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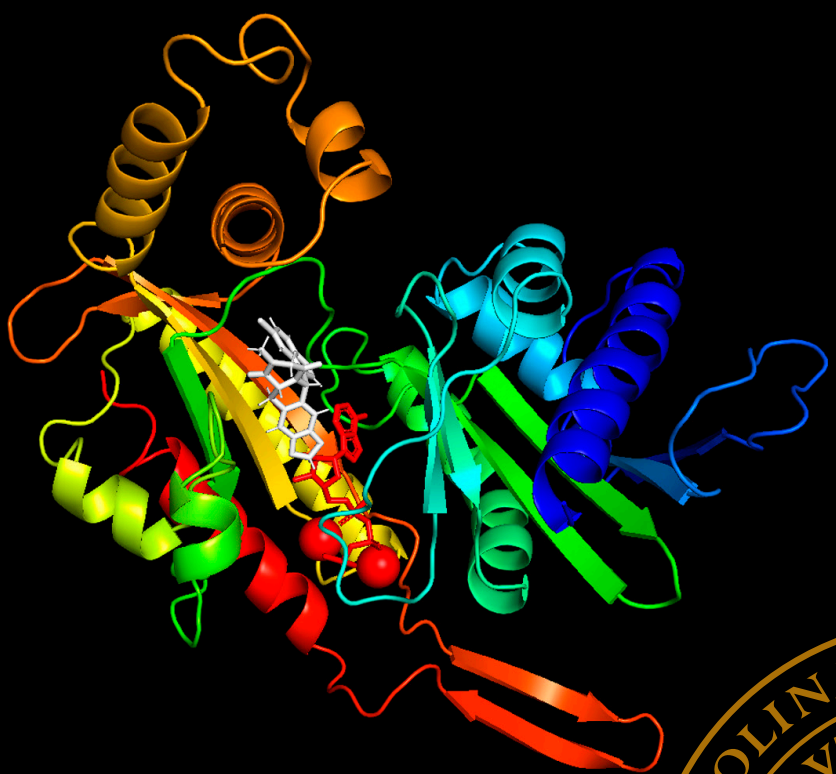

The role of phosphatidylinositol 4-phosphate 5-kinase type I alpha (PIP5K1α) and utility of its inhibitor for targeting metastatic cancer

Martuza Sarwar

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The role of phosphatidylinositol 4-phosphate 5-kinase type I alpha (PIP5K1α) and utility of its inhibitor for targeting metastatic cancer

Prostate cancer (PCa) is the most common cause of cancer-related death in men after lung cancer. Annually, more than 9000 new cases are diagnosed in Sweden and 2500 of them die each year. Metastatic prostate cancer can be treated with castration. However, the disease eventually recurs within 2 years in more aggressive form and becomes insensitive to castration which is termed as castration resistant prostate cancer (CRPC). Currently, effective treatment for CRPC is lacking. Numerous studies have shown that even under castrated level of androgen, the androgen receptor (AR) signaling pathway is constitutively active, which promotes the growth of CRPC cells. Alterations such as AR gene amplification, AR mutation and expression of truncated variants of AR (ARVs) is commonly observed in CRPC patients and through these alterations, CRPC cells become hypersensitive to extremely low level of androgen or become independent of androgens. Furthermore, hyperactivation of the phosphoinositide 3-kinase (PI3K)/Akt pathway is frequently observed in metastatic CRPC patients. The PI3K/Akt pathway can enable the cells to survive in the absence of androgen. In the studies presented in my thesis, I have revealed the importance of a kinase called the phosphatidylinositol 4-phosphate 5-kinase type I alpha (PIP5K1α) in cancer progression and therapeutic resistance. The PIP5K1α kinase acts as the upstream activator of the PI3K/AKT pathway by producing the substrate phosphatidylinositol-4,5-bisphosphate (PIP2) necessary for the activation of the PI3K/AKT signaling pathway. We, for the first time have shown that high level of PIP5K1α and its product PIP2 is a common event in cancer patients and is negatively associated with survival of prostate cancer and breast cancer patients. Furthermore, higher expression of PIP5K1α is associated with the expression of AR variant-7 (AR-V7) which is responsible for enzalutamide resistance in CRPC. We have discovered an inhibitor of PIP5K1α, ISA-2011B through high-throughput kinase screening method. By performing the *in-vitro* and *in-vivo* experiments we have shown that AKT activation can be inhibited by ISA-2011B treatment and by siRNA-mediated knockdown of PIP5K1α. ISA-2011B was effective in reducing tumor size in xenograft mouse models and it has reduced the AR-V7 expression thereby circumventing enzalutamide resistance. Moreover, ISA-2011B inhibited proliferation and induced apoptosis in both luminal A and triple negative (TNBC) breast cancer cell lines. In addition, ISA-2011B showed no cytotoxic effect in normal like MCF-10A breast cancer cell line. Our xenograft study further showed the anticancer effect of ISA-2011B on MDA-MB-231 TNBC tumor. Standard chemotherapeutic agent docetaxel showed almost identical result compared to ISA-2011B in supressing tumor growth. Moreover, we also studied the interconnection between PIP5K1α and immunomodulatory proteins in prostate cancer cells which may play an important role in controlling inflammatory processes.

Our data strongly suggests that PIP5K1α can be developed as a novel drug target in metastatic cancer and ISA-2011B is an important drug candidate for blocking the PI3K / AKT signaling in a subset of patients.

Key words: androgen deprivation therapy, triple negative breast cancer, proliferation, apoptosis, PI3K/AKT.

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Cover photo by Martuza Sarwar. Modelled 3D structure of human PIP5K1α shown with predicted binding site of ISA-2011B (shown as white) at the catalytic core near the ATP (colored in red) binding site.

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To my family and in the memory of my aunt Rowshan Ara Begum and my brother Tanvir Ahmed

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The following studies are included in the thesis and are referred to in the text by their numerals:

1. Semenas J, Hedblom A, Miftakhova R, **Sarwar M**, Larsson R, Shcherbina L, Johansson M, Härkönen P, Sterner O, Persson JL. 2014. The role of PI3K/AKT-related PIP5K1α and the discovery of its selective inhibitor for treatment of advanced prostate cancer. Proceedings of the National Academy of Sciences. 111(35): E3689-E3698.
2. **Sarwar M**, Semenas J, Miftakhova R, Simoulis A, Robinson B, Wingren AG, Mongan NP, Heery DM, Johnsson H, Abrahamsson P-A, Dizeyi N, Luo J, Persson JL. 2016. Targeted suppression of AR-V7 using PIP5K1α inhibitor overcomes enzalutamide resistance in prostate cancer cells. Oncotarget. 7(39):63065-63081.
3. **Sarwar M**, Syed Khaja AS, Aleskandarany M, Karlsson R, Althobiti M, Ødum N, Mongan NP, Dizeyi N, Johnson H, Green AR, Ellis IO, Rakha EA, Persson JL. 2018. The role of PIP5K1α/pAKT and targeted inhibition of growth of subtypes of breast cancer using PIP5K1α inhibitor. Oncogene. 10.1038/s41388-018-0438-2
4. Mandel A, Larsson P, **Sarwar M**, Semenas J, Syed Khaja AS, Persson JL. 2018. The interplay between AR, EGF receptor and MMP-9 signaling pathways in invasive prostate cancer. Molecular Medicine. 24(34):1-13. 10.1186/s10020-018-0035-4
5. **Sarwar M**, Tianyan W, Hedblom A, Heery D and Persson JL. The effect of PIP5K1α inhibitor ISA-2011B is associated to the membrane component of this lipid kinase and lipid membrane receptor FcγRIII receptor. Manuscript.
6. Raposo de Melo A, **Sarwar M** and Persson JL. The effect of Insulin and IL-6 on prostate cancer progression is mediated through PIP5K1α/AKT survival and invasion pathways. Manuscript

List of articles not included in the thesis:

1. **Sarwar M**, Sandberg S, Abrahamsson P-A, Persson JL. 2014. Protein kinase A (PKA) pathway is functionally linked to androgen receptor (AR) in the progression of prostate cancer. Urologic Oncology. 32(1). 10.1016/j.urolonc. 2012.08.019
2. Hedblom A, Laursen KB, Miftakhova R, **Sarwar M**, Anagnostaki L, Bredberg A, Mongan N, Gudas LJ, Persson JL. 2013. CDK1 interacts with RARγ and plays an important role in treatment response of acute myeloid leukemia. Cell Cycle. 12(8):1251-1266.

Abbreviations

ADT (androgen deprivation therapy)

AKT (protein Kinase B)

AMACR (alpha-methylacyl-coA racemase)

AR (androgen receptor)

ARE (androgen response element)

AR-V7 (androgen receptor spliced variant 7)

ARFs (ADP ribosylation factors)

BAD (BCL2 associated agonist of cell death)

BCa (breast cancer)

BTK (Bruton's tyrosine kinase)

CDK1 (cyclin dependent kinase 1)

CTD (C terminal domain)

Cdc42(cell division control protein 42)

CRPC (castration resistant prostate cancer)

DBD (DNA binding domain)

DHT (5α-dihydrotestosterone)

DRE (digital rectal examination)

EGF (epidermal growth factor)

ER (estrogen receptor)

FSH (follicle-stimulating hormone)

FcγRIIIa (Fc Gamma Receptor IIIa)

HER2 (human epidermal growth factor receptor 2)

IP3 (inositol (1,4,5)-trisphosphate)

IL-6 (interleukin-6)

IGF-I (insulin like growth factor 1)

LHRH (luteinizing hormone-releasing hormone)

LH (luteinizing hormone)

MMP-9 (matrix metallopeptidase-9)

NTD (N terminal domain)

PCa (prostate cancer)

PDK 1 (phosphoinositide dependent kinase 1)

PDK 2 (phosphoinositide dependent kinase 2)

PH (pleckstrin homology)

PI3K (phosphoinositide 3-kinase)

PIs (phosphatidyl inositols)

PIP2 (phosphatidylinositol-4,5-bisphosphate/ PtdIns-4,5 P2)

PIP3 (phosphatidylinositol-3,4,5-trisphosphate/ PtdIns-3, 4,5 P3)

PIP5K1α (phosphatidylinositol 4-phosphate 5-kinase type-1 alpha)

PR (progesterone receptor)

PSA (prostate specific antigen)

PTEN (phosphatase and tensin homolog deleted in chromosome 10)

PIK3CA (phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha Isoform)

TNBC (triple negative breast cancer)

TNM (The tumor, node, metastasis)

SH2 (Src homology domain)

SRD5A1 (5α-reductase gene)

Background

Prostate cancer (PCa)

Prostate cancer (PCa) is the second most common form of cancer diagnosed next to lung cancer in men. Approximately, 1.2 million new cases have been reported and about 0.35 million men have died from PCa worldwide (Ferlay J, 2018). According to the current estimation, 77000 men will die as a result of PCa in EU in 2018 (Malvezzi et al., 2018). In Sweden, roughly 10500 men were diagnosed and over 2,300 men died from PCa in 2016, making it the most common form of cancer in men (Socialstyrelsen, 2017). In developed countries such as USA, Nordic countries and western European countries, the disease incidence is higher while uncommon in less developed countries of Asia.

The prostate gland

This walnut shaped gland belongs to male reproductive system and is responsible for production as well as secretion of a mixture of proteolytic enzymes necessary for the liquefaction of semen (Balk, Ko, & Bubley, 2003). Main constituents of this mixture are proteolytic enzymes such as the prostate specific antigen (PSA), prostatic acid phosphatase (PAP) along with citric acid and Zinc. The gland is divided into three histologically unique zones (Aaron, Franco, & Hayward, 2016; McNeal, 1981; Oh WK, 2003). Majority of the PCa cases arises from the peripheral zone (PZ) adjacent to the rectal wall (Aaron et al., 2016) while the transitional zone is the major site for benign enlargement cases (Aaron et al., 2016).The remainder of the gland is called the central zone. The luminal, basal and neuroendocrine cells are the three types of epithelial cells constituting this secretory gland and these cells are enclosed by fibromuscular stroma (Abate-Shen & Shen, 2000).

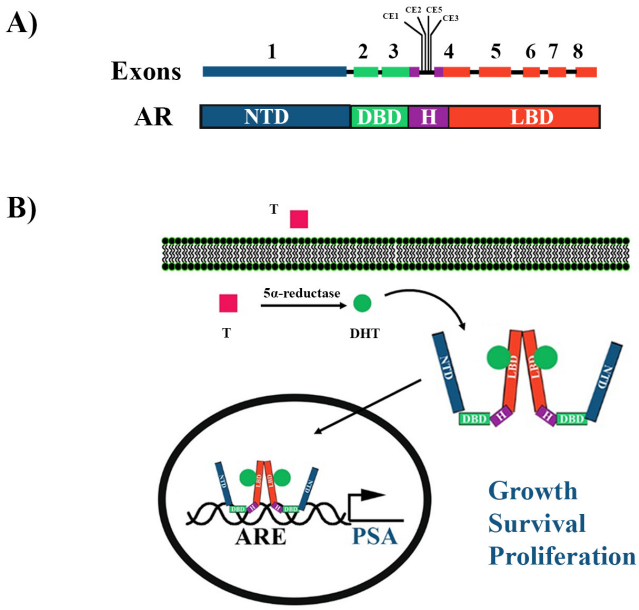

Figure 1. A simplified model of AR structure and signaling pathway. (A) Exon stucture of AR, the exon 1 codes for N- terminal domain (NTD) and the DNA binding domain (DBD) is coded by the exon 2 and part of the exon 3, the hinge region (H) is partly coded by exon 3 and 4. The Ligand binding domain (LBD) is coded by exon 4 to 8. **(B)** The free unbound testosterone (T) is converted by 5α-reductase enzyme to 5α-dihydrotestosterone (DHT). The DHT binds to the LBD, inducing conformational change and dimerization. The dimerized AR then translocates to the nucleus, where the DBD binds to the specific promoter regions containing androgen response elements (AREs) of genes such as PSA. Along with other transcriptional co-regulators, it increases the expression of genes responsible for growth and proliferation of prostate cells. Based on (Greasley, Khabazhaitajer, & Rosario, 2015; Nakazawa, Antonarakis, & Luo, 2014)

Androgen and androgen receptor (AR) signaling in prostate

Studies in human disease and rodents have pointed to the indispensable role of male androgen 5α-dihydrotestosterone (DHT) and the androgen receptor (AR) in the proper development, survival and growth of the cells of the prostate (Heinlein & Chang, 2004). Lack of functional 5α-reductase gene (SRD5A1) in the prostate which converts testosterone to its more potent form DHT, leads to a small or undetectable prostate in humans (Heinlein & Chang, 2004). Inhibition of 5α-reductase enzyme in rat with inhibitors during fetal development shows partial development of prostate (Imperato-McGinley et al., 1985). Absence of prostate gland in AR knockout mice points further to the importance of DHT and AR gene in the development of prostate (Heinlein & Chang, 2004). Furthermore, pioneering work of Charles Huggins and Clarence V. Hodges have established the role of

testicular androgen for PCa tumor growth as surgical removal of testicle (castration) led to tumor regression (Huggins & Hodges, 1941).

Located in the X chromosome (Xq12), the *AR* gene has eight exons and belongs to the nuclear receptor family (Figure 1A). There are three domains of AR, the N-terminal transcriptional activation domain (NTD), DNA-binding domain (DBD) and a C-terminal ligand or hormone binding domain (LBD) (Figure 1A) (P. Lonergan & D. Tindall, 2011). There is also a short domain (of about 50 amino acid long) called the hinge region in between the DBD and LBD. This hinge region facilitates nuclear transport of AR upon ligand binding (P. Lonergan & D. Tindall, 2011). Testosterone is circulated in the body by binding to the serum sex hormone-binding globulin (SHBG) and albumin (Tan, Li, Xu, Melcher, & Yong, 2014) or in the free form. The free unbound testosterone is converted to DHT by the 5α-reductase enzyme intracellularly in the prostate. The DHT bind to the AR, displacing the heat shock proteins (HSPs) and this binding leads to conformational changes of AR which ultimately leads to dimerization and nuclear translocation of AR through interaction with importin-α (Figure 1B) (Tan et al., 2014). In the nucleus, AR induces expression of genes (such as PSA) by binding to the promoter containing androgen response elements (AREs) and bringing other necessary transcriptional co-regulatory proteins (Figure 1B) (Dehm & Tindall, 2007).

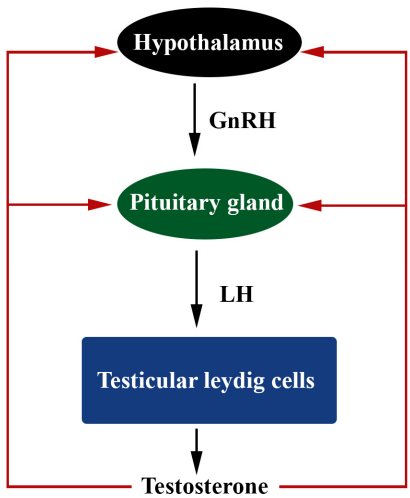

Figure 2. Regulation of testosterone secretion by the hypothalamic–pituitary–gonadal axis. Gonadotropin-releasing hormone (GnRH) secreted from hypothalamus stimulates the release of luteinizing hormone (LH) from the pituitary gland. Androgen is secreted from the Leydig cells of the testicle as a result of LH stimulation. The rise of androgen act as feedback inhibitor of the system (indicated in red arrow) which in turn inhibit the release of GnRH from the hypothalamus.

Regulation of androgen secretion

Testosterone, the major androgen is produced mainly by testicular Leydig cells. The secretion of testosterone is under the regulation of the hypothalamic–pituitary–gonadal axis (Figure 2). The gonadotropin-releasing hormone (GnRH) is secreted in pulses (one and a half hours to two hours) by the hypothalamus. In the anterior pituitary gland, the GnRH stimulates the secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) after binding to the gonadotrophes (Grossmann, Huang, & Tindall, 2001). The LH then stimulates production of androgens from the Leydig cells. Rise in the androgens in turn inhibits the release of GnRH from hypothalamus, thereby acting as the negative feedback for the release of androgen (Figure 2). Because of this feedback inhibition mechanism, luteinizing hormone-releasing hormone (LHRH) agonists or antagonists are used in the clinical setting to inhibit the androgen secretion as a form of medical castration in prostate cancer patients (Watson, Arora, & Sawyers, 2015).

Diagnosis

Most cases are diagnosed as a result of population based screening of blood PSA level and digital rectal examination (DRE). If abnormal PSA (>4μg/ml) concentration is detected and abnormalities of prostate is discovered by DRE; microscopic assessment of tissue is needed for confirmation of PCa (Thompson et al., 2004). Prostate tissues are collected in a grid pattern by transrectal ultrasound guided needle biopsy (Litwin & Tan, 2017). Subsequently, Gleason grades of 1 to 5 are assigned to tissues based upon the microscopic architecture and morphology of the tumor cells compared to healthy cells (Litwin & Tan, 2017). A pathologist sums the two most predominant grades of the tissues which is called the Gleason score. The Gleason score is associated with clinical stage, therapeutic response, metastatic progression and overall survival (Humphrey, 2004). The extent or spread (metastasis) of cancer is evaluated by bone scan, ultrasound, or computerized tomography (CT) scan, or magnetic resonance imaging (MRI) or positron emission tomography (PET) scanning with labelled prostate specific membrane antigen (PSMA). The tumor, node, metastasis (TNM) system is also used for clinical staging of PCa.

Besides PSA, other immunohistological markers such as AR expression and screening for loss of tumor suppressor PTEN expression, expression of alpha-methylacyl-coA racemase (AMACR), proportion of Ki67 positive cells, presence of TMPRSS2:ERG gene fusion provide valuable prognostic information when

combined with PSA and Gleason grading (Kristiansen, 2018). Current imaging modalities such as MRI, PET, CT are not sensitive enough for the detection of micro metastasis present in the bloodstream at early stage during cancer progression (W. Liu et al., 2017). Although not in widespread clinical use, liquid biopsy in the form of circulating tumor cells hold a great promise in detecting these tiny metastases (W. Liu et al., 2017).

Treatment options

The PCa patients are stratified to low, intermediate or high risk groups depending on the Gleason score of their biopsy, PSA concentration and clinical stage of disease (TNM system) (Litwin & Tan, 2017; Mottet et al., 2017). Therapeutic decisions are made depending on which of the risk group their disease fall into and patient's preference. Watchful waiting or active surveillance, surgical removal of the prostate (radical prostatectomy) and radiation therapy are recommended for patients with localized tumors (Litwin & Tan, 2017; Mottet et al., 2017).

Androgen deprivation therapy (ADT) in the form of either surgical removal of the testicles (orchidectomy), or chemical castration with luteinizing hormone-releasing hormone (LHRH) agonists or antagonists or the oral nonsteroidal antiandrogen bicalutamide is used for patients with locally advanced high risk cancer without any metastasis. The LHRH inhibits testosterone secretion from testes thereby decreasing the androgen level. Bicalutamide antagonizes AR action by acting as a competitor for androgen (Watson et al., 2015). Often, ADT is combined with external beam radiotherapy (EBRT). For metastatic PCa, ADT remains the standard first line therapy (Litwin & Tan, 2017). Combined androgen blockage in the form of dual treatment with (LHRH) agonists and antiandrogen is also recommended for metastatic PCa (Chang, Autio, Roach, & Scher, 2014). Patients who do not respond to castration (castration resistant) are treated with chemotherapeutic agent docetaxel or cabazitaxel alone or in combination with ADT (Parker et al., 2015).

Prostate cancer progression from androgen-dependent to castration resistant prostate cancer (CRPC)

Most patients respond well to ADT; however, a subset of patients achieve remissions only for 2–3 years (Watson et al., 2015; Yap et al., 2016). Inevitably, the disease progresses to an incurable stage termed as castration-resistant prostate cancer (CRPC). The CRPC patients are associated with poor prognosis and very few treatment options are available. Numerous studies have pointed that even under the

castrated level of androgen, the AR signaling pathway is constitutively active, which promotes the growth of CRPC cells (He et al., 2018; P. E. Lonergan & D. J. Tindall, 2011; Montgomery et al., 2008; Scher & Sawyers, 2005).

New generation of potent antiandrogen MDV3100 (Enzalutamide) to block nuclear translocation of AR are in clinical use for metastatic CRPC patients. The abiraterone is developed to inhibit residual androgen synthesis by CYP17 enzyme in metastatic CRPC patients. Enzalutamide and abiraterone treatment only increases overall survival by 4 months (Beer et al., 2017) and 3.9 months (de Bono et al., 2011) respectively in CRPC patients compared to the placebo. Inevitably the cancer cells become resistant to these therapies as well (Antonarakis et al., 2014; Attard & Antonarakis, 2016).

Numerous studies have discovered several adaptive mechanisms for the emergence of CRPC. These mechanisms include (1) AR overexpression, (2) AR mutation, (3) activation of AR by growth factors, (4) increase survival by PI3K/AKT pathway and (5) constitutively active truncated variants of AR (ARVs) (Huang, Jiang, Liang, & Jiang, 2018; Karantanos, Corn, & Thompson, 2013; Yuan et al., 2014).

Overexpression of AR due to amplification or mutation in AR may enable the cells to become hypersensitive to extremely low level of androgen. Fluorescence in situ hybridization (FISH) analysis has revealed AR copy number alteration (amplification) in 60% cases of metastatic CRPC patients (Hu, Denmeade, & Luo, 2010; Leversha et al., 2009). In mouse xenograft studies, overexpression of AR in LAPC4 and LNCaP cells increased tumor incidence in castrated mouse compared to control (Charlie D. Chen et al., 2003). Overexpression of AR in LAPC4 cells can convert antagonist to agonist as shown by the increase in PSA expression when treated with bicalutamide (Charlie D. Chen et al., 2003). Point mutation in the LBD of AR has also been reported in CRPC patients with the frequency of 15-20% (Grasso et al., 2012; Jernberg, Bergh, & Wikström, 2017; Mitsiades et al., 2012; Robinson et al., 2015). Mutations such as T878A, W742C, H875Y and F877L can cause AR to be stimulated by progesterone, estrogen, glucocorticoids or even by antiandrogens (Hara et al., 2003; Jernberg et al., 2017; Lorente et al., 2015; Mitsiades et al., 2012).

Several growth factors have been implicated in CRPC. These factors can trans activate the AR or increase the survival of cancer cells. In CWR-R1 cell line, epidermal growth factor (EGF) can activate AR by phosphorylation of nuclear receptor coactivator 2 (TIF2) (Gregory et al., 2004). Furthermore, receptor tyrosine-protein kinase erbB-2 (HER2) can induce androgen independent growth and AR transcriptional activity to increase PSA expression in LNCaP and LAPC-4 cells (Craft, Shostak, Carey, & Sawyers, 1999). In LNCaP cells, EGF can protect apoptosis by phosphorylation and inactivation of BAD (BCL2 associated agonist of cell death) protein (Sastry, Karpova, & Kulik, 2006). This mechanism is

phosphoinositide 3-kinase (PI3K)/AKT dependent as PI3K/AKT inhibitor LY294002 can inhibit BAD phosphorylation. Similarly, IGF-I (insulin like growth factor 1) can also protect androgen independent C4-2 cells from apoptosis in a PI3K/AKT dependent manner (Krueckl et al., 2004). All these receptor pathways converge on the PI3K/AKT, pointing the importance of this pathway in CRPC.

Androgen receptor spliced variant 7 (AR-V7) in CRPC

In total 17 variants of AR (ARVs) lacking LBD has been identified both in cell lines and patients (Ware, Garcia-Blanco, Armstrong, & Dehm, 2014). Interestingly, the cell lines (22Rv1, VCaP and CWR-R1 cells) that are positive for ARVs are in fact androgen independent (Ware et al., 2014). The common salient feature of these variants is that they are constitutively active and can drive expression of AR regulated genes in the absence of androgens. Precise mechanism of how these variants arise has not been fully understood. Proteolysis of wild type AR or alternative splicing could be the cause for the synthesis of these variants (Ware et al., 2014). Of these variants, the AR-V7 which lacks the ligand binding domain to DHT has been implicated strongly for the emergence of CRPC (Hörnberg et al., 2011) as well as resistance to enzalutamide and abiraterone treatment (Antonarakis et al., 2014). In a separate study, AR-V7 was found to be 20 fold higher in CRPC patients compared to the hormone therapy responsive counterparts. (Hu et al., 2009). In AR negative PC-3 cells, AR-V7 transfection showed that it is localized in the nucleus and is constitutively active as shown by the induction of PSA reporter in charcoal stripped medium (androgen depleted) (Chandrasekar, Yang, Gao, & Evans, 2015; Hu et al., 2009). The 22Rv1 cells are positive for both full length AR and AR-V7 and the AR-V7 confers androgen independent growth and resistance to enzalutamide. Knockdown of AR-V7 but not full length AR could inhibit expression of AR regulated PSA gene (Yingming Li et al., 2013).

The phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT)/PTEN pathway in the CRPC

The PI3K/AKT pathway is one of the most aberrantly altered pathway in prostate cancer. This pathway is responsible for controlling cellular growth, proliferation and inhibition of apoptosis (I. Vivanco & C. L. Sawyers, 2002).

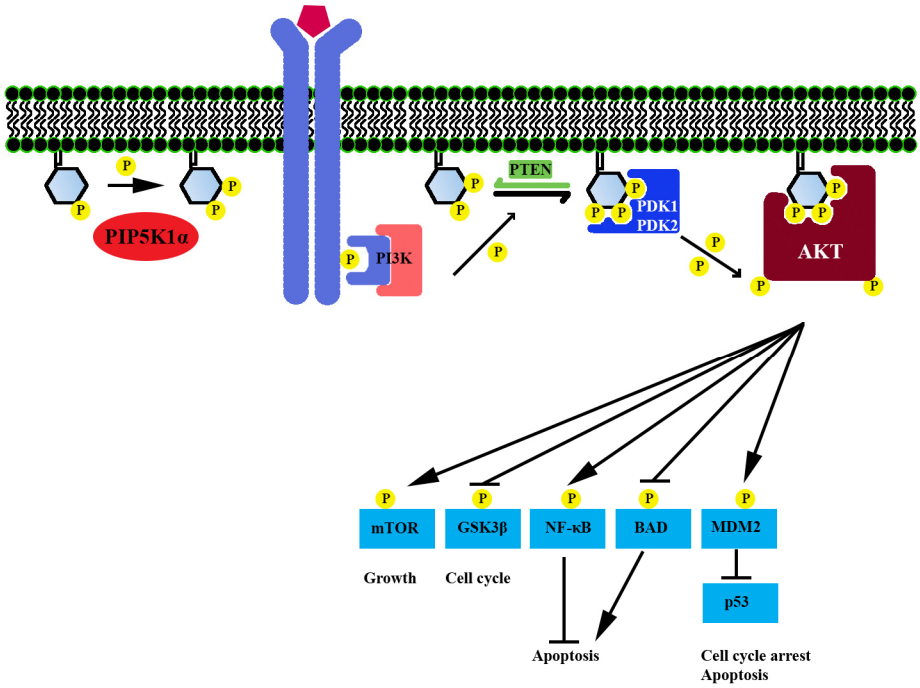

Figure 3. A simplified model of PI3K/AKT signaling pathway. Growth factor binding leads to the activation of the receptor. The activated autophosphorylated residues of the receptor serves as the docking site for the regulatory subunit of the PI3K. The catalytic subunit of PI3K uses the PIP2 (produced by PIP5K1α) to generate PIP3. The PDK1 and PDK gets activated by the PIP3 and in turn activates the serine threonine protein kinase B (AKT) by phosphorylation. The AKT in turn causes the activation (➔) or inhibition (⊣) of numerous targets necessary for growth, cell cycle and survival. The figure is modified from Vivanco and Sawyers et al, (Igor Vivanco & Charles L. Sawyers, 2002).

Typical PI3K/AKT signal transduction pathway is depicted in Figure 3. Upon binding to extracellular growth factors such as EGF (epidermal growth factor) or IGF-1 (insulin like growth factor 1), the tyrosine kinase receptor undergoes autophosphorylation. The regulatory subunit of PI3K binds to the phosphorylated residues of the receptor through its SH2 (Src homology domain) domain (Downward, 2004; Jean & Kiger, 2014). The catalytic subunit of the PI3K complex catalyses the phosphorylation of phosphatidylinositol-4,5-bisphosphate (PtdIns (4,5)P2 or PIP2) at D3 position of the inositol ring to produce phosphatidylinositol-3,4,5-trisphosphate (PtdIns 3, 4, 5 P3) or PIP3 (Figure 4). The PTEN (phosphatase and tensin homolog deleted in chromosome 10) negatively regulates the pathway by dephosphorylating PIP3 to PIP2 (Downward, 2004).

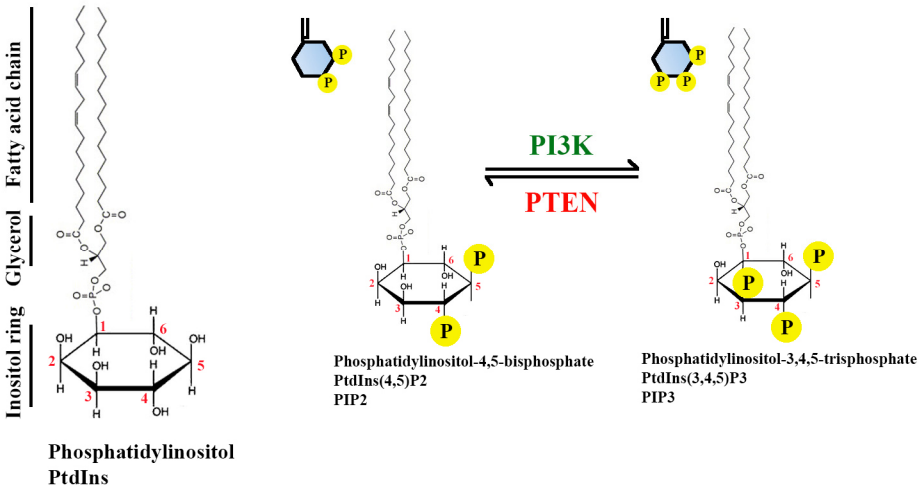

Figure 4. Typical structure of phosphoinositide and PI3K catalyzed reaction. The phosphatidyl insitol (PtdIns) has three components, the fatty acid chains (phosphatidic acid group), glycerol and an inositol ring (the residues are numbered). The PI3K catalyzes the phosphorylation of phosphatidylinositol-4,5-bisphosphate (PtdIns (4,5)P2 or PIP2) at D3 position of the inositol ring to produce phosphatidylinositol-3,4,5-trisphosphate (PtdIns(3, 4,5)P3 or PIP3. The PTEN negatively regulates the pathway by dephosphorylating PIP3 to PIP2. The figure is modified from Vivanco and Sawyers et al, (Igor Vivanco & Charles L. Sawyers, 2002)

The serine-threonine kinase AKT is translocated to the membrane by binding to the PIP3 via its PH (pleckstrin homology) domain. The 3-phosphoinositide dependent kinase 1 and 2 (PDK1 and PDK2) also localizes to membrane by binding the PIP3 through the SH2 domain. The AKT is activated by PDK1 and PDK2 mediated phosphorylation at threonine 308 and serine 473 residue respectively. Activated AKT controls cell cycle, growth, apoptosis by controlling proteins such as p27, mTOR, p53, BAD etc (Downward, 2004; Jean & Kiger, 2014; I. Vivanco & C. L. Sawyers, 2002).

Genomic analysis has shown PTEN loss of function in up to 40% cases of metastatic CRPC patients (Jamaspishvili et al., 2018; Robinson et al., 2015). The gene for catalytic subunit of PI3K (*PIK3A*) has been amplified in 62% cases of PCa (Pearson et al., 2018). Biological consequence of PTEN loss and PIK3CA amplification is the hyper-activation of the AKT pathway and ultimately cell survival and proliferation in CRPC patients even under castrate level of androgen. There is also reciprocal feedback inhibition mechanism between the AKT and AR pathway. Inhibition of AR through castration can induce AKT pathway by AR regulated FKBP5(Carver et al., 2011). Dual inhibition of AR and AKT pathway is necessary for the inhibition of tumor growth (Carver et al., 2011). The AKT pathway may inhibit the ADT mediated apoptosis of prostate epithelial cell by increasing cell survival.

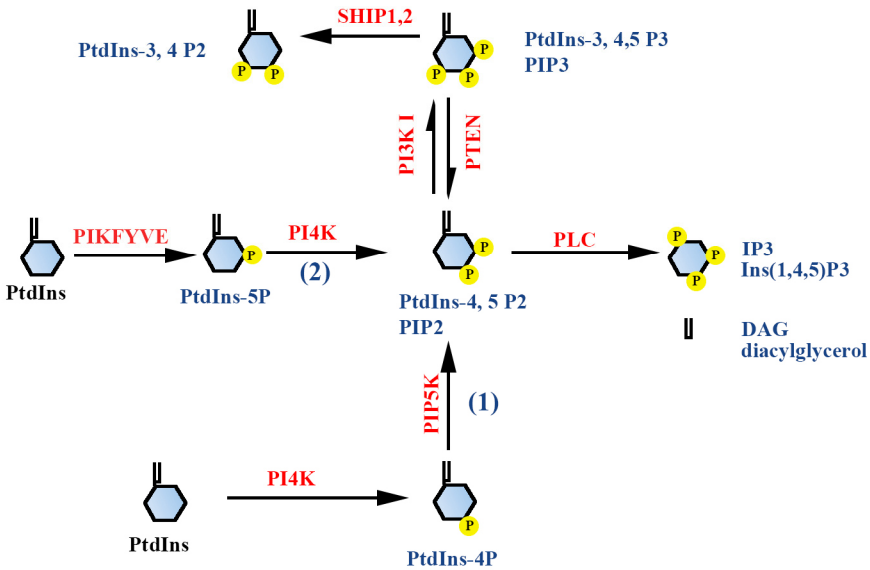

Figure 5. Regulation of intracellular PIP pool. The enzymes are marked with red color and the PIPs are marked with blue. Majority of the PIP2 is synthesized by PIP5K1α which catalyzes the formation of PIP2 from phosphatidyl inositol-4 phosphate (PtdIns-4 P) by phosphorylating at the 5 position of the inositol ring (reaction labelled as 1). The minor pathway is through phosphatidylinositol 5-phosphate 4-kinases (PIP4Ks or PI4K) which phosphorylate phosphatidyl inositol-5 phosphate (PtdIns-5P) at the D-4 position (reaction labelled as 2). The PIP is converted to IP3 and DAG by PLC. While the PI3K generates the PIP3 from PIP2. PTEN converts PIP3 back to PIP2

The phosphatidylinositol 4-phosphate 5-kinase type-1 alpha (PIP5K1α) mediated production of PIP2 is necessary for PI3K cell signaling pathway

The PtdIns (4,5) P2 or PIP2 is a low abundant phosphoinositide and accounts for only 1% of the total membrane phosphoinositides (Mao & Yin, 2007; McLaughlin & Murray, 2005). The PIP2 acts as the substrate for generating second messenger inositol (1,4,5)-trisphosphate [Ins(1,4,5)P3/IP3] and diacylglycerol (DAG) through the action of phospholipase C (PLC), and substrate for the PI3K for generating PIP_3 (van den Bout & Divecha, 2009). The intracellular pool of PIP_2 is maintained by its generation and degradation as exemplified in the Figure 5.

There are two major pathways for the generation of PIP_2. Firstly, via the phosphatidylinositol 4-phosphate 5-kinase type-1 alpha (PIP5K1α) which catalyzes the formation of PIP2 from phosphatidyl inositol-4 phosphate (PtdIns-4P) by phosphorylating at the 5 position of the inositol ring (Oude Weernink, Schmidt, & Jakobs, 2004) (Figure 5). The second major pathway is through phosphatidylinositol 5-phosphate 4-kinases (PIP4Ks) which phosphorylate phosphatidyl inositol-5 phosphate (PtdIns-5P) at the D-4 position (Oude Weernink et al., 2004) (Figure 5). Radioactive phosphorylation assays with [^{32}P] orthophosphate labelling of D-4 and D-5 positions have shown that, PIP2 is produced mainly via PIP5K1α (Oude Weernink et al., 2004). The PIP5K1α expression has been identified in plasma membrane, cytoplasm and nucleus. The kinase is expressed in different tissues, with highest expression level in testis (Table 1).

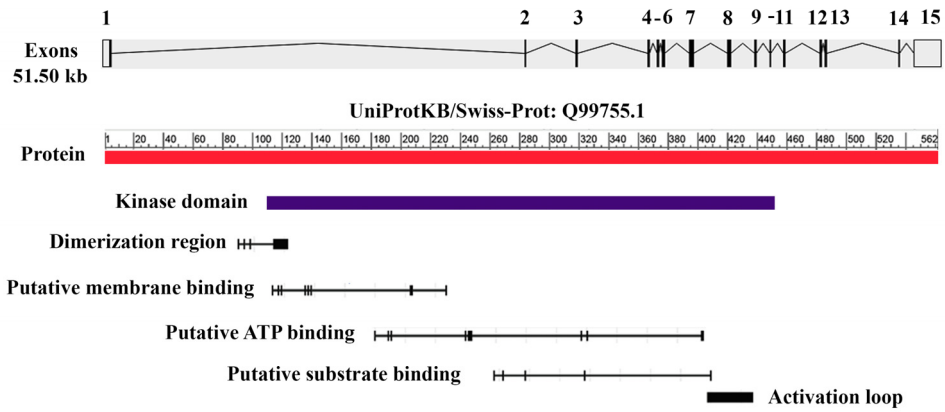

Figure 6. Exons and different regions of the PIP5K1α protein (UniProtKB/Swiss-Prot: Q99755.1).Data retrieved from NCBI gene and protein database.

The PIP5K1α belongs to the type I PIP5Ks. The α (PIP5K1α), β (PIP5K1β) and the γ (PIP5K1γ) are the three genes of the type I PIP5Ks and are in different chromosomes and encodes enzymes of different sizes (Table 2). They all can generate PIP_2 and share a conserved kinase core domain of about 340 amino acid long. Since the major emphasis of the thesis is on identifying the role of PIP5K1α in cancer and the utility of PIP5K1α inhibition as therapeutic choice, the remainder of the discussion will be focused on PIP5K1α. Human PIP5K1α gene has 15 exons and the full length protein runs as 68kDa in denaturing polyacrylamide gel. Important regions and putative sites important for the function of the protein is shown in Figure 6.

Gene knockout mouse of PIP5K1A are subfertile and the morphological change in flagella of sperm was observed (Hasegawa et al., 2012). Interestingly, disorganized F-actin was detected in seminiferous epithelium in PIP5K1A and PIP5K1B double knockout mouse. These studies indicate the importance of the PIP5K1A gene in fertility as well actin polymerization. Reduced response to thrombin action in platelet were also evident in PIP5K1A knockout mouse (van den Bout & Divecha, 2009). Those platelets showed less PLC activation and IP3 concentration as well as less PIP2.

Table 1. Expression of PIP5K1α in differnt human tissues
Protein expression data of different human tissues are retrieved from The Human Protein Atlas database (Ponten, Jirstrom, & Uhlen, 2008; Uhlen et al., 2010).

Expression level	Tissues
High	Testis, appendix, lymph node, tonsil.
Medium	Oral mucosa, epididymis, adrenal gland, cerebral cortex, bone marrow, placenta, ovary, spleen, cervix, uterus, kidney, stomach, hippocampus, pancreas, gallbladder, liver, skin
Low	Prostate, breast, seminal vesicle, bronchus, lungs, cerebellum, thyroid gland, colon, bladder, nasopharynx

Table 2. Human isoforms of type I PIP5Ks
The location, size and features of human PIP5Ks proteins. Data retrieved from NCBI database using the conserved domain search and highlight sequence feature options.

Human PIP5K isoforms	Location	Molecular Wight kDa	Size (amino acid)	Kinase core domain location	Dimerization region	Activation loop
PIP5K1A	1 (1q21.3)	68	562	109-449	91-95, 99, 115-124	405-435
PIP5K1B	9 (9q21.11)	68	540	53-393	35, 39, 43, 59-68	351-381
PIP5K1C	19 (19p13.3)	72	668	103-443	85, 89, 93, 109-118	399-429

Role of PIP2 and PIP5K1α in cellular functions other than signal transduction

The PIP2 is required for cellular functions including exocytosis, endocytosis, mRNA processing, cell cycle progression, focal adhesion and apoptosis (Doughman, Firestone, & Anderson, 2003; Mao & Yin, 2007; McLaughlin & Murray, 2005). PIP5K1α is the predominant kinase which produces PIP2, thus PIP5K1α plays critical role in these PIP2-mediated cellular functions.

Cytoskeletal reorganization

The coordinated polymerization of actin monomers to filaments, stabilization of the filaments and disassembly of the polymers play important roles in processes like migration, adhesion, change in morphology and vesicular trafficking. Elongation of actin filaments is controlled in part by capping proteins, which prevents the access to actin polymerization at barbed ends. Activity of the capping protein such as gelsolin is inhibited by PIP2, thereby promoting branching of actin (Di Paolo & De Camilli, 2006; Lin, Wenegieme, Lu, Chen, & Yin, 1997). The PIP2 also facilitates actin polymerization by activating neuronal wiskott-aldrich syndrome protein (N-WASP) required for chemotaxis of neutrophil (Logan & Mandato, 2012; Srinivasan et al., 2003). Binding of PIP2 induces a conformational change to N-WASP, ultimately leading to the binding and activation of actin-related protein 2 and 3 (Arp2/3) complex. The Arp2/3 is a multi-protein complex necessary for the nucleation of branched actin filament networks in a Cdc42 (cell division control protein 42), a small GTPase of Rho family dependent manner (Di Paolo & De Camilli, 2006; Logan & Mandato, 2012).

Focal adhesion (FA)

Cell to extracellular matrix adhesions or focal adhesion (FA) is important for cell migration and cells response to extracellular signals. Vinculin plays an important role in the FA by interacting with talin and actin and coupling integrin to cytoskeleton (Ezzell, Goldmann, Wang, Parashurama, & Ingber, 1997; Humphries et al., 2007). A conformational change in vinculin is initiated by its interaction with PIP2 at the C-terminal which promotes oligomerization with talin and actin (Chinthalapudi et al., 2014; Short, 2014). In fibroblast cells, vinculin mutants that is unable to bind PIP2 showed slower movement compared to wild type, further proving the importance of PIP2 in cell movement and FA (Short, 2014).

Endocytosis

Clathrin mediated endocytosis is controlled in part by a class of proteins called endocytic clathrin adaptors such as AP-2, AP180, epsin etc (Di Paolo & De Camilli, 2006; Godlee & Kaksonen, 2013). The PIP2 can bind at the PIP2 binding domains present in these adaptor proteins and modulate their activities (Godlee & Kaksonen, 2013). Treatment with PIP2 lowering agent including butanol and ionomycin have shown to disassemble AP-2 and clathrin and reduce transferrin uptake (Godlee & Kaksonen, 2013; Zoncu et al., 2007). The level of PIP2 at the plasma membrane is also correlated positively with secretory vesicles (Di Paolo & De Camilli, 2006).

Apoptosis

PIP5K1α dependent generation of PIP2 protects cells from undergoing apoptosis. The initiator caspase 8 and 9 as well as the effector caspase 3 are necessary for apoptosis. The PIP2 inhibits these caspases directly in HeLa cells (Mejillano et al., 2001). Furthermore, overexpression of PIP5K1α protects from apoptosis whereas the kinase deficient PIP5K1α mutant did not show any protection (Mejillano et al., 2001).

mRNA processing

The PIP2 is needed for mRNA processing processes such as splicing, 3' end processing and polyadenylation (Barlow, Laishram, & Anderson, 2010). A nuclear speckle targeted PIP5KIα regulated-poly(A) polymerase (Star-PAP) has been identified recently and is necessary for 3′-processing of mRNA (Li, Laishram, & Anderson, 2013). Star-PAP forms a multi-protein complex with PIPKIα and this association is necessary for activation of the complex.

Regulation of PIP5K1α activity, localization and expression

Other than modulating the cellular processes that requires the product of PIP5K1α, that is the PIP2, PIP5K1α itself can control vital cellular processes. But the ultimate question is how the activity of PIPKIα is regulated. It is likely that interaction with other proteins facilitate the subcellular localization of PIPKIα to cellular regions necessary for PIP2 generation and this interaction ultimately modulate the activation or inactivation of that protein and PIP5K1α itself.

Role of activation loop and dishevelled

Valuable information can be found in the analysis of the crystal structure. The crystal structure of human PIP5K1α has not yet been resolved (A. Liu, Sui, Wu, & Hu, 2016). Our understanding of its regulation has been enriched from the crystal structure of Zebrafish (*Danio rerio*) PIP5K1α (PDB ID: 4TZ7 or 6CMW)(A. Liu et al., 2016). The human PIP5K1α shares 82% and 77% sequence identity with 4TZ7 and 6CMW respectively. The activation loop is located at the C-terminal end (Figure 7) and the protein usually forms a homodimer (A. Liu et al., 2016; Robinson et al., 2015) . The dimerized form of zPIP5K1α is more active catalytically and more stable, however whether that is the case for human isoform is not yet known.

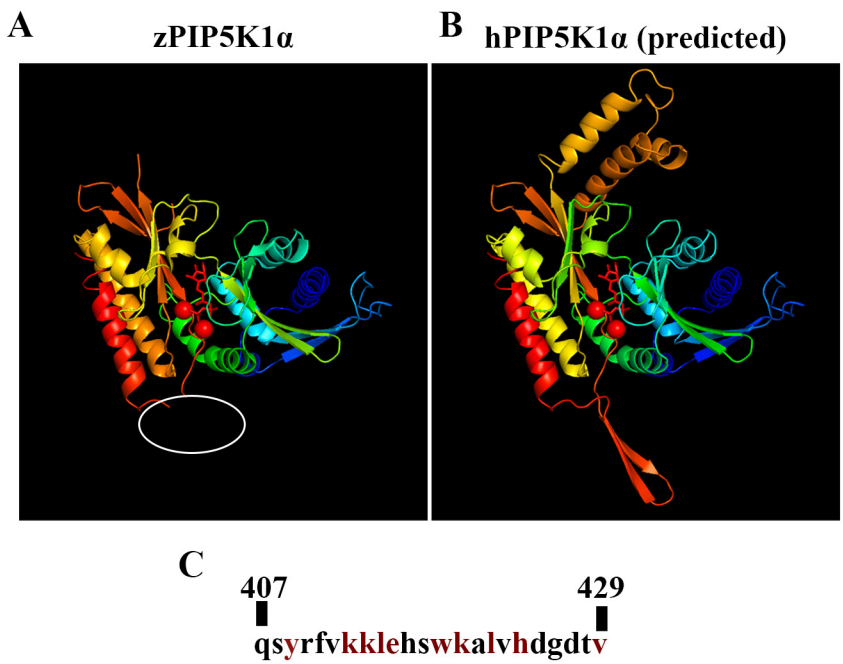

Figure 7. Crystallographic structure of Zebrafish and human PIP5K1α. (A) Crystal structure of zPIP5K1α (PDB ID: 6CMW), the distorted area is circled with white eclipse. **(B)** modelled hPIP5K1α based on zPIP5K1α **(C)** the conserved residues in the activation loop is shown.

It is also revealed that, dishevelled can interact with PIP5K1α and increase its affinity for its substrate PtdIns4P (Robinson et al., 2015). In the crystal structure, this loop (activation loop) is distorted as shown in the Figure 7A indicated by white ellipse. The predicted hPIP5K1α based on z PIP5K1α (PDB ID: 6CMW) is shown

in Figure 7B and conserved residues of the activation loop are shown in Figure 7C. Through NMR (nuclear magnetic resonance) Liu et al, have shown that this activation loop serves as a membrane sensor vital for lipid substrate processing (A. Liu et al., 2016). Mutation in the Leucine (L or Leu) residues severely reduces its activity as a kinase. Since the amino acid residues of this activation loop is also conserved in human PIP5K1α, it is presumable that in human PIP5K1α this activation loop also plays a valuable role in membrane recognition and activity.

Protein-protein interaction

The human Bruton's tyrosine kinase (BTK) is known to interact with PIP5K1α (Saito et al., 2003). Interaction between BTK and PIP5K1α is necessary for translocating PIP5K1α to the membrane for PIP2 generation during B cell receptor activation (Saito et al., 2003). The PIP2 is then used by BTK activated phospholipase C (PLC) for the generation of IP3 and DAG.

Despite having no nuclear localization signal (NLS), the PIP5K1α has been reported to be localized in nuclear speckles and nucleus (W. Li et al., 2013; Mao & Yin, 2007; Mortier et al., 2005). Interaction between PIP2 and syntenin-2 protein through PDZ (postsynaptic density protein, Disc large, Zona occludens) domain is important for nuclear localization of syntenin-2 (Sugi, Oyama, Morikawa, & Jingami, 2008). Mutation in the PDZ domain reduces the nuclear pool of PIP2, pointing the importance of this interaction for maintaining PIP2 level (Mortier et al., 2005). Perhaps other proteins interaction may be necessary for nuclear localization of PIP5K1α and maintain intracellular pool of PIP2. Interestingly, nuclear pool of PIP5K1α has been reported to be post translationally modified by SUMOylation through SUMO-1 and SUMO-2 (Chakrabarti, Bhowmick, Bhargava, Bhar, & Siddhanta, 2013; Chakrabarti et al., 2015). The SUMOylated PIP5K1α fractions increases during apoptosis compared to normal HEK-293 cells. Possibly, post translational modification also play important role in the localization of PIP5K1α in different subcellular fractions.

Regulation by phosphorylation

PIP5K1α has been shown to autophosphorylate itself in vitro in COS-7 cells(Itoh, Ishihara, Shibasaki, Oka, & Takenawa, 2000). The level of autophosphorylation can be enhanced by the addition of phosphatidyl inositols (PIs). The autophosphorylation led to the reduction of PIP5K1α kinase activity (Itoh et al., 2000). But this method of autoregulation by phosphorylation has not been reported *in-vivo* yet.

ARF family and Rho family of GTPases mediated regulation

The ADP ribosylation factors (ARFs) are a class of proteins belonging to family of small GTP-binding proteins of the Ras superfamily. Two members of ARFs such as ARF1 and ARF6 can induce PIP5K1α kinase activity after interaction between them. Forced expression of ARF1 and ARF6 can greatly increase the PIP2 concentration at the plasma membrane in the presence of phosphatidic acid (PA). The Rac1 (Rac family small GTPase 1) protein also can interact with PIP5K1α and can induce kinase activity of PIP5K1α in platelets (Hartwig et al., 1995; Ren & Schwartz, 1998).

Regulation by growth factors

Several important growth factors such as EGF and PDGF have been shown to induce PIP2 synthesis and PIP5K1α kinase activity. The PIP5K1α kinase activity can be induced after EGF stimulation and PIP5K1α is found in complexes with phosphorylated EGF receptor 1 (EGFR) (Oude Weernink et al., 2004; Pike & Casey, 1996). Similarly, in platelets, thrombin can induce PIP2 synthesis (Nolan & Lapetina, 1990; Oude Weernink et al., 2004).

Interlink between PIP5K1α and FcγRIIIa (CD16A) receptor

The FcγRIIIa (CD16a) is a type I transmembrane receptor which has low affinity to IgG. The receptor is important in antibody-dependent cell-mediated cytotoxicity (ADCC) by natural killer (NK) cells and is also expressed by macrophages and endothelial cells (Stern-Ginossar & Mandelboim, 2010). The FcγRIIIa can act as signal transducing receptor in NK cells for inducing the expression of cytokines such as interferon gamma (IFN-γ) and tumor necrosis factor (TNF) (Anegón, Cuturi, Trinchieri, & Perussia, 1988).

After binding to monomeric IgG, FcγRIIIa induces PIP2 hydrolysis to form DAG and IP3 through the action of phospholipase C (Wirthmueller, Kurosaki, Murakami, & Ravetch, 1992). Additionally, activated receptor can induce Syk (spleen tyrosine kinase) family kinases which in turn activates PI3K (Kanakaraj et al., 1994; Rosales, 2017). Galandrini et al. have shown the FcγRIIIa stimulation can lead to activation of Arf6 via PI3K, and Arf6 in turn can induce membrane localization of PIP5K1α and PIP2 production (Galandrini et al., 2005). This interaction is necessary for cytolytic granule-mediated cytotoxicity.

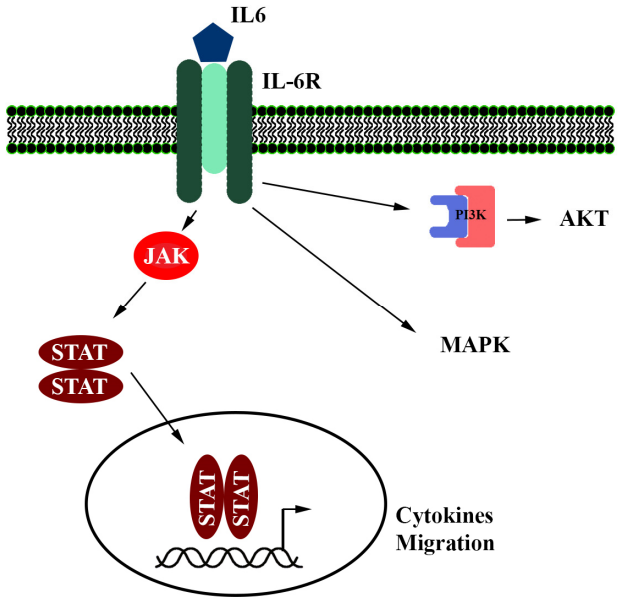

Figure 8. IL-6 signal transduction. The IL6 receptor (IL-6R) is activated upon binding of IL-6. Activation of IL-6 leads to the activation of JAK, which in turn activates the STAT. The activated STAT dimerizes, transports to the nucleus where it activates transcription of genes for cytokines and migration. The activated IL-6R can also serve as the docking site for adaptor proteins that are necessary for PI3K and MAPK pathway, thereby can activate these pathways also.

The interleukin-6 (IL-6) in PCa

Interleukin 6 (IL-6) is critical for regulation of functions such as proliferation, cell survival, tumor immune response and invasion. The classical signal transduction mediated by IL-6 is depicted in Figure 8. Binding of the IL-6 causes the dimerization and activation of the IL-6 receptor (IL-6R). As a consequence of receptor activation, JAK (Janus kinase) is activated by autophosphorylation. The JAK also phosphorylates the IL-6R and STAT3 (Signal transducer and activator of transcription 3) protein. The phosphorylated STAT3 then dimerizes, transports to the nucleus and drives the expression of genes for cell cycle, growth and invasion. The phosphorylated residues on IL-6R also serves as docking site for adaptor proteins of PI3K. The IL-6 pathway activation can also induce the MAPK (mitogen activated protein kinase) pathway, thereby can control proliferation.

In PCa, several components of the IL-6 pathway are altered. Expression of IL-6 regulator NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) is overexpressed in castration resistant LAPC-4 xenograft model (C. D. Chen & Sawyers, 2002; Culig, Steiner, Bartsch, & Hobisch, 2005). In CRPC patients, IL-6

serum levels were higher than the ADT responders (Drachenberg, Elgamal, Rowbotham, Peterson, & Murphy, 1999; Wise, Marella, Talluri, & Shirazian, 2000). In AR negative DU-145 cells, IL-6 stimulation was sufficient for AR responsive reporter activation in androgen independent fashion when after transfection with AR vector (Hobisch et al., 1998). This induction can be abrogated by MAPK inhibitor and antiandrogens.

Table 3. Molecular subtypes of breat cancer
Data for the table are retrieved and modified from Dai et al. (Dai et al., 2015)

Subtype	IHC marker expression
Luminal A	**ER^+PR^+ $HER2^-KI67^-$**
Luminal B	**$ER^+PR^+HER2^-KI67^+$**
	ER^+PR^+ $HER2^+KI67^+$
HER2	**ER^-PR^- $HER2^+$**
Normal-like	**ER^+PR^+ $HER2^-KI67^-$**
Basal/ Triple negative (TNBC)	**ER^-PR^- $HER2^-$, basal marker$^+$**

Triple negative breast cancer (TNBC) and AKT

Over 2 million (2,088,849) new cases of breast cancer (BCa) will be diagnosed and over 0.6 million (626,679) females will die from breast cancer in 2018 (Bray et al., 2018). It is the second most diagnosed cancer when both sexes are combined after lung cancer. Gene expression profiling have identified five subtypes of breast cancer namely the luminal A, luminal B, HER2 over-expression, basal and normal-like tumors (Table 3). Immunohistochemistry (IHC) markers such as ER (estrogen receptor), PR (progesterone receptor), HER2 (Receptor tyrosine-protein kinase erbB-2) and ki67 can provide valuable prognostic information and help dictate therapies. While hormonal therapy, surgery and radiation therapy as well as targeted therapy (anti HER2 antibodies) can improve the survival of the Luminal and HER2 subtype patients, treatment of the triple negative breast cancer (TNBC) still remains a challenge. Triple negative breast cancer patients have worst prognoses than the other subtypes with overall survival of 13 months (Delaloge & DeForceville, 2017). Chemotherapy is the only option in TNBC treatment.

Numerous studies have suggested an important role of PI3K/AKT pathway in TNBC. Activating mutation in PIK3CA and inactivating mutation in PTEN is a

common feature in TNBC cases (Bianchini, Balko, Mayer, Sanders, & Gianni, 2016; Shah et al., 2012; The Cancer Genome Atlas et al., 2012). Cossu-Rocca et al. have shown that 57% of *PIK3CA* mutated cases had higher pAKT expression as determined by immunohistochemistry analysis (Cossu-Rocca et al., 2015). In the same study PTEN inactivation frequency was over 11%. Most recently, a multicenter double blind trial with ipatasertib, an Inhibitor of AKT in combination with paclitaxel, have shown clinical benefit (overall survival) compared to paclitaxel alone. In stratified patients with *PIK3CA/AKT1/PTEN* status, the overall survival benefit was 6.9 months in ipatasertib arm(Dent et al., 2018; Kim et al., 2017).

Rationale for targeting PIP5K1a

Role of PI3K/AKT pathway in controlling growth, survival through inhibition of apoptosis and migration has been well established not only in BCa and PCa but also in other types of malignancies. Cancer cells, particularly in advanced metastatic stage relies on or get addicted to the PI3K/AKT pathway. Components of this pathway such as the catalytic subunit PIK3CA amplification or activating mutation and PTEN inactivation has been frequently reported in advance stage cancer. Biological consequence of these alteration will lead to over-production of PIP3 from PIP2. PTEN inactivation would lead to less degradation of PIP3. Since PIP5K1α is the major kinase responsible for PIP2 generation, and PIP5K1α act on upstream of AKT, PIP5K1α represents a novel drug target for inhibiting the PI3K/AKT pathway. Since PIP5K1α and PIP2 mediates cytoskeletal reorganization, endocytosis and apoptosis, targeting PIP5K1α may also result in inhibition of cancer cell's migratory ability and can induce apoptosis. Yamaguchi et al. have shown that invadopodium (protrusions formed at the invasive front) formation in TNBC cell line MDA-MB-231, can be inhibited by PIP5K1a knockdown or by injecting anti PIP2 antibodies into the cells (Yamaguchi et al., 2010).

Aims of my PhD thesis

The role of PI3K/AKT pathway in survival and invasion of cancer cells is well established. Several preclinical drugs targeting this pathway are already in late stage of clinical trials. The protein kinase PIP5K1α acts as the upstream activator by generating the substrate (PIP2) necessary for triggering the pathway. However, the expression, function and utility of PIP5K1α as a druggable target have not been studied in detail in prostate cancer and breast cancer. The overall aim of my thesis is to study the role of PIP5K1α and its interlinked cellular pathways in the progression and metastasis of prostate cancer and breast cancer, and to develop and characterize the PIP5K1α inhibitor for treatment of advanced metastatic prostate and breast cancer

The thesis is based on the following specific aims:

1. Study the clinical importance of PIP5K1α expression in subtypes of prostate cancer and breast cancer.
2. Elucidate the role of PIP5K1α and its associated PI3K/AKT and AR in progression of prostate cancer and breast cancer.
3. Study the molecular pathways and underlying mechanisms that regulate PIP5K1α in prostate cancer.
4. Characterize the inhibitory effect and mode of action of PIP5K1α inhibitor in cell line-based and in xenograft mouse model.

Materials and methods

The materials and methods are described in detail in the papers 1-6. Only the methods that may need further explanation are mentioned here.

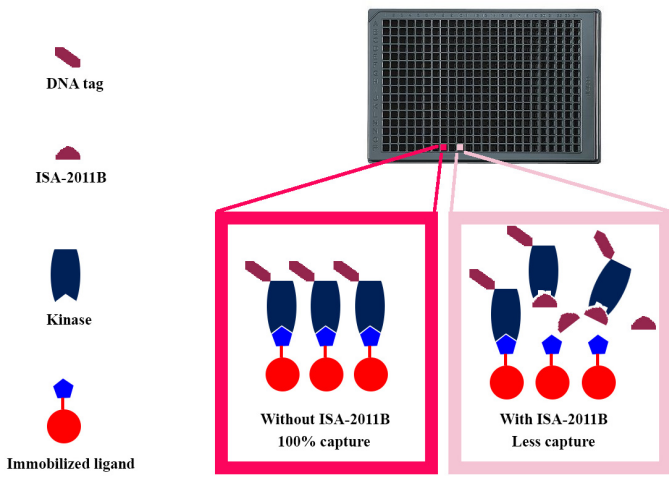

Figure 9. Kinome scan method. Inhibitors that binds to the active site will lead to less kinase capture on the immobilized ligand. The DNA tag attached to the kinases is used for quantitative measurement of possible interactions with qPCR.

Discovery of ISA-2011B as PIP5K1α inhibitor

The ISA-2011B (CAS 1395347-24-6) is tetrahydroisoquinoline derivative and has the chemical formula $C_{22}H_{18}ClN_3O_4$. It was synthesized via Pictet-Spengler approach at the department of organic chemistry, Lund University. It showed anti-proliferative effect on metastatic cell lines from various malignancies. ISA-2011B was discovered to be an inhibitor for PIP5K1α through high-throughput KINOMEscan® Assay Platform (DiscoverX Corporation) against 450 human

kinases. The method relies on active site-directed competition binding assay depicted in the Figure 9 and does not involve ATP. Molecules that has potential of binding directly or indirectly in the active site of the kinase would prevent binding of the kinase to the immobilized ligand. Without potential inhibitors the kinase would be captured on the plate whereas the kinase capture would be less with the inhibitors. The DNA tag attached to the kinases is used for quantitative measurement of possible interactions with qPCR. With ISA-2011B, nearly 80% of the PIP5K1α did not get captured. This data proves that ISA-2011B binds competitively to the ligand binding site of PIP5K1α. Furthermore, *in-vitro* cell based assay showed that ISA-2011B treatment can downregulate PIP5K1α and inhibit AKT activation as shown by western immunoblotting with anti pAKT antibody (phospho Ser 473).

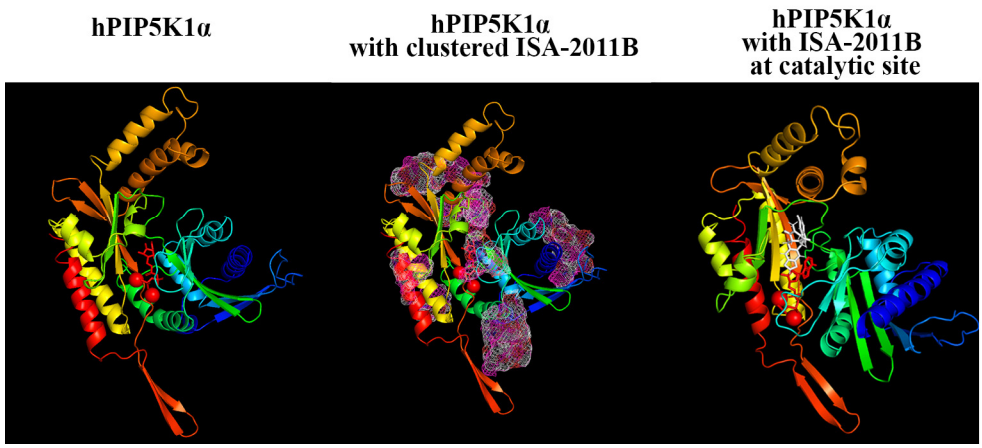

Figure 10. Predicted model of hPIP5K1α and possible binding site of ISA-2011B on hPIP5K1α. (Left pannel) The modelling of hPIP5K1α was performed with SWISS-MODEL workbench based on the crystal structure of zebrafish PIP5K1α (PDB ID: 6CMW) (Waterhouse et al., 2018). **(Middle pannel)** Possible binding sites for ISA-2011B was modeled with SwissDock software (Grosdidier, Zoete, & Michielin, 2011). Note the 2 calcium ions and one ATP is shown in red color and the possible bindind site of ISA-2011B is shown in mesh format. **(Right pannel)** one of the possible binding site at the catalytic core near the ATP binding site is shown (ISA-2011B shown in white).

Modelling of human PIP5K1α and clustering ISA-2011B on the modeled PIP5K1α

Modelling of human PIP5K1α was performed with SWISS-MODEL workbench with target–template alignment function (Waterhouse et al., 2018). The template of zebrafish PIP5K1α (PDB ID: 6CMW, Crystal structure of zebrafish PIP5K1α with

bound ATP/Ca2+) was used to model the human PIP5K1α (hPIP5K1α). The predicted model of hPIP5K1α is visualized with PyMol software and a side by side comparison with zPIP5K1α is shown in Figure 7. Possible binding sites for ISA-2011B on modelled hPIP5K1α were evaluated with SwissDock server (Grosdidier et al., 2011). ISA-2011B showed many possible binding sites on the modelled structure of hPIP5K1α. Possibly the binding of ISA-2011B near the ATP binding site is the reason for its inhibitory function. The ISA-2011B clustering on hPIP5K1α is shown in the Figure 10.

Immunohistochemistry and scoring

Antigen retrieval was achieved by heating in a pressure cooker in pH6 or with target retrieval buffer (DAKO) or at high pH buffer using a PT-link module (DAKO). An automated staining procedure (Ventana Inc, Tucson, AZ) was used for immunohistochemical (IHC) staining with Envision Flex reagents in an Autostainer Plus system (DAKO). The tissue micraoarray (TMA) sections (4μm) were stained with primary antibodies. For secondary antibody incubation and visualization, Dako real envision detection system was used. Counterstaining of cell nuclei was done with Hematoxylin. Stained TMA's were scanned with ScanscopeCS (ScanscopeCS, Aperio, Vista, CA). Three different scientist and a board certified pathologist evaluated and scored the tissues from 1(negative) to 3 (very strong intensity) depending on the intensity. Staining was deemed to be positive if more than 10% cells in the core show staining. The semi quantitative H-scoring method was used to score the breast cancer TMA's in Paper 3. The H-score was obtained by multiplying the percentage of stained tumor cells (minimum 0 and maximum 100) and the staining intensity (0 was negative, 1 was weak, 2 was moderate and 3 was strong staining).

Invasion assay

For evaluating the effect of PIP5K1α inhibitor ISA-2011B on invasion a basement membrane (8 μm pore size) and the colorimetric cell invasion assay Kit (Merck, KGaA) was used. Fifty thousand ($5x10^4$) PC-3 cells were cultured in the invasion chamber with or without ISA-2011B (50μM). 10% FBS with or without ISA-2011B was used as chemoattractant. After 48 hours, invaded cells were stained, dissolved in 10% (vol/vol) acetic acid and quantified on ELISA plate reader by reading of OD at 560 nm. For determining the outcome of PIP5K1A overexpression, transfected cells (1.25×10^5) were cultured in the high sensitivity non-cross-linked collagen invasion assay (Merck, KGaA) chamber with 8 μm pore size. PIP5K1A

overexpressing PNT1A cells were serum starved for 96 hours prior to seeding into the invasion chambers. As chemoattractant 10% FBS supplemented RPMI-1640 medium was used. After 48 hours, invaded cells were counted under the microscope.

Migration assay

The MDA-MB-231 cells were transfected with or without the PIP5K1A overexpressing vector for 24 hours prior to seeding in the Boyden trans-well chambers (8 μm) (BD Biosciences). In brief, $1.5x10^5$ cells were cultured in the trans-well chambers and 20% of FBS was utilized as chemo-attractant. After a day, migrated cells were fixed, stained and calculated under the microscope. For Paper 5, $5x10^4$ C4-2/C4-2ΔPIP5K1A cells were seeded. Cells were serum starved for 24 hours before seeding. The RPMI-1640-media supplemented with 50% serum was utilized as chemo-attractant. After 20 hours, the migrated cells were fixed, stained and calculated under the microscope.

Spheroid assay

Equal number of cells was seeded in polyhema-coated ultra-low attachment culture dishes in spheroid formation medium (DMEM F-12, B27 (x2) supplement, 40 ng/ml EGF and 40 ng/ml FGFβ). After 10 days of culture with or without ISA-2011B (50μ), the number of spheroids were counted, and photographs were documented with an Olympus AX70 microscope using NIS Elements F 2.20 software. For immunofluorescence analysis of spheroids, the spheroids were fixed with 4%PFA then permeabilized and stained with antibodies.

Co-culture and spheroid assay

C4-2/ C4-2ΔPIP5K1A cells and U-937 cells were cultured in mono- or co-culture at ratios 1:1.5 in standard RPMI-1640-media supplemented with 10% serum. After 24 hours, the cells were separated by washing and trypsinization and then counted. Each cell line was seeded separately (monoculture) or together (coculture) in spheroid formation medium for 10 days.

Mouse xenograft

Animal experiments were performed after the approval by Swedish Regional Ethical Animal Welfare Committee.

Paper I

Effect of PIP5K1A gene knock down was investigated in NRMI nude mice. Equal amount (2×10^6) of PIP5K1A knocked down PC-3 cells were injected in the flanks (7 mouse/group) subcutaneously (SC) and tumor growth were monitored for 20 days.

Tumor growth inhibition after treatment of ISA-2011B was evaluated in BALB/c nude mice. Four million PC-3 cells were implanted and as tumor volume reached 50 mm^3, mice were randomized (6 mouse/group) into four different groups. Intraperitoneal injections were carried out every second day with either vehicle (control) or ISA-2011B (40 mg/kg) or docetaxel (10 mg/kg) or in combination of ISA-2011B and docetaxel for a total of 20 days. The electric slide calliper was utilized to measure the tumor diameter and the equation (a x b2/2; a=larger and b=smaller diameters) was applied for calculation of volume.

Paper 2

Two million 22Rv1 cells with or without AR-V7 transfection were injected to the flanks of NMRI nude mice (Taconic Europe, Lille Skensved, Denmark) and randomized into different groups and tumor growth were monitored. Mice were treated as soon as their tumor volume reached 150 mm^3 with or without ISA-2011B (40 mg/Kg) every second day and their tumor growth were monitored for a total of 15 days.

Paper 3

In the female BALB/c nude mice, 4×10^6 MDA-MB-231 cells were implanted subcutaneously. The mouses were randomized into different groups after their tumor reached 50 mm^3 in volume and treated with vehicle (control), docetaxel (10 mg/kg) and ISA-2011B (40 mg/kg) every other day with intraperitoneal injection. Tumor growth was monitored for a total of 24 days.

Summary of results and discussion

Paper 1

In this study we wanted to know whether PIP5K1α expression increases in the cancer patients and if this holds true, whether there is any utility of targeting PIP5K1α with our newly discovered inhibitor ISA-2011B against PIP5K1α *in-vitro* and *in-vivo.*

Elevated levels of PIP5K1α and its product PIP2 were frequently observed in prostate tumors from PCa patients and were associated with poor disease-free survival. The *PIP5K1A* gene amplification was common event in metastatic PCa patients. The *PIP5K1A* high patient had either homozygous deletion or mRNA downregulation of *PTEN* gene. These data suggest that PIP5K1α is upregulated in PCa and it represents a novel target in cancer cells, since inhibition of PIP5K1α may block the cancer cell survival pathways which are related to activation of AKT pathway and inactivation of PTEN tumor suppressor gene.

The novel compound ISA-2011B was established as a binding partner for PIP5K1α using high-throughput screening against 460 human kinases. The inhibitory effect of ISA-2011B on cell proliferation was further validated by *in-vitro* cell based assay by using various types of PCa cell lines including metastatic androgen independent (PC-3 cells, 22RV1 cells) and dependent cells (LNCaP cells). Our *in-vivo* data in PTEN null cells clearly showed that ISA-2011B treatment can sufficiently reduce the tumor growth nearly to the same level as docetaxel, a standard chemotherapeutic agent used for metastatic PCa.

Furthermore, we showed that AKT activation can be inhibited by ISA-2011B and by siRNA-mediated knockdown of PIP5K1α in PC-3 cells. Conversely, AKT activity can be improved by overexpression of PIP5K1α. Since we observed that PIP5K1α was highly expressed in primary and metastatic tumors in PCa patients, we wanted to mimic the conditions *in-vivo*, thus we induced overexpression of PIP5K1α in non-malignant (PNT1A) and malignant cell lines (PC-3 cells). We also wanted to evaluate the effect of PIP5K1α overexpression on proliferation, invasion and survival in these cells. We found that, PNT1A cells become invasive and gained proliferative ability as a result of PIP5K1α overexpression leading to increased AKT activity. Previous reports indicated that CDK1 can interact with AR and this

interaction is important for AR stabilization (Koryakina, Knudsen, & Gioeli, 2015). Our data suggest that ISA-2011B can downregulate the AR by reducing CDK1.

Our data points to the usability of targeting PIP5K1α with our newly discovered inhibitor against PIP5K1α.

Paper 2

Second generation antiandrogen enzalutamide (MDV3100) is recommended for post docetaxel metastatic CRPC patients. This drug exerts its effect by binding to the ligand binding site of AR.

However, patients become resistant to this therapy due to the expression of AR variant-7 (AR-V7) which was enhanced by enzalutamide. Enzalutamide induces apoptosis in PCa cells expressing full lengths AR which contain the ligand binding domain, but not in PCa cells expressing AR-V7, leading to the expansion of PCa cells containing AR-V7 (Antonarakis et al., 2014; Y. Li et al., 2013). In this study, we wanted to investigate if there was any correlation between AR-V7 and PIP5K1α expression in prostate cancer patients. And if ISA-2011B might be able to induce apoptosis in PCa cells expressing AR-V7 through depleting PIP5K1α, which might overcome enzalutamide resistance.

We found that high PIP5K1α expressing tumors also had elevated expression of AR-V7. AR-V7 high metastatic patients had a trend of higher PIP5K1α, though it was not statistically significant due to small number of patients in this cohort.

To better recapitulate the clinical situation in patients (AR-V7 overexpression), we introduced AR-V7 overexpression in enzalutamide resistant 22Rv1 cells. An enhancement of PIP5K1α expression was observed in AR-V7 overexpressed cells. Level of cyclin E and A2 was also enhanced as a result of AR-V7 enhancement. In xenograft model, AR-V7 overexpressing cells formed larger tumors compared to control cells. Isolated tumors from these mice also confirmed increased proliferation marker (ki67), PIP5K1α and extracellular matrix degrading MMP9 (matrix metallopeptidase 9). ISA-2011B was effective in reducing tumor size in xenograft mouse model and it reduced the AR-V7 expression thereby circumventing enzalutamide resistance. The ISA-2011B treatment was similarly effective *in-vitro* while enzalutamide was ineffective in reducing transcriptional activity of AR-V7. Our subcellular fractionation and immunoprecipitation data points to the importance of protein-protein complex between PIP5K1α-CDK1 and AR-V7 for the stability of AR-V7. By comparing the effect of PI3K inhibitor LY294002 to ISA-2011B, we showed that effect of ISA-2011B on AR/AR-V7 was partly independent of AKT pathway.

Paper 3

Of all the subtypes of breast cancer, treatment of the triple negative breast cancer (TNBC) patients remains a challenge as chemotherapy is the only choice. Lack of our understanding of this highly heterogeneous disease and putative driver oncogenic alteration has hampered development of targeted therapies. Numerous studies indicated the vulnerability of TNBC against therapies targeting the PI3K/AKT pathway. In our study we wanted to find out whether we could suppress the growth of TNBC cells with our PIP5K1α inhibitor.

In TNBC cohort, PIK3CA mutation was associated positively with PIP5K1α and PIP5K1α expression (protein and mRNA level) was associated with poor survival. Same association was observed in Luminal A type patients. Amplification or mRNA upregulation was most frequently observed in TNBC patients.

ISA-2011B inhibited proliferation and induced apoptosis in both luminal A and TNBC cell line, while ISA-2011B showed no cytotoxic effect in normal like MCF-10A cells. Our xenograft study further verified the anticancer effect of ISA-2011B. Standard chemotherapeutic agent docetaxel showed almost the identical result compared to ISA-2011B in suppressing tumor growth.

We also revealed a positive cooperation between PIP5K1α and ERα in regulating its target gene activation by using luciferase-based promoter activity assay. ERα target gene cyclin D1 could be repressed as early as 2 hours with ISA-2011B. Identical to the prostate cancer cells, ISA-2011B treatment or by gene knockdown of PIP5K1α was able to repress AKT activation.

Our data suggest that ISA-2011B may have great potential to target breast cancer cells which express elevated level of PIP5K1α/AKT and ERα. Since there is no effective therapy for treatment of TNBC tumors, it will be of great importance to develop ISA-2011B for treatment of TNBC.

Paper 4

In this study we revealed the possible interconnection between EGFR and AR in PCa. In order to further examine the clinical importance of EGFR expression in PCa, we examined EGFR mRNA expression in BPH tissues, primary PCa tissues and PCa metastatic lesions. We found that EGFR expression was significantly higher in metastatic lesions compared with BPH tissues. There was a trend that EGFR expression was increased in metastatic lesions compared to primary prostate tumors. However, the statistical significance was not achieved. By using The Cancer

Genome Atlas (TCGA) Prostate Adenocarcinoma Provisional database, we found that AR correlates with EGFR mRNA expression in primary PCa tissues.

To investigate whether AR overexpression may lead to upregulation of EGFR in PCa cell lines, we induced AR overexpression in VCaP cells expressing endogenous AR. We found that AR overexpression and ligand (DHT) induction were necessary to induce EGFR level. We also induced AR expression in PC-3 cells which do not express functional AR and examined AR expression on EGFR in PC-3 cells. We found that expression of AR alone did not have any effect on EGFR expression. Similar to what was observed in VCaP cells, AR expression with its ligand DHT greatly increased EGFR expression. These data suggest that AR pathway is linked to EGFR pathways in PCa. Also, AR overexpression alone was able to induce MMP-9 expression in PCa cells.

Additionally, we showed that ISA-2011B was able to repress the induction of MMP-9 and EGFR by inhibiting AR. Our data suggest that ISA-2011B can be applied as an effective inhibitor to impede the AR-associated EGFR and MMP-9 pathways.

Paper 5

In immune cells, the FcγRIIIa receptor mediated signal transduction is linked to PIP5K1α. The FcγRIIIa receptor upon stimulation, induces PIP2 production via PIP5K1α which is necessary for the signaling cascade. However, this association has not been evaluated in prostate cancer. In this study we wanted to evaluate interconnection between FcγRIIIa and PIP5K1α in prostate cancer and if there are any functional consequences for this interaction.

CRISPR-CAS9 gene editing was used to generate a shortened (truncated) version of PIP5K1α (ΔPIP5K1A) in C4-2 cells. Nuclear expression was unaffected but the cytosolic expression of PIP5K1α reduced dramatically in the C4-2ΔPIP5K1A cells. The cytoplasmic MMP-9 expression was almost diminished in C4-2ΔPIP5K1A cells. Interestingly, pAKT was also upregulated in these cells, probably through other compensatory mechanism. Upregulation of pAKT could have suggested that these cells might survive or proliferate better than the wild type. However, proliferation assay revealed that C4-2ΔPIP5K1A cells had decreased proliferation than the normal cells and was linked with a loss of full length PIP5K1α. C4-2ΔPIP5K1A cells formed less spheroids and were also smaller in size than the C4-2 cells. We propose that the cytoplasmic PIP5K1α is important for maintaining stem cell like properties of these cells. ISA-2011B completely diminished the cells ability to form these stem cell like spheroids. Another interesting observation in the C4-2ΔPIP5K1A cells derived spheroids was the downregulation of pAKT. In PC-3 cells, FcγRIIIa knockdown showed less PIP5K1α in the membrane and cytoplasm.

Similar effect was also observed in FcγRIIIa knockdown C4-2 and in C4-2ΔPIP5K1A cells suggesting clear association between FcγRIIIa and cytoplasmic PIP5K1α. The AR expression was downregulated in FcγRIIIa knockdown cells and even more so in FcγRIIIa knocked down C4-2ΔPIP5K1A cells. We also studied the interaction between FcγRIIIa and PIP5K1α in a coculture of immune cells (U937 cells) and cancer cells. Cocultured C4-2ΔPIP5K1A cells formed less spheroids than the normal cells. Our data suggests cytoplasmic PIP5K1α is essential for growth and progression of castration resistant PCa possibly through AR and FcγRIIIa signaling.

Paper 6

Inflammatory factors including cytokines often increase survival of cancer cells and development of neuroendocrine phenotype of prostate cancer (PCa), an invasive and castration resistant form of PCa. The aim was to investigate whether PIP5K1α protein expression may be regulated in response to the key inflammatory factors secreted by the prostate cancer cells including insulin and IL-6. The proliferation promoting and apoptosis inhibiting ability of insulin and IL-6 by triggering the activity of PI3K/AKT pathway is well known. We examined the influence of insulin and IL-6 alone or in combination on the expression of PIP5K1α/PI3K/AKT pathway in LNCaP cells. We also examined whether PIP5K1α inhibitor, ISA-2011B, might abrogate the cancer-promoting effects of insulin and IL-6. LNCaP cells were cultured with insulin, IL-6, and ISA2011B. Immunoblots were used to evaluate the role of PIP5K1α. Our preliminary data suggest that insulin or IL-6 may abrogate the effect of ISA-2011B on PIP5K1α.

We also examined the effect of insulin and IL-6 alone or in combination on LNCaP cells in which PIP5K1α was depleted via si-RNA-mediated knockdown.

The effects of insulin and IL-6 treatments on PIP5K1α and pAKT and their association with survival and invasiveness of PCa cells will be further investigated in the near future.

Conclusions and future directions

The PIP5K1α is highly expressed in cancer patients. The high expression of PIP5K1α may be due to gene amplification but may also be due to its protein modification. Higher levels of PIP5K1α mRNA and protein expression are associated with poor survival in men with prostate and females with breast cancer. However, the prognostic value of PIP5K1α needs to be evaluated in several more and much larger patient cohorts. Nevertheless, PIP5K1α emerges as a novel target, as PIP5K1α produces PIP2 which serves as substrate for PI3K/AKT pathway. Further, PIP5K1α may regulate the AR and AR-V7, since the nuclear PIP5K1α was found in complexes with AR and AR-V7, and PIP5K1α is required for protein stabilization of AR and AR-V7.

Future analysis of this association must be carried out with patient samples who have enzalutamide or abiraterone resistance.

Through many approaches such as gene knockdown or overexpression or using our newly discovered PIP5K1α inhibitor, we have shown that PIP5K1α can activate the PI3K/AKT pathway and the consequence of this activation is the increased proliferation, survival, invasion and migration of cancer cells. We could reverse these highly relevant cellular activities in cancer cells by using the PIP5K1α inhibitor ISA-2011B both *in-vitro* and *in-vivo*.

Taken together our data suggest that ISA-2011B has a great potential to serve as a therapeutic compound for treating cancer patients with constitutively active PI3K/AKT pathway. In order to further show the selective effect of ISA-2011B on cancer which express elevated levels of PIP5K1α/PI3K/AKT, we should examine the effect of ISA-2011B on lung cancer, colon cancer, pancreatic cancer and leukemia models as well, as the aggressive subsets of these cancer often have high levels of PI3K/AKT and EGFR and PTEN mutation. Although the toxicological assay of ISA-2011B have been performed in mice and rats, the similar studies should be performed in larger animal models. To further identify the precise binding between ISA-2011B and PIP5K1α, crystal structure of human PIP5K1α needs to be resolved.

Populärvetenskapling sammanfattning

Prostatacancer (PCa) är den vanligaste orsaken till cancerrelaterad död hos män efter lungcancer. Årligen upptäcks i Sverige drygt 9000 nya fall och 2500 män dör i sjukdomen varje år. Även globalt utgör sjukdomen ett stort folkhälsoproblem, inom EU uppskattas att 77000 män dör i prostatacancer år 2018. Prostatcancer i tidigare utvecklingsstadium kan behandlas med hormonblockare som inhibiterar androgenreceptor (AR), vilket leder till regression av tumören. Men en tredje del av behandlade patienter får återfall inom ca 2 år i mer aggressiv cancerform som blir okänslig för denna behandling. Patienten utvecklar kastrationsresistant prostatatcancer (CRPC). I nuläget saknas effektiv behandling för CRPC.

Det manliga könshormonet testosteron och dihydrotestosteron (DHT) har en stor betydelse i prostatacancer cellernas tillväxt. AR binder med DHT och färdas in i cellkärnan som sätter igång cancercellernas tillväxt. Vid CRPC, dock dominerar andra signalvägar som är nödvändiga för att cancerceller ska kunna klara sig utan testosteron.

I mitt avhandlingsarbete, har vi identifierat proteinet PIP5K1α som har i laboratorieförsök visat sig främja tillväxten av de aggressiva prostatacancrar och bröstcancer celler. PIP5K1α proteinet är en kinas som producera lipid PIP2. Våra studier har visat att PIP5K1α har stor betydelse för bröst- och prostatatumörtillväxt och spridning. Felaktig nivå i PIP5K1α aktiverar PI3K/AKT signalväg och öka tumörcellernas tillväxt, överlevnad och spridning. Felaktighet i PIP5K1α gör att cancercellerna blir resistenta mot de existerande behandlingsformerna. När vi undersökte prover från patienter med prostatacancer kunde vi se att de som hade höga nivåer av PIP5K1α i sin prostata, samtidigt hade de höga nivåer av en kortare form av AR, AR-V7, vars uttryck är stark relaterad till enzalutamide resistans hos PC patienter.

Vi har upptäckt att en läkemedelskandidat, ISA-2011B kunde hämma PIP5K1α, och blockera AKT signalväg, som är starkt överaktivt i prostatacancerceller, och är kopplat till deras överlevnadsfunktion. ISA-2011B blockerar PIP5K1α, den blir med andra ord göra cancerceller verkningslös och de tappar sina förmåga att överleva, växa och sprida sig. Studierna som ingår i denna avhandling har också

visat att bröstcancer också har felaktigheter i PIP5K1α uttrycket. ISA-2011B behandling av bröstcancerceller gör att cancercellerna blir mindre spridningsbenägna vilket minskar risken för metastaser. Eftersom dessa cancerceller delar samma signalvägar (PIP5K1α/PI3K/AKT) för att styra sin tillväxt och överlevande har vi visat att de går att behandla med ISA-2011B. Vi har även kunnat visa att PIP5K1α spelar en viktig roll vid reglering av inflammatorisk process. Behandlingen av cancerceller med insulin tillsammans med cytokinen och interleukin 6 (IL-6) negativ påverkar effekt av ISA-2011B på PIP5K1α/AKT och AR.

Slutligen, våra studier har visat att ISA-2011B har en stor förmåga att blockera PI3K/AKT signalväg därmed är den en viktig läkemedelskandidat. Vi hoppas att i framtiden kunna kontrollera cancertillväxten på ett effektivare sätt än vi kan idag och ge cancerpatienter skräddarsydd behandling.

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About the thesis and the author

Prostate cancer (PCa) is the most common cause of cancer-related death in men after lung cancer. Metastatic prostate cancer can be treated with castration. However, the disease eventually recurs within 2 years in more aggressive form and becomes insensitive to castration which is termed as castration resistant prostate cancer (CRPC). Currently, effective treatment for CRPC is lacking. In my thesis I studied the role of a kinase called the phosphatidylinositol 4-phosphate 5-kinase type I alpha (PIP5K1α) in cancer progression and therapeutic resistance. Through many laboratory methods, we established that this kinase acts as the activator of the PI3K/AKT pathway. Activation of the PI3K/AKT pathway is responsible for resistance to many anticancer therapies. We have discovered an inhibitor called ISA-2011B against the PIP5K1α kinase. Our data strongly suggests that PIP5K1α can be developed as a novel drug target in metastatic cancer and ISA-2011B is an important drug candidate for blocking the PI3K / AKT signaling in a subset of patients.

Martuza Sarwar was born in Bangladesh. He has a background in medical molecular biology. In his spare time, he enjoys the company of his family, loves watching football, cricket, enjoys carp fishing and loves playing badminton with friends.

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