

## Understanding the functional discrepancy of *Pim-1* in cancer

Allal Ouhtit<sup>1</sup>, Sukalp Muzumdar<sup>1</sup>, Ishita Gupta<sup>1</sup>, Somya Shanmuganathan<sup>1</sup>, Yahya Tamimi<sup>2</sup>

<sup>1</sup>Department of Genetics, College of Medicine and Health Sciences, Sultan Qaboos University, PO Box 35, PC 123, Al Khoud, Sultanate of Oman, <sup>2</sup>Department of Biochemistry, College of Medicine and Health Sciences, Sultan Qaboos University, PO Box 35, PC 123, Al Khoud, Sultanate of Oman

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### 1. ABSTRACT

The *Pim-1* gene encodes for a serine/threonine protein kinase proto-oncogene involved in cytokine signaling as well as in various pathways regulating cell cycle and apoptosis. *Pim-1* kinase plays an important role in the development of various tumors mainly, prostate cancer, Burkitt's lymphoma, oral cancer and various other hematopoietic lymphomas. This review will focus on the importance and mechanisms of *Pim-1* in prostate cancer and the potential clinical relevance of its various inhibitors.

### 2. INTRODUCTION

The calcium/calmodulin-dependent kinases (camks), including CAMKI, CAMKII and CAMKIV, are involved in various cellular responses mediated by hormones, neurotransmitters and other signaling events (1). Activated camks upon phosphorylation, are involved in synchronizing fluctuations of calcium levels depending on the suitable cellular response. Camks are partly controlled by the intracellular calcium receptor calmodulin (cam). Camkii, camki and camkiv, consist of an auto-regulatory domain that inhibits enzymatic activity in the absence of calcium/cam (2). Calcium/cam binding activates CAMKII, while camki and camkiv contain an activation loop that has to undergo phosphorylation

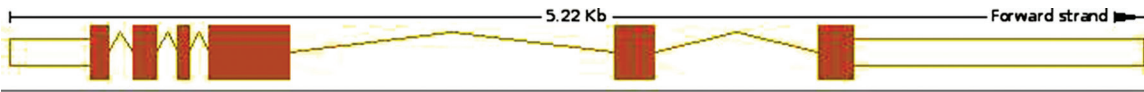
of a threonine residue by camk kinase (camkk) for activation. The CAMK group consists of the *Pim* kinases that constitute a family of three vertebrate serine/threonine protein kinases (*Pim-1*, *Pim-2* and *Pim-3*) (3), enzymes involved in phosphorylation of the hydroxyl group of serine and threonine.

The *Pim-1* oncogene was initially discovered as a pro-viral insertion site for the Moloney murine leukemia virus (MuLV) (4), which was mutated in more than 25% of murine T-cell lymphomas. The human homolog of this gene named *hpim* is located in the vicinity of 6p21 (4). Although primarily situated in the cytoplasm, *Pim-1* is occasionally found within the nucleus (5,6,7).

Ongoing studies in our laboratory, aim to shed light on the discrepancy and unravel the mechanisms of action of *Pim-1* in prostate cancer. Here we discuss the current knowledge of these mechanisms and the progress in understanding the multiple roles of *Pim-1*.

### 3. STRUCTURE OF PIM-1

In mammals, *Pim-1* oncogene encodes a serine/threonine protein kinase proto-oncogene



**Figure 1.** Structure of *Pim-1*.

located on the short arm of chromosome 6 (6p21.2.), encompassing 5kb of DNA including 5 introns and 6 exons (Figure 1).

Human *Pim-1* consists of 313 amino acids and encompasses 94-97% amino acid homology to murine and rats. The active site of *Pim-1* is located between amino acids 38-290 and contains multiple conserved motifs important for its function, including a glycine loop motif, a phosphate-binding site and a proton acceptor site. Mutations such as K67M leads to the complete inhibition of *Pim-1* kinase activity (8). The crystal structure of *Pim-1* shows that phosphorylation of *Pim-1* is needed for its stability and not for regulating kinase activity (9).

The *Pim-1* serine/threonine kinase has two variant isoforms derived from two alternative transcription initiation sites. The smaller, 33kDa isoform was first reported in 1988 by Telerman *et al.* (10). The murine 44kDa protein is produced through translational initiation at a non-conventional CUG codon upstream of the usual start codon; However this has not been independently confirmed in humans (11). The multifactorial regulation of *Pim-1* expression includes cytokines, such as IL-12 (12) and IFN- $\alpha$  (13), growth factors (14), and hormones such as gonadotropin (15). Additionally, being a stress-phase expressed kinase, it is also expressed in conditions such as hepatic ischemia (16), hypoxia (17), and in response to infections by *H. pylori* (18) and Epstein-Barr virus (19).

#### 4. FUNCTIONS OF PIM-1

Physiologically, *Pim-1* is expressed in both malignant and normal cells. The following sections will discuss the role of *Pim-1* in both normal and malignant cells.

##### 4.1. Physiological functions of *Pim-1* in normal cells

Physiologically, *Pim* kinases are involved in the growth and survival of leukocytes, transcriptional regulation as well as various other signaling pathways. In various tissues, *Pim-1* and *Pim-2* are expressed at low levels, but cytokine-driven

activation (including interleukins 2, 3 and 7, granulocyte-macrophage colony stimulating factor, interferon- $\alpha$  and  $\gamma$  and erythropoietin) leads to a strong leukocytic induction, mediated through the JAK/STAT pathway (3). *Pim-1* is also part of a negative feedback loop in the JAK/STAT pathway; it is involved in the stabilization of a suppressor of JAK/STAT pathway – *Socs1*. In fact, both *Socs1* and *Pim-1,2* knockout mice exhibit prolonged JAK/STAT signaling upon IL-4 stimulation (20). Although *Pim-1* knockout mice have a very mild phenotype, triple knockout mice for all the *Pim* genes exhibit a life-long reduction in body size, due to reduced cellular proliferation (21). Additionally, *Pim* triple knockout mice also exhibit impaired response to hematopoietic growth factors (21, 22). *Pim* kinases appear to share multiple common substrates such as BAD, p21<sup>WAF1/CIP1</sup> and *Cot/Tp1-2* substrates with Akt/PKB and other AGC kinases, thus playing a redundant role in regulating hematopoietic cell proliferation and survival (25) (3).

*Pim* kinases phosphorylate various proteins involved in the regulation of nuclear transcription and cell cycle, including the transcriptional repressors (HP1) and activators (NFATc1 and c-Myb), the nuclear transcription protein (p100), the cell cycle regulators (p21, Cdc25A phosphatase and C-TAK1/MARK3/Par1A) and the pro-apoptotic proteins (BAD on Ser-112) (3) (25-30). Heterochromatin-associated protein 1 (HP1) and *Pim-1* associated protein (PAP1) are involved in the silencing of chromatin and mRNA splicing regulation respectively, processes which are important components of the nuclear transcription regulation mechanisms (31, 32). While the co-activator p100 activates c-Myb (26), signals from T-cell receptors are relayed *via* the NFATc1 protein (27). *Pim* kinases can induce apoptotic resistance upon removal of growth factors. Since *Pim* kinases play critical roles in both cell growth and survival, they provide a distinct survival benefit during tumorigenesis (3).

##### 4.2. Physiological functions of *Pim-1* in cancer

*Pim* kinases are known to be involved in promoting early transformation, cell growth and cell

survival (7). *Pim-1* kinase has found to be involved in the development of various tumors mainly, prostate cancer (PC), Burkitt's lymphoma, oral cancer and various hematopoietic lymphomas (8, 34). As a downstream effector of the VEGF-A/Flk1 pathway, *Pim-1* might also play a role in angiogenesis and vasculogenesis (33).

### 4.2.1. Physiological functions of *Pim-1* in prostate cancers

The link between *Pim-1* kinase and PC was first established by Dhanasekaran *et al*, who showed its significantly aberrant expression in PC (7). Studies have shown that interleukin-6 promotes activation of *Pim-1* kinase by activating Janus-activated kinase/signal transducers and other signaling transcription components involved in the onset of PC (35). *Pim-1* overexpression in high grade prostatic intra-epithelial neoplasia (HG-PIN) is considered as an initiating event in PC development, thus qualifying *Pim-1* as a biomarker in PC (36). It has been observed that cells displaying strong *Pim-1* expression have abnormal mitotic spindles, chromosomal disaggregation and amplified centrosomes, characteristics responsible for the transformation of androgen-dependent prostate cells to androgen-independent prostate carcinoma cells (6). A synergism between the expression levels of *Pim-1* and *Myc* was also postulated due to their co-regulation in PC (7). In addition, although *Pim-1* was generally overexpressed in prostatic neoplasia, its down-regulation significantly correlated with poor prognostic outcomes of the disease based on the frequency of PSA recurrence (7).

Androgen receptors (AR), critical players in PC phosphorylated by multiple kinases, were regulated by *Pim-1* in PC cells (37). The *Pim-1* kinase isoforms, *Pim-1S* and *Pim-1L*, are up-regulated during PC and play a key role in maintaining the stability and the transcriptional activity of AR, through Ser-213 and Thr-850 phosphorylation. While, only the long isoform is capable of AR phosphorylation at Thr-850 leading to AR stabilization, only the short isoform can promote degradation of AR through phosphorylation at Ser-213 (38).

### 4.2.2. Physiological functions of *Pim-1* in other cancers

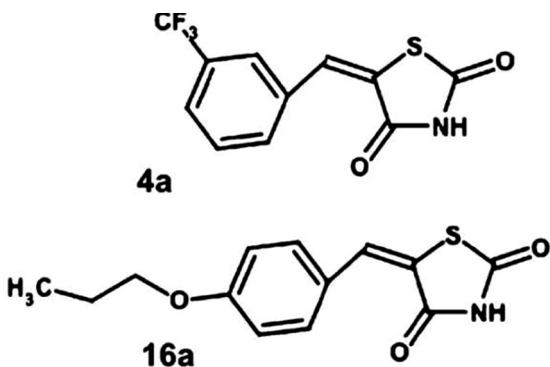
*Pim-1* is also over-expressed in various hematopoietic and lymphoid malignancies, gastric carcinomas, squamous cell carcinomas

of the head and neck and colorectal cancers (6). *Pim-1* chromosomal translocations and somatic hyper-mutations were identified in non-Hodgkin lymphomas. Moreover, *Pim-1* is involved in non-IG/BCL6 translocations, a characteristic feature in B-cell non-Hodgkin lymphomas (39). In some cases of non-Hodgkin lymphoma and chronic lymphocytic leukemia, *Pim-2* is overexpressed (3, 40). Mutated *Pim-1* was detected in approximately 50% of cases of diffuse large B-cell lymphomas, and also altered in primary lymphomas of the central nervous system (41). Overexpression in various large B cell lymphomas suggests *Pim-1* as a vital predictive and prognostic marker. *Pim-1* and the anti-apoptotic protein A1 promote BCR/ABL-dependent leukaemogenesis by cumulative growth of BCR/ABL-mutated cells. They also promote cell cycle and support the BCR/ABL-mediated cell protection from apoptosis (6, 42).

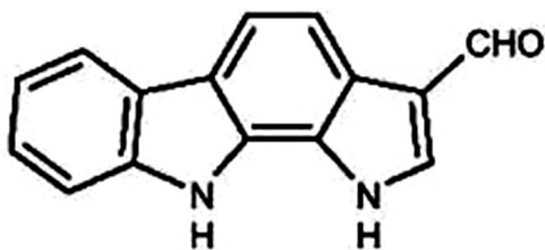
*Pim-1* plays a major role in the preservation and transformation of Epstein–Barr virus (EBV) infected B-cell lymphocytes, a characteristic linked to Burkitt's lymphoma and helps in stimulating survival of mutated cells. In combination with the components of the STAT3 signaling pathway, c-Myc and Bcl-2, *Pim-1* is upregulated to promote the transformation and growth of blastoid variant mantle cell lymphoma (MCL-BV). Also, *Pim-1* can induce phosphorylation and up-regulation of the RUNX protein, which is mutated on the site of *PIM* (6p21-23), a characteristic found in the translocation t (12;21)-positive Acute Lymphoid Leukemia (ALL) (6, 40). *Pim-1* expression is present in normal gastric mucosa and gastric carcinoma cell lines, suggesting its involvement in *H. pylori* related diseases, such as gastritis and gastro-intestinal tumors (18). In human pancreatic duct epithelial cells expressing KRas, *Pim-1* is up-regulated and contributes to the onset of pancreatic ductal adenocarcinoma (14). In solid tumors such as intrahepatic cholangiocarcinoma (ICC) (43) and non-small cell lung carcinomas (NSCLC) (44), there is a gain of 6p, region where *Pim-1* is expressed.

## 5. INHIBITORS OF PIM-1 KINASE

*Pim-1* kinase's oncogenic and pro-survival ability is associated with chemo-resistance in hormone-refractory PC, which are severely aggressive. Hence, studies are now focusing on the identification and development of *Pim* kinase inhibitors that can guide the establishment of targeted therapeutic strategies against cancer (35).



**Figure 2.** Novel benzylidene-thiazolidine-2,4-diones shown to inhibit Pim-1 kinase. Reproduced with permission from (45).



**Figure 3.** 1,10-dihydropyrrolo(2,3-a)carbazole-3-carbaldehyde (DHPCC-9). Reproduced with permission from (46).

As matter of fact, one of the classes of inhibitors, benzylidene-thiazolidine-2,4-diones, can abrogate the activity of the Pim-1 kinase *in vitro* in the 22Rv1 and DU145 PC cell lines. The loss of Pim-1 kinase activity was determined by measuring the levels of phosphorylated Bad, a pro-apoptotic protein phosphorylated by Pim-1, thus abrogating its activity. Pim-1 has also been shown to interact with various other cell cycle-regulating proteins to promote cell cycle checkpoint progression, and escape from apoptosis. FACS analysis revealed that, the inhibitors were also able to override the activity of Pim-1 and induce a cell cycle arrest at G<sub>1</sub>, (Figure 2) (45).

Another small molecule, inhibitor of Pim-1 kinase, is the DHPCC-9, with general cytotoxicity can reverse the anti-apoptotic effect exhibited by the Pim-1 kinase in malignant cells (46). This effect is mediated through the inhibition of the phosphorylation of Pim-1 substrates such as Bad, which uses the same mechanism as that utilized by the novel benzylidene-thiazolidine-2,4-diones

discussed earlier. In addition, DHPCC-9 is able to block the promotion of cell migration and invasion caused by overexpression of the Pim-1 kinase. In fact, NFAT1c transfected cells treated with DHPCC-9, inhibited cell motility (Figure 3), suggesting that Pim-1-promoted cell migration and invasion is, likely at least partially, mediated through the NFATc1 (46).

Further, a selective small molecule inhibitor of Pim kinase family, an imidazo (1,2-*b*) pyridazine derivative, SGI-1776 is specific for Pim kinases with lower affinity. It can inhibit cell cycle and induce apoptosis in PC cells and cause molecular changes which include targeting Pim kinase substrates, Bad and p21 at their specific phosphorylation sites Ser-112 and Thr-145 respectively. In addition, to the above, several studies have shown that SGI-1776 induces cytotoxic effects in androgen-independent prostate tumors but not in androgen-dependent tumors. This inhibitor in combination with chemotherapy has found to inhibit Pim kinase in prostate cancer by inducing taxane sensitivity and can prevent resistance to taxane by inhibition of MDR1 activity (35).

The Pim-1 kinase is also a known target for immunotherapy of cancer, with monoclonal antibodies directed against Pim-1 being tested in both *in vitro* and *in vivo* models. The antibody used was shown to react with both the 33kDa and the 44kDa isoforms of the protein as well with a novel 37kDa Pim-1 detected in the study which is most probably a splicing variant or is generated through post-translational modification. This monoclonal antibody was able to induce most of the effects expected of a Pim-1 kinase inhibitor including reduction in phosphorylation levels of pro-apoptotic protein such as Bad and Akt thus causing their inhibition. In addition, the anti-Pim-1 mAb was shown to have a synergistic effect (using 2-way ANOVA,  $P < 0.001$ ) with chemotherapy, specifically with cisplatin and epirubicin, as it also inhibited the growth of chemo-resistant cancer cells, through abrogation of the Pim-1 mediated phosphorylation of BCRP/ABCG2, otherwise responsible for the induction of chemo-resistance (47).

## 6. SUMMARY AND PERSPECTIVE

The Pim-1 kinase has been shown to play an important role in the prostate cancer in general



and in the progression of pre-malignant HG-PIN to malignant prostatic carcinomas in particular. Further investigation into possible mutations/polymorphisms in the *Pim-1* gene, which could putatively cause overexpression of the gene and thus increase susceptibility to prostatic cancer, is needed. Research showing the link between single nucleotide polymorphisms in the *Pim-1* gene and an increased incidence of non-small cell lung cancer in Korean patients can be taken as a starting point for any such endeavor (48). Mutations have also been detected in the *Pim-1* kinase gene in 50% of B-cell diffuse large cell lymphomas, thus, furthering the case for the existence of such mutations in prostatic cancer, especially since *Pim-1* kinase is known to play a major role in the progression of this cancer (49).

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**Send correspondence to:** Allal Ouhtit, Department of Genetics, College of Medicine and Health Sciences, Sultan Qaboos University, PO Box 35, PC 123, Al Khoud, Sultanate of Oman. Tel: 00968-2414-3464, Fax: 968-2441-3419, E-mail: aouhtit@squ.edu.om