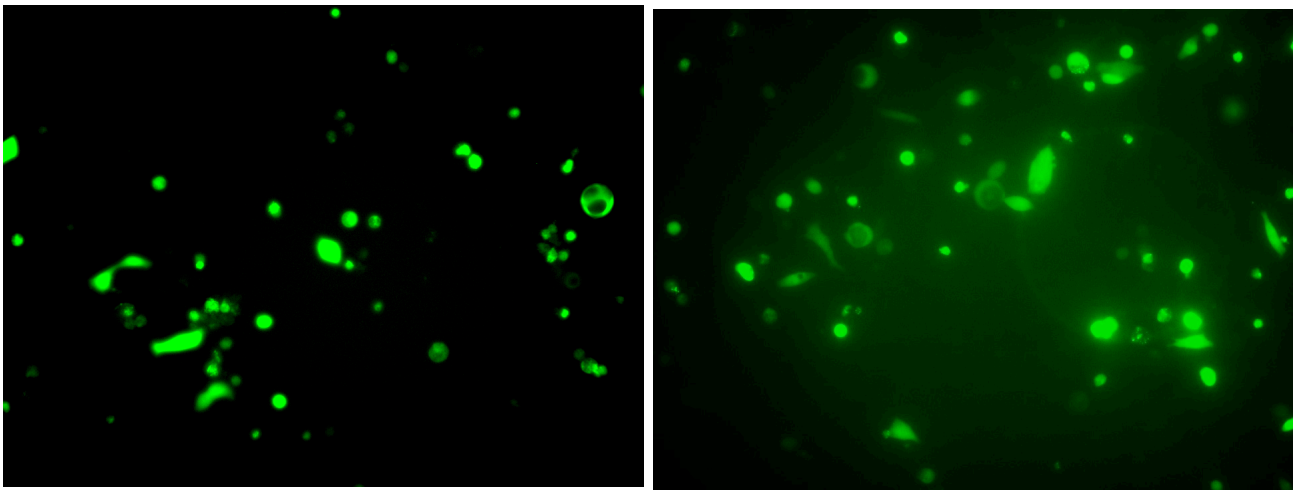


Figure 7: PCSD1-GFP–Luciferase cells grown on HS5/HS27 stromal layer. 20x.
Left: 7 day growth period. Right: 14 days of growth. Transitional cells noted.

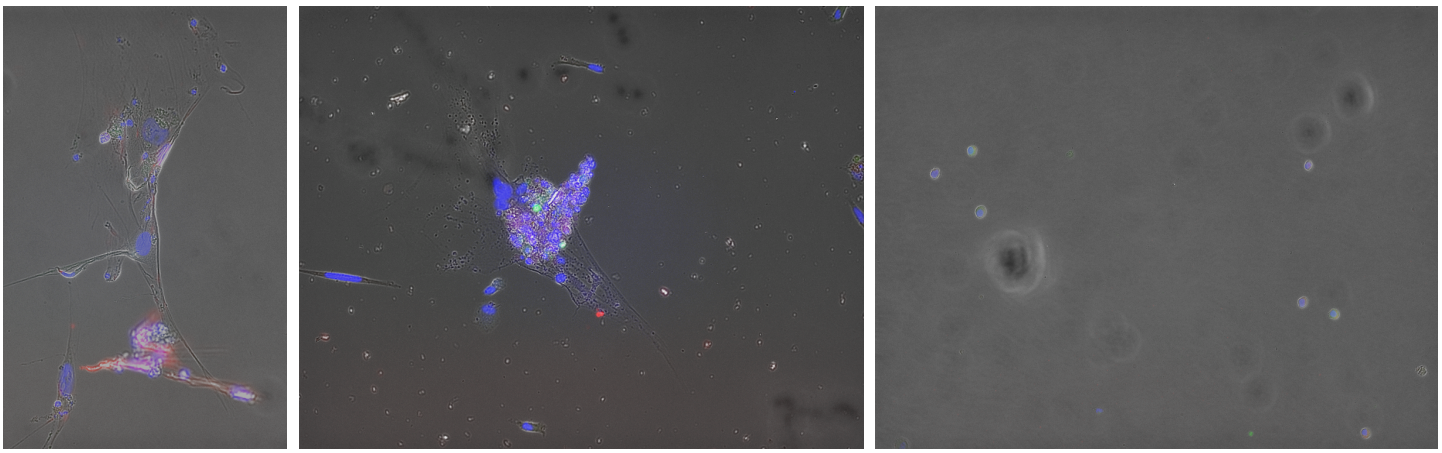


Figure 8 (Left): HS5/HS27 cells only. (Middle): PCSD1 cells grown on HS5/HS27. (Right): PCSD1 cells only. Blue – DAPI nuclear stain. Red – PSMA stain. Green – GFP. 20x magnification.

Importance of Communication – the Need of a PDX model repository

The following is a free-access website created for the CAMJ lab to aid in patient-derived xenograft (PDX) research collaboration and patient understanding regarding prostate cancer research.

https://sites.google.com/ucsd.edu/pdxmodelsofprostatecancer/home

The website consists of four pages outlined below:

Home Page

What is a PDX Model?

Patient-derived xenograft (PDX) model is a type of biological model composed of patient-derived tumor fragments that are grafted to immunocompromised mice.

What makes PDX Models special?

PDXs can maintain the original histology, as well as the molecular and genetic characteristics of the source tumor[42].

What is PCSD1?

With support of the Leo and Anne Albert Charitable Trust we developed a new series of PDX mouse models: the PCSD series (Prostate Cancer San Diego: PCSD1, PCSD4, PCSD5, PCSD13, and PCSD17) of in vivo patient-derived xenograft (PDX) models which closely recapitulate the bone metastatic disease in patients.
In addition, we have created and continue to grow the The Leo and Anne Albert Bone Metastatic Prostate Cancer Research Center BIOBANK.

Is there a list of Prostate Cancer PDX Models?

Yes, several literature reviews have been published on this matter. However, you can find a simplified table of the various prostate cancer PDX models that were developed and in use today, here.

Biobank

GOAL

To maintain and grow a BioBank of patient prostate cancer bone metastasis specimens, xenografts and organoids to use for genomics, basic research, pre-clinical testing of novel therapies and for sharing with the scientific community for translational research on bone metastatic prostate cancer.

Prostate cancer bone metastases are not often surgically removed and, thus, have been challenging to study and to develop models to test new therapies. However, in cases in which the bone metastasis has caused or is about to cause a pathologic fracture, orthopaedic surgical repair is performed and the tumor tissue is removed. In collaboration with UCSD surgeons: Drs. Anna Kulidjian and Christopher Kane, and with the support of the Leo and Anne Albert Charitable Trust, we began collecting surgical patient prostate cancer bone metastasis specimens which we used to develop a new series of patient-derived xenograft (PDX) mouse models: the PCSD series (Prostate Cancer San Diego: PCSD1, PCSD4, PCSD5, PCSD13, and PCSD17) of in vivo patient-derived xenograft (PDX) models which closely recapitulate the bone metastatic disease in patients.

The best characterized is the PCSD1 model which replicates a number of properties of the patient's bone metastatic cancer. My group has used whole exome sequencing, genome-wide CNV analysis, single cell RNASeq, microarray expression profiling, RT-PCR, qPCR, FACS, immunohistochemistry and microCT scanning to show that our PDX model closely reproduced bone metastatic disease in prostate cancer patients. We have shown that the bone microenvironment specifically promotes resistance to androgen deprivation therapy with either bicalutamide or enzalutamide treatment.

We use our PDX models to test novel therapies, including CART immunotherapies, to overcome therapy resistance of bone metastatic prostate cancer.

The PCSD BIOBANK consists of the surgical patient specimens, the PCSD series of PDX and patient derived organoids (PDOs) models of prostate cancer bone metastasis already generated from the biospecimens that we have collected and new patient specimens that we continue to collect using the UCSD Moores Cancer Center Biorepository. Thus far, we have 20 surgical specimens of prostate cancer bone metastases, PCSD1-20, numbered in the chronological order in which they were received. Full urologic and orthopaedic donor patient clinical information is available from a de-identifed database. Specimens from five patients who spanned the spectrum of different treatment stages have established serially transplantable xenografts: PCSD1, PCSD4, PCSD5, PCSD13 and PCSD17. Matched patient whole blood, sera, and urine are also available along with FFPE prostatectomy tissue on the patients who had this procedure in prior treatment.

CAMJ Lab

Who we are

We are a small but fearless team of researchers commanded by Dr. Christina Jamieson at UC San Diego.

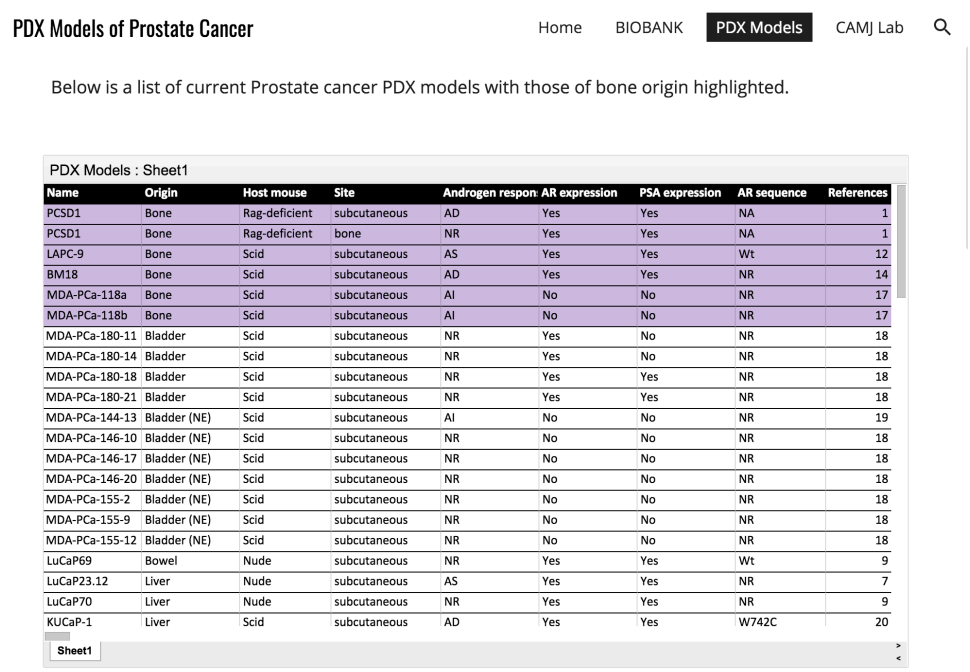
Our Focus

Dr. Jamieson's research is focused on elucidating mechanisms of therapy resistance in bone metastatic urologic cancers using patient-derived xenograft (PDX) models and 3D in vitro models. She is applying her expertise in genome-wide analyses of prostate cancer tumors and primary patient-derived tumor models to translate next generation genomics on prostate cancers

into practical applications and novel therapies that will improve and, she hopes, eventually transform patient care.

PDX Models

Figure 9: Screenshot of PDX Models tab showing the bone-metastatic prostate cancer cell lines highlighted in purple.



PDX Models of Prostate Cancer

Home BIOBANK PDX Models CAMJ Lab

Below is a list of current Prostate cancer PDX models with those of bone origin highlighted.

PDX Models : Sheet1

Name	Origin	Host mouse	Site	Androgen respon	AR expression	PSA expression	AR sequence	References
PCSD1	Bone	Rag-deficient	subcutaneous	AD	Yes	Yes	NA	1
PCSD1	Bone	Rag-deficient	bone	NR	Yes	Yes	NA	1
LAPC-9	Bone	Scid	subcutaneous	AS	Yes	Yes	Wt	12
BM18	Bone	Scid	subcutaneous	AD	Yes	Yes	NR	14
MDA-PCa-118a	Bone	Scid	subcutaneous	AI	No	No	NR	17
MDA-PCa-118b	Bone	Scid	subcutaneous	AI	No	No	NR	17
MDA-PCa-180-11	Bladder	Scid	subcutaneous	NR	Yes	No	NR	18
MDA-PCa-180-14	Bladder	Scid	subcutaneous	NR	Yes	No	NR	18
MDA-PCa-180-18	Bladder	Scid	subcutaneous	NR	Yes	Yes	NR	18
MDA-PCa-180-21	Bladder	Scid	subcutaneous	NR	Yes	Yes	NR	18
MDA-PCa-144-13	Bladder (NE)	Scid	subcutaneous	AI	No	No	NR	19
MDA-PCa-146-10	Bladder (NE)	Scid	subcutaneous	NR	No	No	NR	18
MDA-PCa-146-17	Bladder (NE)	Scid	subcutaneous	NR	No	No	NR	18
MDA-PCa-146-20	Bladder (NE)	Scid	subcutaneous	NR	No	No	NR	18
MDA-PCa-155-2	Bladder (NE)	Scid	subcutaneous	NR	No	No	NR	18
MDA-PCa-155-9	Bladder (NE)	Scid	subcutaneous	NR	No	No	NR	18
MDA-PCa-155-12	Bladder (NE)	Scid	subcutaneous	NR	No	No	NR	18
LuCaP69	Bowel	Nude	subcutaneous	NR	Yes	Yes	Wt	9
LuCaP23.12	Liver	Nude	subcutaneous	AS	Yes	Yes	NR	7
LuCaP70	Liver	Nude	subcutaneous	NR	Yes	Yes	NR	9
KUCaP-1	Liver	Scid	subcutaneous	AD	Yes	Yes	W742C	20

Sheet1

Prostate Cancer Organoids

Background

Despite the prevalence of prostate cancer research, in vitro studies have been hard to establish and even harder to equate to in-vivo conditions until recent years. With development of organoid-based cultures we are getting closer to in-vivo-like conditions within the comfort of an incubated petri-dish. Three-dimensional organoid cultures have proven to be of value in increasing our understanding of the biology of disease and offer the potential of regenerative and genetic therapies. Wang et al summarized the use of organoids in urological research in 2017 however, several questions remain: First - how are PCa organoid cultures defined, validated and studied? And second – is there a validated pathway to testing biopharmaceuticals on in-vitro organoid cultures? The goal of this literature review is to further define organoid research as it relates to prostate cancer.

Methods

A systemic electronic literature search was conducted to identify any publications relating to the use of Organoid cell culture as it relates to prostate cancer using PubMed (http://www.pubmed.gov/) and Cochrane Library (http://www.cochranelibrary.com/) from January 1997 to December 2017. Several combinations of the following search terms were used to identify pertinent publications: 'Prostate Spheroids", "Organoid", "3D Cell Culture", "prostate cancer", and "malignancy". Only peer-reviewed published articles were included in the analysis of current state of research pertaining to prostate cancer organoids. The research was categorized into groups based on final conclusions including how the development of organoid cell culture impacts in vitro cancer research, how the authors quantified the research and what conditions were used to maintain cultures. Exclusion criteria: papers not peer reviewed.

A brief history of organoids:

1997	**The term "organoid" used to describe 3-dimensional prostate cancer cell culture. The 3D growth model was composed of CaP+ bone stroma (in collagen gel or microgravity-simulated growth conditions) by Chung et al** [43]**.**
2001	Lang et al. cultures 3D human prostate tissue in Matrigel[44].
	Rhee et al. shows effect of estrogen on 3D in-vitro culture by measuring PSA expression[45].
2005	Festuccia et al. uses a 3D organoid model to draw conclusions about prostatic neoplasia vs cancer. Tests the effect of DHT on cell culture by cell counting[46].
2007	Li et al. demonstrates self-renewal and multilineage differentiation from single adult prostate stem/progenitor cells in a specific in vitro microenvironment[47].
	Tyson et al. demonstrates that organoid culture conditions can be varied, records effect of extracellular calcium on organoid morphology[48].
2013	Zhang et al. uses specifically defined organoid conditions in order to test importance of CECAM gene group in CaP[49].
2014	**Karthaus et al. defines growth conditions to produce organoids that are genetically stable, reconstitute prostate glands in recombination assays, and can be experimentally manipulated**[50]**.**
	Gao et al. performs whole exome sequencing to show mutations in FOXA1 and PIK3R1, as well as of DNA repair and chromatin modifier pathways linked advanced disease[51].
	Chua et al masters single cell organoid development [52].
2015	Gao et al. and Vela et al. publish reviews on organoid development[53], [54].
	Robinson et al. recognizes the need for complex 3D imaging techniques, implements Markov fields (mathematics) to help cell tissue culture studies [55].
	Harma et al. uses 3D organoid cultures to study AKT-inhibitors in prostate cancer [56].
	Agarwal et al. demonstrates two types of luminal progenitor cells in CaP[57].
	Lee et al. uses organoid cultures to further study the origin of CaP.[58]
2016	**Drost et al. further describes the culture protocol that allows the growth of both the luminal and basal prostatic epithelial lineages, as well as the growth of advanced prostate cancers**[59]**.**
	Park et al. implies that distinct subtypes of prostate cancer may arise from luminal and basal epithelial cell types subjected to the same oncogenic insults. Provides a platform for the functional evaluation of oncogenes in basal and luminal epithelial populations of the human prostate [60].
2017	Zhang et al continues to hunt down the "Cell of origin" for CaP by studying Normal Human Prostate cells (NHPs)[61].
	Unno et al. Prostate cancer organoids provide a useful pre-clinical model for the evaluation of new candidate cancer genes, cancer disparities, and potentially for testing of novel therapeutic agents[62].
	Blattner et al. and Shoag et al. study SPOP mutations in mouse organoids to link them to increased proliferation[63], [64].
	Pan et al. tests gambonic acid on prostate organoids in hopes of validating its effects on in-vivo CaP[65].

Growth Conditions for Organoid culture

Although various methodologies have been described since 1997, the most cited method was described by Karthaus et al and had the following requirements.
For murine cells, "[We] embedded dissociated cells of wild-type murine prostate epithelium in Matrigel and added ''generic'' organoid medium containing the growth factors EGF, Noggin, and R-spondin1 (ENR). We also included the Alk3/4/5 inhibitor A83-01 to inhibit TGF-b pathway signaling to prevent a proliferative block in prostate cells." [50].
Human prostate organoid cultures required additional growth factors including: Ffibroblast growth factor-10 (FGF10), FGF2, prostaglandin E2 (PGE2), nicotinamide and the p38 inhibitor SB202190.
Most recently, Ma et al. demonstrated a very precise protocol for organoid cultures of LnCAP and C4-2B cell lines with Matrigel and organoid medium containing 50× diluted B27, 1.25 mM N-acetyl-L- cysteine, 5 ng/ml EGF, 100 ng/ml Noggin, 500 ng/ml recombinant R-spondin 1, 500 nM A83-01, 10 ng/ml FGF10, 5 ng/ml FGF2, 1 μM PGE2, 10 mM nicotinamide, 10 μM SB202190, 1 nM DHT, and 10 μM Y-27632 dihydrochloride [66].

Present "Future" Directions

Now that organoid growth conditions have been well established and studied for both murine and human prostate cancer cells, several questions remain regarding the quantification of organoid-bound-cells. How do we confirm prostatic lineage withing organoids? How can we study organoid responses to therapeutics? A wide array of quantification and qualification techniques has been used across many research groups including: qrtPCR, rtPCR, Western Blotting, Whole Exome Sequencing, immunohistochemistry (IHC), immunofluorescence (IF), flow cytometry, or via size measurement obtained via light microscopy.
Properties of cells and organoids that were often quantified were organoid diameter at various timepoints, organoid morphology as being hollow vs filled, and organoid edges as being smooth or irregular. Some of markers that were studies across many laboratories included PSA, PSMA, Cytokeratins (CK4, CK14, CK8, CK18) to distinguish basal vs luminal cell types, p63, CD44, CD49. The table below demonstrates the various experimental conditions and their check-points for prostate organoid research.

Author	**Experimental Conditions, checks, and outcomes**
Lang et al. 2001[44]	- Human primary cultures in Matrigel - Dihydrotestosterone (DHT) and Estrogen increased spheroid-forming efficiency as measured by number of spheroids per high-power field.
Rhee et al. 2001[45]	- Prostate cancer cells (LNCaP) cultured in a three-dimensional rotating-wall vessel - Assessed effect of DHT and 17-beta-estradiol (17-13-E2) at various concentrations (1-100nM) on cell growth (calculated via cell counts) and PSA secretion (measured in ng/mL) - both DHT and 17-13-E2 lead to increase in cell counts of LNCaPs and associated cells
Festuccia et al. 2005 [46]	- Showed the differential expression of epithelial and prostatic markers in 30 CaP, 6 high grade prostate intraepithelial neoplasia (PIN) and 6 BPH-derived primary cell cultures - Growth response study of DHT (0.01-10uM) on "organoids" based on cell number.
Li et al 2007[47]	- Murine prostate stem cells plated in vitro in laminin containing Matrigel medium form clonogenic spheroid structures or "prostate spheres". - Addition of DHT to culture medium drives differentiation to a luminal cell-like phenotype as validated via qRTPCR for K5 and K8 and AR.

Beltran et al. 2011[67]	- Neuroendocrine prostate cancer (NEPC) is an aggressive subtype of prostate cancer that most commonly evolves from preexisting prostate adenocarcinoma (PCA). - Used next-generation RNA sequencing and oligonucleotide arrays, profiled 7 NEPC, 30 PCA, and 5 benign prostate tissue (BEN) samples and validated findings in tumors from a large cohort of patients (37 with NEPC, 169 with PCA, and 22 with BEN) using immunohistochemistry and FISH. - Enhanced sensitivity of NEPC (and MYCN overexpressing PCA) to Aurora kinase inhibitor (PHA-739358) both in vitro and in vivo , with complete suppression of neuroendocrine marker expression following treatment assessed with immunoblotting.
Zhang et al. 2013 [49]	- Prostate cancer cells were grown on Matrigel - Inhibition of CEACAM1 with antibodies inhibited tubule formation by over 50% while the remaining tubules were stunted. - Inhibition of CEACAM20 with antisense oligonucleotides inhibited tubule formation and stunted the growth of acini. - For inhibition assays, soluble-CEACAM1 or antibodies were added to medium. Colonies with or without tubule formation were counted with an inverted light microscope. Measured: colony numbers, colonies with tubules and mean fluorescence intensity.
Gao et al. 2014 [51]	- Developed a long-term organoid culture of prostate cancer from biopsy specimens and circulating tumor cells that recapitulate molecular diversity of CaP subtypes and retained the histology, grade and genomic alterations properties of the original cancer - Whole exome sequencing shows a low mutational burden, consistent with genomics studies, but with mutations in FOXA1 and PIK3R1, as well as of DNA repair and chromatin modifier pathways. - Cell viability assays included treatment of 5000 organoids with DHT, Enzalutamide, everolimus. Cells were counted using a CellTiter-Glo Luminescent Cell Viability Assay.
Chua et al. 2014[52]	- Organoids generated from CARNs (castration-resistant Nkx3.1-expressing cells) or normal prostate epithelia within Matrigel exhibit tissue architecture containing luminal and basal cells, undergo long-term expansion in culture and exhibit functional androgen receptor signaling - Analyzed effect of DHT, dimethylsulphoxide (DMSO) [no effect], and Enazlutamide [minimal effect on organoid formation]. Treatment with enzalutamide and MK-8669 inhibited organoid formation as observed with light microscopy and qRT-PCR.
Karthaus et al. 2014 [50]	-Established organoids from mice prostate and human postate - measured by dose-dependent induction of PSA mRNA by DHT and reduced expres- sion of NKX3.1 and PSA upon DHT withdrawal via qRT-PCR
Harma et al 2015 [56]	- Testing of betulins and abietane derivatives organotypic model system of advanced, castration-resistant prostate cancers. - Preliminary screen of 93 compounds done in 2D cultures with Epithelial cells, EP156T and LNCaP and PC-3. From those, 25 betulin derivatives were tested with 3D cultures and effect on cell cycle progression, mitosis, proliferation and unspecific cytotoxicity, cell motility and tumor cell invasion monitored. - Testing was done via qRT-PCR, WB, organoid size and organoid complexity studied.
Akerfelt et al 2015 [68]	-Developed a platform of microtissues from prostate cancer cells, combined with cancer associated fibroblasts (CAFs) - Chemical perturbation of selected proteins that are associated with tumorigenesis; wingless-type MMTV integration site family (WNT), β-catenin signaling, and γ-secretase protein complex (Notch), FAK, AKT kinase (AKT), phosphoinositide-3-Kinase (PI3K) and mammalian target of rapamycin (mTOR) and GMCSF. Results were monitored by cell counting/organoid counting and motion detection [Fig 10]

	-Showed that focal adhesion kinase (FAK) inhibitors specifically blocked tumor growth and invasion concurrently with fibroblast spreading and motility.
Kwon et al 2015[69]	- Sca-1 (stem cell antigen-1) identifies a small population of murine prostate luminal cells that reside in the proximal prostatic ducts adjacent to the urethra. - In the in vitro prostate organoid assay, a small fraction of the Sca-11 luminal cells are capable of generating budding organoids that are morphologically distinct from those derived from other cell lineages. - Enzalutamide treatment did not impact formation of organoids as verified by cell counting.
Blattner et al 2017 [63]	- Using these models and human prostate cancer samples, showed that SPOP mutation activates both PI3K/mTOR and androgen receptor signaling, uncoupling the normal negative feedback between these two pathways. - To test SPOP mutation, the researchers checked the percentage of mutation-positive organoids, relative protein expression and PTEN PAKT staining.
Unno, et al 2017[62]	-used human prostate cancer as a model, to assess feasibility of engineering defined genetic alterations in well-known cancer driver genes to transform benign prostate epithelial organoids derived from African American men. - Benign human prostate organoids transduced with lentiviruses expressing MYC, shPTEN, shTP53 and AR, to mimic prostate cancer development. - Organoids expressing MYC, shPTEN, shTP53 and AR (denoted MPPA); MYC, shPTEN and shTP53 (MPP); or MYC (M) were significantly larger, had higher proliferation rates and demonstrated pathologically transformed morphology compared to organoids transduced with control lentivirus. - Alterations in MYC, PTEN and TP53 also affected the rate of organoid basal-to-luminal differentiation in vitro as checked by microscopy, IHC, and qRTPCR, and viability measured with CellTiter.
Pan et al 2017[65]	-Explored effect of gambogic acid (GA) on the growth and cell death of castrate resistant CaP with PTEN- and p53- to show that GA reduced cells viability in both cell types - LAPC and benign prostate cancer cells treated with GA(50-150nM) for 24 and 48hrs then cell viability was determined by cell proliferation assay. Cells were also counted via microscopy, FACS, WB and cell titer glo assay performed.
Dai et al. 2017 [70]	- Bromodomain inhibitors (JQ1 and I-BET) show good results in early trials outcomes in early clinical trials. - Pathologically, prostate cancer–associated SPOP mutants fail to interact and promote the degradation of BET proteins, leading to increased levels in SPOP-mutant prostate cancer. - prostate cancer cell lines and organoids derived from individuals harboring SPOP mutations are more resistant to BET-inhibitor-induced cell growth arrest and apoptosis studied via cell proliferation assays.
Ma et al 2017[66]	-Reconfirm that LNCaP and C4-2B cells are able to form organoids under the defined organoid culture conditions (Matrigel) -Tested the response of organoids vs 2D cultured to interleukin-17A treatment, showing that treatment differently from the cells in the monolayer culture. The mRNA levels were analyzed by qRT-PCR.

Conclusion

It appears that several research groups are using the prostate cancer organoid models to test therapeutics as well as explore prostate cancer genomics and origins. Despite having a well outlined protocol of cell growth media the protocol for ascertaining organoid responses to testing conditions remains variable with qRT-PCR of known cell bio markers being the most frequently utilized testing parameter. It is evident that 2D cultures differ from 3D cultures in their relative

expression of mRNA [64] elucidating the fact that culture conditions have to be controlled prior to analyzing therapeutics. However, methodology demonstrated by Gao, Clevers, and Sawyers allow for recapitulation of histological and morphological traits of cancers leading us to believe they are more accurate than traditional 2D cell cultures for testing therapies.

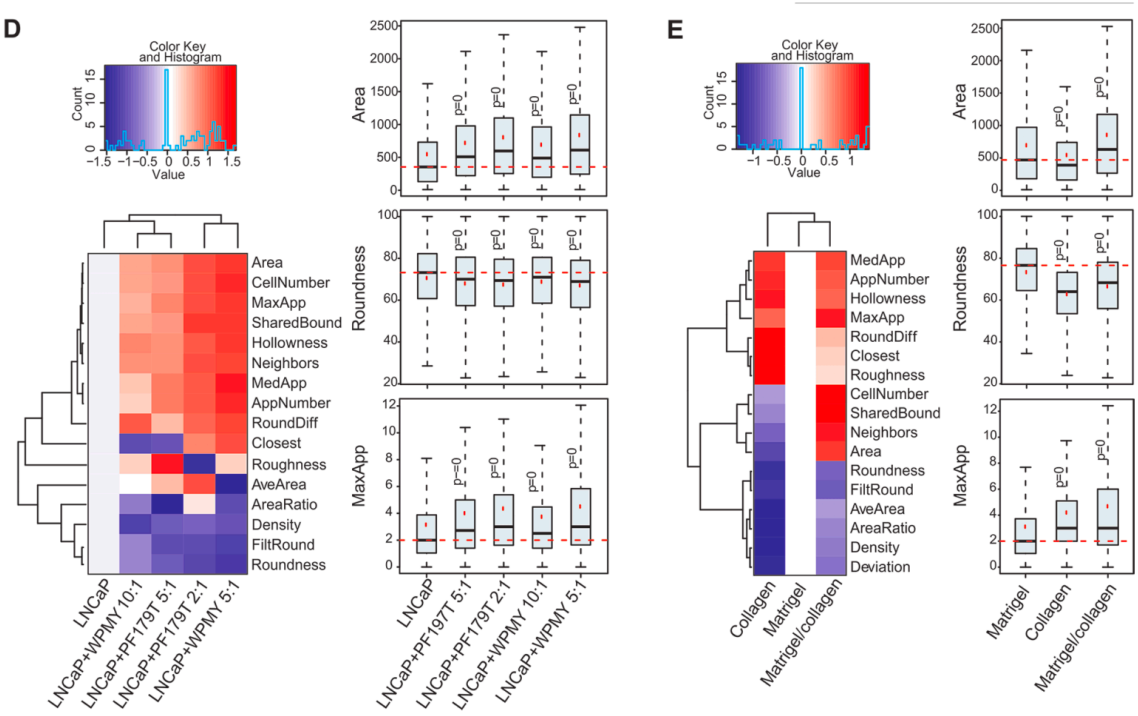


Figure 10. Example of the various physical parameters of organoids studied by Akerfelt in order to categorize differences in organoids.

[1] R. Siegel, J. Ma, Z. Zou, and A. Jemal, "Cancer statistics, 2014," *CA. Cancer J. Clin.*, vol. 64, no. 1, pp. 9–29, Jan. 2014.

[2] N. V Desireddi *et al.*, "Improved stage and grade-specific progression-free survival rates after radical prostatectomy in the PSA era.," *Urology*, vol. 70, no. 5, pp. 950–5, Nov. 2007.

[3] O. Raheem *et al.*, "A novel patient-derived intra-femoral xenograft model of bone metastatic prostate cancer that recapitulates mixed osteolytic and osteoblastic lesions.," *J. Transl. Med.*, vol. 9, no. 1, p. 185, Oct. 2011.

[4] E. Godebu *et al.*, "PCSD1, a new patient-derived model of bone metastatic prostate cancer, is castrate-resistant in the bone-niche.," *J. Transl. Med.*, vol. 12, no. 1, p. 275, Oct. 2014.

[5] E. Nemoto *et al.*, "Wnt5a signaling is a substantial constituent in bone morphogenetic protein-2-mediated osteoblastogenesis.," *Biochem. Biophys. Res. Commun.*, vol. 422, no. 4, pp. 627–32, Jun. 2012.

[6] A. Kikuchi, H. Yamamoto, A. Sato, and S. Matsumoto, "Wnt5a: its signalling, functions and implication in diseases," *Acta Physiol.*, vol. 204, no. 1, pp. 17–33, Jan. 2012.

[7] F. Jin *et al.*, "Regulation of prostate cancer cell migration toward bone marrow stromal cell-conditioned medium by Wnt5a signaling.," *Mol. Med. Rep.*, vol. 8, no. 5, pp. 1486–92, Nov. 2013.

[8] M. Okamoto *et al.*, "Noncanonical Wnt5a enhances Wnt/β-catenin signaling during

osteoblastogenesis.," *Sci. Rep.*, vol. 4, no. 1, p. 4493, Mar. 2014.
[9] E. R. Shamir and A. J. Ewald, "Three-dimensional organotypic culture: experimental models of mammalian biology and disease," *Nat. Rev. Mol. Cell Biol.*, vol. 15, no. 10, pp. 647–664, Oct. 2014.
[10] S. Wang, D. Gao, and Y. Chen, "The potential of organoids in urological cancer research," *Nat. Rev. Urol.*, vol. 14, no. 7, pp. 401–414, May 2017.
[11] L. Huang *et al.*, "The role of Wnt5a in prostate gland development," *Dev. Biol.*, vol. 328, no. 2, pp. 188–199, Apr. 2009.
[12] S. H. Allgeier *et al.*, "WNT5A selectively inhibits mouse ventral prostate development," *Dev. Biol.*, vol. 324, no. 1, pp. 10–17, Dec. 2008.
[13] T.-J. Zhang, B. G. Hoffman, T. Ruiz de Algara, and C. D. Helgason, "SAGE reveals expression of Wnt signalling pathway members during mouse prostate development.," *Gene Expr. Patterns*, vol. 6, no. 3, pp. 310–24, Mar. 2006.
[14] T. P. Yamaguchi, A. Bradley, A. P. McMahon, and S. Jones, "A Wnt5a pathway underlies outgrowth of multiple structures in the vertebrate embryo.," *Development*, vol. 126, no. 6, pp. 1211–23, Mar. 1999.
[15] X. Liang, H. Li, D. Fu, T. Chong, Z. Wang, and Z. Li, "MicroRNA-1297 inhibits prostate cancer cell proliferation and invasion by targeting the AEG-1/Wnt signaling pathway.," *Biochem. Biophys. Res. Commun.*, vol. 480, no. 2, pp. 208–214, Nov. 2016.
[16] X. Shu *et al.*, "Genetic variants of the Wnt signaling pathway as predictors of aggressive disease and reclassification in men with early stage prostate cancer on active surveillance.," *Carcinogenesis*, vol. 37, no. 10, pp. 965–971, Oct. 2016.
[17] S. Thiele, T. D. Rachner, M. Rauner, and L. C. Hofbauer, "WNT5A and Its Receptors in the Bone-Cancer Dialogue," *J. Bone Miner. Res.*, vol. 31, no. 8, pp. 1488–1496, Aug. 2016.
[18] J.-C. Tseng, C.-Y. Lin, L.-C. Su, H.-H. Fu, S.-D. Yang, and C.-P. Chuu, "CAPE suppresses migration and invasion of prostate cancer cells via activation of non-canonical Wnt signaling," *Oncotarget*, vol. 7, no. 25, pp. 38010–38024, Jun. 2016.
[19] D. T. Miyamoto *et al.*, "RNA-Seq of single prostate CTCs implicates noncanonical Wnt signaling in antiandrogen resistance," *Science (80-.).*, vol. 349, no. 6254, pp. 1351–1356, Sep. 2015.
[20] S. Thiele *et al.*, "WNT5A has anti-prostate cancer effects in vitro and reduces tumor growth in the skeleton in vivo.," *J. Bone Miner. Res.*, vol. 30, no. 3, pp. 471–80, Mar. 2015.
[21] G. T. Lee *et al.*, "Prostate cancer bone metastases acquire resistance to androgen deprivation via WNT5A-mediated BMP-6 induction.," *Br. J. Cancer*, vol. 110, no. 6, pp. 1634–44, Mar. 2014.
[22] S. Zhao *et al.*, "MiR-26a inhibits prostate cancer progression by repression of Wnt5a.," *Tumour Biol.*, vol. 35, no. 10, pp. 9725–33, Oct. 2014.
[23] D. Zheng *et al.*, "Role of WNT7B-induced noncanonical pathway in advanced prostate cancer.," *Mol. Cancer Res.*, vol. 11, no. 5, pp. 482–93, May 2013.
[24] A. S. S. Khaja, L. Egevad, L. Helczynski, P. Wiklund, T. Andersson, and A. Bjartell, "Emphasizing the role of Wnt5a protein expression to predict favorable outcome after radical prostatectomy in patients with low-grade prostate cancer.," *Cancer Med.*, vol. 1, no. 1, pp. 96–104, Aug. 2012.
[25] R. M. Kypta and J. Waxman, "Wnt/β-catenin signalling in prostate cancer," *Nat. Rev. Urol.*, vol. 9, no. 8, pp. 418–428, Aug. 2012.
[26] S. Takahashi *et al.*, "Noncanonical Wnt signaling mediates androgen-dependent tumor growth in a mouse model of prostate cancer.," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 108, no. 12, pp. 4938–43, Mar. 2011.
[27] A. S. Syed Khaja *et al.*, "Elevated level of Wnt5a protein in localized prostate cancer tissue is associated with better outcome.," *PLoS One*, vol. 6, no. 10, p. e26539, Oct. 2011.
[28] H. Yamamoto *et al.*, "Wnt5a signaling is involved in the aggressiveness of prostate cancer and expression of metalloproteinase.," *Oncogene*, vol. 29, no. 14, pp. 2036–46, Apr. 2010.
[29] Q. Wang *et al.*, "A novel role for Wnt/Ca2+ signaling in actin cytoskeleton remodeling and cell

motility in prostate cancer.," *PLoS One*, vol. 5, no. 5, p. e10456, May 2010.
[30] B. T. MacDonald, K. Tamai, and X. He, "Wnt/beta-catenin signaling: components, mechanisms, and diseases.," *Dev. Cell*, vol. 17, no. 1, pp. 9–26, Jul. 2009.
[31] Q. Wang *et al.*, "Hypomethylation of WNT5A, CRIP1 and S100P in prostate cancer.," *Oncogene*, vol. 26, no. 45, pp. 6560–5, Oct. 2007.
[32] M. Verras and Z. Sun, "Roles and regulation of Wnt signaling and β-catenin in prostate cancer," *Cancer Lett.*, vol. 237, no. 1, pp. 22–32, Jun. 2006.
[33] R. H. Giles, J. H. van Es, and H. Clevers, "Caught up in a Wnt storm: Wnt signaling in cancer.," *Biochim. Biophys. Acta*, vol. 1653, no. 1, pp. 1–24, Jun. 2003.
[34] Z. Zhong, M. Shan, J. Wang, T. Liu, Q. Shi, and D. Pang, "Decreased Wnt5a Expression is a Poor Prognostic Factor in Triple-Negative Breast Cancer.," *Med. Sci. Monit.*, vol. 22, pp. 1–7, Jan. 2016.
[35] J. Cai *et al.*, "MicroRNA-374a activates Wnt/β-catenin signaling to promote breast cancer metastasis," *J. Clin. Invest.*, vol. 123, no. 2, pp. 566–79, Jan. 2013.
[36] M. Jönsson, J. Dejmek, P.-O. Bendahl, and T. Andersson, "Loss of Wnt-5a protein is associated with early relapse in invasive ductal breast carcinomas.," *Cancer Res.*, vol. 62, no. 2, pp. 409–16, Jan. 2002.
[37] S. Zhang *et al.*, "Abstract 1193: Treatment of breast cancer xenografts with paclitaxel enriches for cancer stem cells that can be targeted by a ROR1-specific antibody," *Cancer Res.*, vol. 76, no. 14 Supplement, pp. 1193–1193, Jul. 2016.
[38] M. Y. Choi *et al.*, "Pre-clinical Specificity and Safety of UC-961, a First-In-Class Monoclonal Antibody Targeting ROR1.," *Clin. Lymphoma. Myeloma Leuk.*, vol. 15 Suppl, no. 0, pp. S167-9, Jun. 2015.
[39] M. P. O'Connell *et al.*, "Hypoxia induces phenotypic plasticity and therapy resistance in melanoma via the tyrosine kinase receptors ROR1 and ROR2.," *Cancer Discov.*, vol. 3, no. 12, pp. 1378–93, Dec. 2013.
[40] A. H. Grossmann *et al.*, "The small GTPase ARF6 stimulates β-catenin transcriptional activity during WNT5A-mediated melanoma invasion and metastasis.," *Sci. Signal.*, vol. 6, no. 265, p. ra14, Mar. 2013.
[41] M. P. O'Connell *et al.*, "The orphan tyrosine kinase receptor, ROR2, mediates Wnt5A signaling in metastatic melanoma," *Oncogene*, vol. 29, no. 1, pp. 34–44, Jan. 2010.
[42] T. Inoue, N. Terada, T. Kobayashi, and O. Ogawa, "Patient-derived xenografts as in vivo models for research in urological malignancies," *Nat. Rev. Urol.*, vol. 14, no. 5, pp. 267–283, May 2017.
[43] L. W. Chung, H. E. Zhau, and T. T. Wu, "Development of human prostate cancer models for chemoprevention and experimental therapeutics studies.," *J. Cell. Biochem. Suppl.*, vol. 28–29, pp. 174–181, 1997.
[44] S. H. Lang, M. Stark, A. Collins, A. B. Paul, M. J. Stower, and N. J. Maitland, "Experimental prostate epithelial morphogenesis in response to stroma and three-dimensional matrigel culture.," *Cell Growth Differ.*, vol. 12, no. 12, pp. 631–40, Dec. 2001.
[45] H. W. RHEE *et al.*, "PERMANENT PHENOTYPIC AND GENOTYPIC CHANGES OF PROSTATE CANCER CELLS CULTURED IN A THREE-DIMENSIONAL ROTATING-WALL VESSEL," *Vitr. Cell. Dev. Biol. - Anim.*, vol. 37, no. 3, p. 127, 2001.
[46] C. Festuccia *et al.*, *International journal of oncology.*, vol. 26, no. 5. University of Crete, Faculty of Medicine, Laboratory of Clinical Virology, 2005.
[47] L. Xin, R. U. Lukacs, D. A. Lawson, D. Cheng, and O. N. Witte, "Self-Renewal and Multilineage Differentiation In Vitro from Murine Prostate Stem Cells," *Stem Cells*, vol. 25, no. 11, pp. 2760–2769, Nov. 2007.
[48] D. R. Tyson, J. Inokuchi, T. Tsunoda, A. Lau, and D. K. Ornstein, "Culture requirements of prostatic epithelial cell lines for acinar morphogenesis and lumen formation in vitro: Role of extracellular calcium," *Prostate*, vol. 67, no. 15, pp. 1601–1613, Nov. 2007.
[49] H. Zhang, A. Eisenried, W. Zimmermann, and J. E. Shively, "Role of CEACAM1 and

CEACAM20 in an In Vitro Model of Prostate Morphogenesis," *PLoS One*, vol. 8, no. 1, p. e53359, Jan. 2013.
[50] W. R. Karthaus *et al.*, "Identification of multipotent luminal progenitor cells in human prostate organoid cultures," *Cell*, vol. 159, no. 1, pp. 163–175, 2014.
[51] D. Gao *et al.*, "Organoid cultures derived from patients with advanced prostate cancer," *Cell*, vol. 159, no. 1, pp. 176–187, 2014.
[52] C. W. Chua *et al.*, "Single luminal epithelial progenitors can generate prostate organoids in culture," *Nat. Cell Biol.*, vol. 16, no. 10, pp. 951–961, Oct. 2014.
[53] D. Gao and Y. Chen, "Organoid development in cancer genome discovery," *Curr. Opin. Genet. Dev.*, vol. 30, pp. 42–48, 2015.
[54] I. Vela *et al.*, "Prostate cancer organoids: a potential new tool for testing drug sensitivity," *Expert Rev Anticancer Ther*, vol. 15, no. 3, pp. 261–263, 2016.
[55] S. Robinson, L. Guyon, J. Nevalainen, M. Toriseva, M. Åkerfelt, and M. Nees, "Segmentation of Image Data from Complex Organotypic 3D Models of Cancer Tissues with Markov Random Fields," *PLoS One*, vol. 10, no. 12, p. e0143798, Dec. 2015.
[56] V. Härmä *et al.*, "Optimization of Invasion-Specific Effects of Betulin Derivatives on Prostate Cancer Cells through Lead Development," *PLoS One*, vol. 10, no. 5, p. e0126111, May 2015.
[57] S. Agarwal *et al.*, "Identification of Different Classes of Luminal Progenitor Cells within Prostate Tumors," *Cell Rep.*, vol. 13, no. 10, pp. 2147–2158, 2015.
[58] S. H. Lee and M. M. Shen, "Cell types of origin for prostate cancer," *Curr. Opin. Cell Biol.*, vol. 37, pp. 35–41, Dec. 2015.
[59] J. (Hubrecht) Drost *et al.*, "Organoid culture systems for prostate epithelial tissue and prostate cancer tissue," *Nat. Protoc.*, vol. 11, no. 2, pp. 347–358, 2016.
[60] J. W. Park *et al.*, "Prostate epithelial cell of origin determines cancer differentiation state in an organoid transformation assay.," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 113, no. 16, pp. 4482–7, Apr. 2016.
[61] D. Zhang *et al.*, "Developing a Novel Two-Dimensional Culture System to Enrich Human Prostate Luminal Progenitors that Can Function as a Cell of Origin for Prostate Cancer.," *Stem Cells Transl. Med.*, vol. 6, no. 3, pp. 748–760, Mar. 2017.
[62] K. Unno *et al.*, "Modeling African American prostate adenocarcinoma by inducing defined genetic alterations in organoids," *Oncotarget*, vol. 8, no. 31, pp. 51264–51276, 2017.
[63] M. Blattner *et al.*, "SPOP Mutation Drives Prostate Tumorigenesis In Vivo through Coordinate Regulation of PI3K/mTOR and AR Signaling," *Cancer Cell*, vol. 31, no. 3, pp. 436–451, Mar. 2017.
[64] J. Shoag *et al.*, "SPOP mutation drives prostate neoplasia without stabilizing oncogenic transcription factor ERG," no. 10, pp. 1–6.
[65] H. Pan, L. Lu, X. Wang, B. Li, K. Kelly, and H. Lin, "Gambogic acid induces cell apoptosis and inhibits MAPK pathway in PTEN−/−/p53−/− prostate cancer cells in vitro and ex vivo," *Chin. J. Integr. Med.*, pp. 1–8, Jun. 2017.
[66] L. Ma *et al.*, "Organoid culture of human prostate cancer cell lines LNCaP and C4-2B," *Am J Clin Exp Urol*, vol. 5, no. 3, pp. 25–33, 2017.
[67] H. Beltran *et al.*, "Molecular characterization of neuroendocrine prostate cancer and identification of new drug targets.," *Cancer Discov.*, vol. 1, no. 6, pp. 487–95, Nov. 2011.
[68] M. Åkerfelt *et al.*, "Automated tracking of tumor-stroma morphology in microtissues identifies functional targets within the tumor microenvironment for therapeutic intervention," *Oncotarget*, vol. 6, no. 30, pp. 30035–30056, Oct. 2015.
[69] O.-J. Kwon, L. Zhang, and L. Xin, "Stem Cell Antigen-1 Identifies a Distinct Androgen-Independent Murine Prostatic Luminal Cell Lineage with Bipotent Potential," *Stem Cells*, vol. 34, no. 1, pp. 191–202, Jan. 2016.
[70] X. Dai *et al.*, "Prostate cancer-Associated SPOP mutations confer resistance to BET inhibitors through stabilization of BRD4," *Nat. Med.*, vol. 23, no. 9, pp. 1063–1071, 2017.