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Abstract (no less than 200 words)

Pim-3 is a member of Provirus integration site for Moloney murine leukemia virus (Pim) family proteins that exhibits serine/threonine kinase activity. Similar to other Pim kinases (Pim-1 and Pim-2), Pim-3 is involved in many cellular processes including cell proliferation, survival, and protein synthesis. Although Pim-3 is expressed in normal vital organs, it is overexpressed particularly in tumor tissues of endoderm-derived organs including the liver, pancreas, and colon. Silencing of Pim-3 expression can retard *in vitro* cell proliferation of hepatocellular, pancreatic, and colon carcinoma cell lines, by promoting cell apoptosis. Pim-3 lacks any regulatory domains similarly as Pim-1 and Pim-2 do, and therefore, Pim-3 can exhibit its kinase activity once it is expressed. Pim-3 expression is regulated at transcriptional and post-transcriptional levels, by transcription factors such as Ets-1 and post translational modifiers such as translationally controlled tumor protein (TCTP), respectively. Pim-3 could promote growth and angiogenesis of human pancreatic cancer cells *in vivo* in an orthotopic nude mouse model. Furthermore, a Pim-3 kinase inhibitor inhibited cell proliferation when human pancreatic cancer cells were injected into nude mice, without inducing any major adverse effects. Thus, Pim-3 kinase may serve as a novel molecular target for developing targeting drugs against pancreatic and other types of cancer.

Key words: Serine/threonine kinase; Pancreatic cancer; Ets-1; Translationally Controlled Tumor Protein; c-Myc; Vascular endothelium growth factor; Apoptosis; Cell cycle

Core tip: The present review describes the advanced knowledge on the roles of Pim-3 in pancreatic cancer development and progression, and provides a new idea for Pim-3 as a therapeutic target in human pancreatic cancer.

1. Introduction

Pancreatic cancer is the fourth leading cause of cancer related deaths in the USA ^[1], Patients usually suffer from non-specific abdominal discomforts in the primary stages, which often delay early diagnosis and treatment. Furthermore, even in the initial evolutionary phase of disease development, pancreatic cancer cells tend to undergo invasion and metastasis. Therefore, complete removal of tumors by surgical procedures is often impossible. Another major stumbling block in treating pancreatic cancer is its frequent resistance to the treatments of chemotherapy and radiotherapy. Consequently, pancreatic cancer has an exceptionally poor prognosis with an overall 5-year survival rate of less than 5% ^[2, 3]. Thus, a novel molecular targeted therapy will be a required therapeutic option for human pancreatic cancer treatment.

Malignant lesions of the pancreas show a ductal, an acinar, or an endocrine lineage. Nearly 80 % of pancreatic carcinomas are classified as pancreatic ductal adenocarcinoma (PDAC) ^[4]. An activating mutation in a key proto-oncogene, K-ras, has been observed in most PDACs and is presumed to be the first significant event involved in pancreatic carcinogenesis ^[4]. The development and progression of PDAC are associated with additional multiple genetic and epigenetic alterations in several proto-oncogenes, tumor-suppressor genes, and signaling pathways. Pim-3 kinase has essential roles in the regulation of signal transduction cascades. Moreover, its expression is enhanced in human pancreatic cancer cell lines and blocking of its expression induced apoptosis and decreased chemoresistance in human pancreatic cancer^[5, 6].

Provirus integration site for Moloney murine leukemia virus (Pim) family is a proto-oncogene, which belongs to the group of calcium/calmodulin-regulated kinase and exhibits serine/threonine kinase activity ^[7]. The Pim family consists of three members, Pim-1, Pim-2, and Pim-3 ^[8]. The Pim-1 gene was first discovered as a proviral insertion site in Moloney murine leukemia virus ^[9]. A subsequent study demonstrated that Pim-1 transgenic mice are predisposed to the development of experimental T cell lymphoma in cooperation with c-Myc and N-Myc ^[10]. Pim-2 was similarly identified as a proviral integration site in Moloney murine leukemia virus-induced T cell lymphomas ^[11] and can

synergize with c-Myc-induced lymphomagenesis^[8]. Pim-3 was first identified as a novel gene, which is induced by membrane depolarization or forskolin in rat PC12 pheochromocytoma cells, and was designated as kinase induced by depolarization (KID-1)^[12]. Subsequently, KID-1 was renamed Pim-3 due to its high sequence similarity with other Pim family proteins, Pim-1 and Pim-2. Although Pim-3 can be detected in several normal tissues including those of the brain and heart, it is expressed in high levels in tumor tissues of various organs particularly those of endoderm-derived organs such as the pancreas, liver, colon and stomach^[5, 13, 14].

In this review, we aim to highlight the pathophysiological roles of Pim-3 in the development and progression of cancer, particularly pancreatic cancer. Moreover, by considering the sequence similarity of Pim-3 with other Pim kinases, we were able to rationalize and predict the possible functions of Pim-3 by extrapolating from the data established for other Pim family members, particularly Pim-1. We further discuss the potential of Pim-3 as a novel molecular target for antineoplastic therapy.

2. Structure of Pim-3 protein

The open reading frame of human Pim-3 mRNA encodes a protein consisting of 326 amino acids with a calculated molecular weight of 35,861 (Figure 1)^[13]. Human Pim-3 protein shares a high percentage of sequence homology with other members of the Pim family; Pim-3 and Pim-1 are 71% identical at the amino acid level, and Pim-3 and Pim-2 are 44.0% identical^[14-17].

The crystal structure of the Pim-3 protein has not yet been established, but several research groups have independently reported the crystal structure of Pim-1 and Pim-2 in the free form as well as in complex with their inhibitors^[18-22]. The Pim-1 kinase adopts a two-lobe kinase fold connected by a hinge region (residues 121–126)^[18]. The N-terminal lobe is composed of antiparallel β -sheets while the C-terminal lobe is composed mainly of α -helices (Figure 1). The adenosine triphosphate (ATP)-binding site is located in a deep intervening cleft between the two lobes and the hinge region. The Pim family proteins have no regulatory domains. Moreover, the ATP binding pocket in Pim-1

remains open irrespective of the presence or the absence of ATP ^[18], indicating a continuous maintenance of an active state conformation. Similar findings have been reported for the structure of Pim-2 kinase ^[20]. Thus, this may account for a good correlation between protein expression levels and overall kinase activity in the case of Pim-1 and Pim-2 ^[15]. Given a high sequence similarity (Figure 1 and NCBI Reference Sequence: NP_001001852.2), it is highly likely that Pim-3 kinase can adopt a similar three dimensional active conformation. Importantly, several residues believed to confer specificity in Pim-1 kinase are also conserved within Pim-2 and Pim-3 proteins.

3. Mechanisms underlying control of Pim-3 expression

Pim-3 mRNA is detected in several normal human tissues including the heart, brain, lung, kidney, spleen, placenta, skeletal muscle, and peripheral blood leukocytes, but not in colon, thymus, liver, and small intestine ^[13]. Pim-3 is expressed in endothelial cells ^[23]. Focal cerebral ischemia enhances Pim-3 mRNA expression in the peri-infarction cortex at early time points ^[24]. Similarly, ischemia reperfusion injury enhances intra-cardiac Pim-3 expression through p38-mediated signaling pathway ^[25]. In the mouse embryo, *Pim-3* gene expression is detected in the liver, kidneys, lungs, thymus, central nervous system, periphery of the pancreas, secretory epithelium of the stomach and intestinal epithelium ^[26].

Pim-3 mRNA is found to be expressed in a panel of human Ewing's family tumor cell lines ^[27] and nasopharyngeal carcinoma cell lines ^[28]. Likewise, we revealed that Pim-3 protein is scarcely detected in adult normal endoderm-derived organs such as liver, pancreas, colon, and stomach, but its expression is augmented in premalignant and malignant lesions of these organs (Table 1) ^[5, 13, 29, 30]. Pim-3 protein is mostly detected in the cytoplasm of these tumors. In the liver, aberrant expression of Pim-3 protein is also observed in the precancerous lesions such as regenerative nodules and adenomatous hyperplasia ^[13]. Similarly, in the colon and the stomach, Pim-3 protein is detected at higher levels in adenoma tissues compared with adenocarcinoma tissues ^[29, 30]. These observations suggest that Pim-3 plays a crucial role in the initial phase of carcinogenesis.

Pim-3 expression is regulated mainly at transcriptional and post-transcriptional levels. We will discuss the regulatory mechanisms at these two levels in detail.

3.1. Transcriptional regulation of Pim-3 expression

The EWS/Ets fusion proteins are pathognomonic for Ewing's sarcoma (EWS). These fusion proteins arise from the chromosomal translocations that combine a portion of the amino-terminal region of EWS to one of the five members of Ets family transcription factors, friend leukemia integration transcription factor (FLI), Ets-related gene (ERG), FEV, Ets translocation variant 1 (ETV1), Ets translocation variant 4 (ETV4/E1AF) ^[31]. Deneen and colleagues demonstrated that EWS/Ets fusion proteins can enhance Pim-3 gene transcription ^[27] in NIH 3T3 cells.

We have determined the 5'-flanking region of the human *Pim-3* gene, in order to elucidate the molecular mechanisms underlying constitutive Pim-3 expression in human pancreatic cancer cells. The human Pim-3 gene contains a canonical TATA box and putative binding sites for several known transcription factors, such as signal transducer and activator of transcription (Stat)3, Sp1, Ets-1, and nuclear factor NF- κ B and NF-1 ^[32]. Pim-3 expression is enhanced in murine embryonic stem cells by leukemia inhibitory factor (LIF)/gp130-dependent signaling and the Stat3 transcription factor ^[33]. In contrast, the transfection of dominant negative form of Stat3 failed to inhibit the promoter activity of *Pim-3* gene in human pancreatic cancer cells ^[32]. We further demonstrated that the region between -264 and -164 bp is essential for constitutive *Pim-3* gene expression. This region contains one NF- κ B, two Sp1, and two Ets-1 binding sites. *Pim-1* gene can be induced by CD40-mediated signaling in an NF- κ B-dependent manner ^[34]. However, the mutation in NF- κ B binding site of the *Pim-3* gene failed to reduce promoter activities in human pancreatic cancer cells ^[32]. Further examination has revealed that two Sp1 binding sites and the distal Ets binding site are crucial for the constitutive *Pim-3* gene expression in human pancreatic cancer cells. The crucial roles of Ets-1 in constitutive *Pim-3* gene expression are further supported by our observations that the overexpression of Ets-1 enhances Pim-3 expression, whereas the transfection of dominant negative form of Ets-1

or Ets-1 small interfering RNA (siRNA) reduces Pim-3 expression in human pancreatic cancer cells ^[32]. As the expression of both Ets-1 and Sp1 is enhanced in various types of cancer, including pancreatic cancer ^[35, 36], Ets-1 and Sp1 may act cooperatively to induce constitutive *Pim-3* gene expression as observed with their other target genes ^[37].

3.2. Post-transcriptional regulation of Pim-3 expression

Pim kinase mRNAs have multiple copies of AUUUA sequences in their 3' untranslated regions (UTR), a typical characteristic sequence of mRNA with a short half-life. Moreover, GC-rich sequences are present in the 5' UTR of Pim mRNAs and frequently require cap-dependent translation. Indeed, the overexpression of eukaryotic translation initiation factor 4E (eIF4E) leads to an increase in Pim-1 protein levels, indicating that Pim-1 mRNA is translated in a cap-dependent manner ^[38]. Moreover, eukaryotic translation initiation factor, eIF4E, can bind a stem-loop-pair sequence present in the 3' UTR of Pim-1 mRNA, which allows nuclear export and translation of Pim-1 transcript ^[39]. Since Pim-3 mRNA shows analogous sequences as Pim-1 mRNA, the translation of Pim-3 mRNA can be regulated in a similar way.

Similar to Pim-1, Pim-3 can autophosphorylate some of their serine residues but whether this has any functional significance is yet to be elusive ^[19]. Moreover, Pim-1 and Pim-3 have been shown to bind to the serine/threonine protein phosphatase 2A (PP2A), resulting in their dephosphorylation, ubiquitination, and proteasomal degradation ^[40, 41].

3'UTR of Pim-1 harbors multiple binding sites for miRNAs including miRNA-33 ^[42], miRNA-16 ^[43], miRNA-1 ^[44], miRNA-328 ^[45], and miRNA-210 ^[46]. The miRNAs are generally highly conserved evolutionarily. ^[42] They can bind to the putative target sites present in 3'UTR of *Pim-1* gene and can directly inhibit its expression at the post-transcriptional level, thereby blocking proliferation and growth of cancer cells and smooth muscle cells. The relevant analysis for the structure of human *Pim-3* mRNA indicates that 3' UTR of *Pim-3* gene harbors multiple binding sites for miRNAs (www.ebi.ac.uk; www.microrna.org). It will be interesting to know whether Pim-3 translation can be regulated in a similar manner.

We have identified a translationally controlled tumor protein (TCTP/TPT1) that interacts with Pim-3 by using yeast two-hybrid screening ^[47]. TCTP was aberrantly expressed and co-localized with Pim-3 in human pancreatic cancer cells. Mapping studies have confirmed that the co-localization is due to the interaction between the amino acids in the C-terminal fold of Pim-3 and the amino acids in the N-terminal sequence of TCTP. Pim-3 had no effect on TCTP expression or phosphorylation; however, overexpression of TCTP increased Pim-3 expression in a dose-dependent manner. Moreover, RNAi-mediated ablation of TCTP expression reduced Pim-3 protein but not mRNA via ubiquitin-proteasome degradation pathway. The resultant reduced Pim-3 expression eventually inhibited tumor growth *in vitro* and *in vivo* by arresting cell cycle progression and enhancing apoptosis. Furthermore, TCTP and Pim-3 expression were significantly correlated in pancreatic adenocarcinoma specimens, and in tumors from patients showing high expression levels of TCTP and Pim-3 obtained at an advanced stage of cancer. Thus, TCTP-mediated enhancement of Pim-3 protein expression may be involved in the regulation of cell cycle progression and apoptosis in pancreatic carcinogenesis ^[47].

4. Biological functions of Pim-3

Treatment with Pim-3 shRNA can decrease *in vitro* proliferation of various types of cancer cells by inducing apoptosis ^[5, 13, 29]. The major function of Bad, a pro-apoptotic BH3-only protein, is to regulate apoptosis. Unphosphorylated Bad binds and eventually inactivates anti-apoptotic family members, primarily Bcl-X_L and also Bcl-2. Phosphorylation of Bad at Ser¹¹², Ser¹³⁶, and Ser¹⁵⁵ impairs its binding to Bcl-X_L and Bcl-2 and the translocation of Bad from the surface of mitochondria to the cytosol is guided by the protein 14-3-3. The presence of unbound Bcl-X_L maintains a mitochondrial membrane potential and inhibits apoptosis ^[48, 49]. Pim-1 and Pim-2 can phosphorylate Bad at Ser¹¹², while Akt phosphorylates Ser¹³⁶ and Ser¹⁵⁵. The phosphorylation of Bad can result in its inactivation and subsequent inhibition of apoptosis ^[50, 51]. Elevated levels of Pim-3 increases the amount of Bad phosphorylated at

Ser¹¹² and inhibits apoptosis, while Pim-3 shRNA treatment dephosphorylates Ser¹¹² and promotes apoptosis (Figure 2) [5, 29]. Therefore, similar to Akt and other Pim kinases, Pim-3 can modulate apoptosis by phosphorylating the pro-apoptotic molecule, Bad. Moreover, *Pim-3* gene transduction increased Bcl-2 expression, suppressed apoptosis as evidenced by reduced caspase-3 activation, and eventually protected against hepatic failure in D-galactosamine-sensitized rats, which received lipopolysaccharide [52]. Similarly, the transfection of *Pim-3* gene into cardiomyocytes attenuated ischemia/reperfusion injury-induced cell death through a p38 mediated MAPK signaling pathway [25]. Erythropoietin can protect renal cells from apoptosis by activating Stat5 and this anti-apoptotic effect is also mediated by Pim-3 [53].

Pim-3 shows a high sequence identity with Pim-1 even at their kinase domains (Figure 1). Both Pim-1 and Pim-3 bind to a consensus peptide substrate (AKRRRRHPSGPPTA) with a remarkable high affinity ($Kd = 40\text{--}60$ nM), whereas the binding affinity of this peptide for Pim-2 is relatively weak (640 nM) [19]. Therefore, Pim-1 and Pim-3 can phosphorylate the same or a similar set of substrates, and the evaluation of Pim-1 characteristics and functions can provide useful insights to decipher the major biological functions of Pim-3. In addition to Bad, Pim kinases can phosphorylate a wide range of cellular proteins. These include transcription factors (Stat [54], c-Myc [55], Myb [56], runt-related transcription factor (Runx) 1 and Runx3), cell cycle regulators (p^{21CIP} and p^{27KIP1}, Cdc25A, and Cdc25C), signaling pathway intermediates (suppressor of cytokine signaling 1 (SOCS1) [57], SOCS3 [58], and MAP3K5 [59]), and regulators of protein synthesis (eukaryotic translation initiation factor 4B (eIF4B)) [60].

Pim-1 can phosphorylate Cdc25A, thereby increasing its phosphatase activity and the activity of cyclin D1-associated kinases, which can result in cell cycle progression [61]. Pim-1 phosphorylates Cdc25C-associated kinase 1 (C-TAK1), which can potently inhibit Cdc25C and can promote cell cycle progression at the G₂/M phase [62]. Pim-1 can phosphorylate the threonine residue of p21, another molecule involved in cell cycle progression. Its phosphorylation leads to its relocation to the cytoplasm resulting in enhanced protein stability and eventually increased cell proliferation [63, 64]. All Pim

kinases including Pim-3 can phosphorylate CDK inhibitor, p27 at its threonine residues, thereby inducing the binding of p27 to 14-3-3 protein, resulting in its nuclear export and proteasome-dependent degradation ^[65]. Moreover, Pim-1 phosphorylates and inactivates forkhead transcription factors, FoxO1a and FoxO3a, resulting in depressed *p27* gene transcription, which leads to cell cycle progression (Figure 2) ^[65]. Similarly, the transfection with Pim-3 shRNA reduced G1 population of human pancreatic cancer cells compared with the cells transfected with scramble shRNA ^[51]. Moreover, a small-molecule Pim-3 kinase inhibitor markedly retarded *in vitro* growth of human pancreatic cancer cell lines by inducing G2/M arrest ^[66], suggesting a potential role for Pim-3 in cell cycle progression. Consistently, cell cycle progression is accelerated in hepatocytes of transgenic mice, which express human Pim-3 cDNA selectively in hepatocytes ^[67] and downregulation of Pim-3 decreased the amounts of Cdc25C, cyclin B1, and phospho-p21 (Our unpublished data). Thus, Pim-3 can promote cell cycle progression and eventually contribute to carcinogenesis by modulating the functions of these regulatory molecules involved in cell cycle progression.

Mice deficient in all three Pim kinases are designated as triple knockout (TKO) mice. TKO mice have reduced body size at birth and throughout the postnatal period of their life and they are viable and fertile ^[68]. However, TKO mouse-derived embryonic fibroblasts (MEFs) show depressed AMP-dependent protein kinase (AMPK) activity, grow slowly in culture medium, and have decreased rates of 5'-cap-dependent protein synthesis ^[69]. Transduction of *Pim-3* gene alone into these MEFs can reverse AMPK activation, increase protein synthesis, and drive the growth to a similar level as wild-type MEFs. Moreover, Pim-3 expression can markedly increase the levels of c-Myc and the peroxisome proliferation-activated receptor γ co-activator 1 α (PGC-1 α), enzymes capable of regulating glycolysis and mitochondrial biogenesis ^[69]. Similarly, Pim-1 and Pim-2 phosphorylate serine and threonine residues of c-Myc protein ^[55]. Furthermore, Pim-1 can act as a co-activator of Myc by phosphorylating Ser¹⁰ of histone H3 on the nucleosome at the Myc-binding sites ^[70]. Thus, Pim-3 can augment the rate of protein synthesis by modulating AMPK, c-Myc and PGC-1 α (Figure 2).

Pim-1 and Pim-3 together play a significant role in maintaining the self-renewal capacity of mouse embryonic stem (ES) cells *in vitro* [33]. ES cells overexpressing Pim-1 and Pim-3 have a greater capacity to self-renew and display a greater resistance to LIF deprivation as evidenced by a clonal assay. On the contrary, ablation of Pim-1 and Pim-3 genes increases the rate of spontaneous differentiation in a self-renewal assay and impairs the growth of undifferentiated ES cell colonies with increased rate of apoptosis [33].

Pim-3 is highly expressed at the cellular lamellipodia in endothelial cells and is co-localized with focal adhesion kinase (FAK). In addition, Pim-3 shRNA treatment impairs endothelial cell spreading, migration, and proliferation, leading to a reduction in tube-like structure development in a Matrigel assay [23]. However, TKO mice did not display any apparent abnormal phenotypes in embryogenesis and vascular development [68].

Pim-3 expression is detected in the β cells located in the pancreatic islets [71]. Pim-3-deficient mice exhibit an increased glucose tolerance and insulin sensitivity. Moreover, Pim-3 can negatively regulate insulin secretion by inhibiting the activation of Erk1/2 via SOCS6 [71]. In contrast, the inhibition of another survival kinase, Akt, can induce hyperglycemia [72, 73].

The switch from latent phase to productive viral reactivation (lytic phase) is crucial for sustaining viral multiplication in infected host cells. Findings from recent clinico-epidemiological study indicated the importance of lytic reactivation in the development and progression of Kaposi's sarcoma (KS) [74]. Latency-associated nuclear antigen (LANA) is presumed to be a novel regulator of the life cycle of γ herpes virus including Kaposi's sarcoma herpes virus (KSHV). Pim-1 and Pim-3 contribute to the viral reactivation of KSHV by phosphorylating LANA, and thereby promote KS progression [74].

5. Roles of Pim-3 in cancer development and progression, particularly in pancreas

Pim-3 can contribute to cancer development and progression by acting on tumor

cells and tumor microenvironments. The primary activities of Pim-3 on tumor cells include the delivery of survival signaling, the regulation of cell cycle progression, protein synthesis, and Myc activation (Figure 3). In addition to its effects on tumor cells, Pim-3 can have profound impacts on tumor microenvironments, especially neovascularization process (Figure 3). In the following sections, we will discuss the roles of Pim-3 in carcinogenesis, with a focus on these two aspects.

5.1. Effects of Pim-3 on tumor cells

Forced expression of Pim-3 can promote anchorage-independent growth whereas co-expression of a kinase-dead Pim-3 mutant can attenuate EWS/FLI-mediated NIH 3T3 tumorigenesis in immunodeficient mice ^[27]. These observations suggest the involvement of Pim-3 in cancer development and progression.

Pim-3 can prevent apoptosis in pancreatic cancer cells by phosphorylating Bad a pro-apoptotic molecule on the serine residues (Ser¹¹², Ser¹³⁶, or Ser¹⁵⁵), which in turn prevents Bcl-X_L binding and promotes Bad translocation from the surface of the mitochondria to the cytosol by the protein 14-3-3 ^[48, 49]. Among the serine residues present in Bad, Ser¹¹², but not Ser¹³⁶ and Ser¹⁵⁵, is abundantly phosphorylated in human pancreatic cancer cell lines. Moreover, the ablation of endogenous Pim-3 reduces the population of phosphorylated Bad followed by an enhancement of apoptosis, whereas Pim-3 overexpression produces exactly the opposite phenotypes. These observations suggest that Pim-3 has a crucial role in preventing apoptosis of human pancreatic cancer cells.

Cell survival can be regulated by Wnt/ β -catenin and Stat3 signaling pathways. An integrative molecular screening by using siRNA identified Pim-3 as a new regulator of Wnt/ β -catenin signaling^[75]. Thus, Pim-3 can positively regulate the Wnt/ β -catenin signaling pathway in the colorectal cancer cell lines (DLD-1 and SW480) ^[75]. Moreover, Pim-3 is a positive regulator of Stat3 signaling in the prostate cancer cell line (DU-145) and in the pancreatic cancer derived cell line (MiaPaCa2) ^[56]. Thus, Pim-3 can promote cancer cell survival by modulating Wnt/ β -catenin and/or Stat3 signaling pathways.

Pim-1 can promote cell cycle progression by phosphorylating and modulating the functions of molecules involved in cell cycle progression. Moreover, Pim kinases positively regulate transcription factors controlling the expression of genes implicated in cell cycle progression ^[65]. Since Pim-3 shares a high sequence identity with Pim-1, it is possible that Pim-3 can perform similar regulatory functions as Pim-1. Treatment with Pim-3 shRNA showed a marked reduction in G1 population of human pancreatic cancer cells while scramble shRNA had few effects ^[5]. Furthermore, a small-molecule Pim-3 kinase inhibitor markedly retarded the *in vitro* growth of human pancreatic cancer cell lines by inducing G2/M arrest ^[66]. These findings indicate that Pim-3 may have a major influence in cell cycle progression of cancer cells.

Pim-1 and Pim-2 help in cell survival by suppressing myc-induced apoptosis ^[10, 11]. Transgenic mice expressing *Eμ*(immunoglobulin heavy-chain enhancer)-*Pim-1* and *Eμ-Myc* succumb to lymphoma in utero or around birth ^[76]. On the contrary, *Eμ-Myc* transgenic mice that are deficient in *Pim-1* and *Pim-2* genes develop lymphoma slowly with time ^[8]. Thus, Myc-driven tumorigenesis depends on physiological levels of Pim-1 and Pim-2 expression. Several mechanisms have been proposed to explain the cooperation between Myc and Pim kinases. Myc recruits Pim-1 to the E-boxes of the Myc target genes such as Fos-related antigen 1 (*FOSL1* (*Fra-1*)) and DNA-binding protein inhibitor *ID2*, and Pim-1 phosphorylates Ser¹⁰ of histone H3 on the nucleosome at the Myc-binding sites thereby, acting as a co-activator of Myc ^[70]. An expression profile analysis demonstrated that about 20 % of the Myc-regulated genes are also under the control of Pim-1 ^[70]. Moreover, Pim-1 and Pim-2 phosphorylate c-Myc protein at its serine and threonine residues ^[55]. This results in stabilization and subsequent enhancement of the transcription activities of c-Myc protein. Furthermore, Pim-3 can enhance c-Myc mRNA expression through the activation of PGC-1 α ^[69]. The enhanced expression of c-Myc and PGC-1 α may account for enhanced glycolysis. Thus, Pim kinases can promote tumorigenesis by modulating the activities of c-Myc and promoting Warburg effects ^[10, 11].

5.2 Roles of Pim-3 in tumor microenvironments

One of the basic characteristic features of tumor tissues is the abundance of newly formed vasculature for supply of nutrients and oxygen to the growing tumor cells and elimination of metabolic wastes and carbon dioxide. Pim-3 is abundantly expressed at mRNA and protein levels at the cellular lamellipodia and is co-localized with FAK in endothelial cells ^[23]. Pim-3 shRNA treatment impaired endothelial cell spreading, migration, and proliferation, leading to a reduction in tube-like structure formation in a Matrigel assay ^[23]. Moreover, tumor necrosis factor (TNF)- α transiently increases Pim-3 mRNA expression through TNF receptor-1 (TNFR1) pathway in endothelial cells (ECs) and eventually promotes EC spreading and migration ^[77]. Constitutive Pim-3 overexpression in gastric cancer tissues can induce angiogenesis ^[30].

Tumor-associated neovasculature formation is regulated by various angiogenic factors. Notably, vascular endothelial growth factor (VEGF) has an important role in tumor-associated vasculature formation ^[78, 79]. Although most pancreatic cancer tissues are hypovascular, elevated levels of VEGF are sometimes detected in pancreatic cancer cells^[80]. Earlier studies have demonstrated that Pim-3 overexpression was responsible for increased VEGF expression and the growth of pancreatic cancer *in vivo* in an orthotopic nude mouse model ^[81]. The lack of any vascular phenotypes in Pim-3-deficient mice indicates that Pim-3 is dispensable for normal vasculature formation. However, given distinctive gene expression profiles of tumor-associated endothelial cells (ECs) ^[82], Pim-3 may have distinct roles in the tumor-associated endothelial cells.

6. Pharmacological characterization of Pim-3 Inhibitors

It is obvious from our discussions that aberrant activation and expression of Pim kinases are associated with various types of cancer. Enhanced expression of Pim-2 kinase is detected in hematologic malignancies and prostate cancer. Additionally increased Pim-1 expression is observed in pancreatic cancer, squamous cell carcinoma, gastric cancer, colorectal cancer, hepatocellular carcinoma ^[83-85], bladder carcinoma ^[86], and liposarcoma ^[87]. In contrast, Pim-3 expression is selectively overexpressed in malignant

lesions of endoderm-derived organs such as the liver ^[13], pancreas ^[5], colon ^[29], and stomach ^[30]. Furthermore, lack of apparent phenotypes in TKO mice suggests that Pim kinases are dispensable for the maintenance of normal functions of vital organs. Collectively, Pim kinases can be good candidate molecules for targeted cancer therapy. Examples of Pim-1 inhibitors include an anti-Pim-1 antibody and a cell penetrating peptide, both of which suppresses tumor growth *in vivo* in xenograft mouse models transplanted with human cancer cell lines ^[88, 89].

The crystal structure of Pim-3 has not yet been reported. However, the crystal structure of Pim-1 and Pim-2 has been resolved and revealed the presence of a unique hinge region that connects the two lobes of the protein kinase domain ^[18-20]. As a result, ATP binds to Pim kinases in a fundamentally different way from how it binds to other protein kinases ^[18, 19]. Thus, it may be possible to design compounds, which will selectively inhibit Pim kinases but no other serine/threonine kinases ^[16].

Several independent research groups have developed small-molecule inhibitors against Pim kinases including flavonol quercetargetin ^[90], imidazole[1,2-b]pyridazines ^[91, 92], bezylindene-thiazolidine-2,4-dione ^[93-95], 3,5-disubstituted indole derivatives ^[96], pyrazolo[3,4-g]quinoxaline derivatives ^[97], 1,6-dihydropyrazolo[4,3-c]carbazoles and 3,6-dihydropyrazolo[3,4-c]carbazole derivatives ^[98], and pyrrolo[2,3-a]carbazole and pyrrolo[2,3-g]indazole derivatives ^[99-101]. Among them, 1,6-dihydropyrazolo[4,3-c]carbazoles, 3,6-dihydropyrazolo[3,4-c]carbazoles, and pyrrolo[2,3-g]indazoles can inhibit Pim-3 activities ^[98, 100]. In our previous studies we have demonstrated that derivatives of stemonamide synthetic intermediates can inhibit Pim-3 as well as Pim-1 and Pim-2 activities and can reduce tumor growth *in vivo* in mouse xenograft models using human pancreatic cancer cell line without causing major adverse side-effects ^[102, 103].

The substrates preferred by Pim-1 and Pim-3 ^[19] are very similar in identity. Therefore, designing of isoform specific inhibitors that will differentiate and preferentially bind to one Pim member over the other is extremely challenging. Indeed, pyrrolo[2,3-a]carbazole has low nano molar binding affinity for Pim-1 and Pim-3 kinases

but only weakly inhibits Pim-2 (IC_{50} for Pim-1, $0.57 \pm 0.04 \mu\text{M}$; IC_{50} for Pim-2, $> 10 \mu\text{M}$; IC_{50} for Pim-3, $0.04 \pm 0.01 \mu\text{M}$)^[104]. Similar pharmacological observations have been recorded with phenanthrene derivatives^[77]. However, it will be interesting to find out if an inhibitor which specifically inhibits the action of one Pim member will provide any additional advantage over a multi-Pim kinase inhibitor.

Akt, similar to Pim kinases can phosphorylate a similar set of substrates, such as Bad, thereby initiating the proliferation of cancer cells^[105]. Akt is aberrantly activated in various types of tumors and Akt inhibitors have been extensively investigated^[72]. An Akt inhibitor, “GSK690693,” has exhibited potent antitumor activity in pre-clinical trials on animals^[105]. Akt is a key signaling protein and Akt-2 is directly involved in insulin receptor signaling pathway. Consequently, the genetic disruption of Akt kinase genes results in severe phenotypic changes, such as neonatal mortality, severe growth retardation and reduced brain size^[106-108] and Akt-2 inhibition induces severe hyperglycemia^[105]. The use of Akt inhibitors for anticancer treatment is seriously limited because of these shortcomings. In contrast, Pim kinases including Pim-3 are not involved in insulin receptor signaling pathway, and the inhibition of Pim kinases hardly shows any detrimental effects on normal glucose metabolism. Thus, Pim kinases are more effective molecular targets than Akt for targeted cancer therapy, and are particularly useful for treating pancreatic cancer which gets frequently complicated by hyperglycemia.

7. Future Perspectives

Pim-3 kinase is aberrantly expressed in malignant lesions but not in normal tissues of endoderm-derived organs such as liver, pancreas, colon, and stomach^[5, 13, 29, 30], and contributes to tumorigenesis by inhibiting apoptosis of tumor cells and promoting cell cycle progression. Moreover, genetic deficiency of *Pim-3* gene does not result in apparent changes in phenotypes, suggesting that Pim-3 may be physiologically dispensable. Unlike Akt kinases^[72], Pim kinases are not involved in insulin receptor signaling pathway; therefore, the inhibition of Pim kinases has very little influence on

glucose metabolism. Indeed, inhibition of Pim-3 kinase activities slows the growth or even causes regression of pancreatic tumors in mice without causing hypoglycemia [66, 102, 103]. Since Pim-3 kinase is constitutively active, once it is expressed aberrantly, inhibition of Pim-3 can be used for inhibiting cancer progression. Furthermore, there is accumulating evidence to suggest that Pim-3 plays a vital role in the interaction between tumor cells and their surrounding stroma. Further studies on these aspects will unravel the novel pathophysiological roles of Pim-3. Nevertheless, strategies to inhibit Pim-3 activity warrant an intensive investigation for the discovery, and development of new targeted anti-cancer therapeutics.

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References

- 1 **Siegel R**, Naishadham D, Jemal A. Cancer statistics, 2012. *CA Cancer J Clin* 2012; **62**(1): 10-29. [PMID: 22237781 DOI: 10.3322/caac.20138]
- 2 **Li D**, Xie K, Wolff R, Abbruzzese JL. Pancreatic cancer. *Lancet* 2004; **363**(9414): 1049-1057. [PMID: 15051286 DOI: 10.1016/S0140-6736(04)15841-8]
- 3 **Jemal A**, Siegel R, Xu J, Ward E. Cancer statistics, 2010. *CA Cancer J Clin* 2010; **60**(5): 277-300. [PMID: 20610543 DOI: 10.3322/caac.20073]
- 4 **Vincent A**, Herman J, Schulick R, Hruban RH, Goggins M. Pancreatic cancer. *Lancet* 2011; **378**(9791): 607-620. [PMID: 21620466 DOI: 10.1016/S0140-6736(10)62307-0]
- 5 **Li YY**, Popivanova BK, Nagai Y, Ishikura H, Fujii C, Mukaida N. Pim-3, a proto-oncogene with serine/threonine kinase activity, is aberrantly expressed in human pancreatic cancer and phosphorylates bad to block bad-mediated apoptosis in human pancreatic cancer cell lines. *Cancer Res* 2006; **66**(13): 6741-6747. [PMID: 16818649 DOI: 10.1158/0008-5472.CAN-05-4272]
- 6 **Xu D**, Cobb MG, Gavilano L, Witherspoon SM, Williams D, White CD, Taverna P, Bednarski BK, Kim HJ, Baldwin AS, Baines AT. Inhibition of oncogenic Pim-3 kinase modulates transformed growth and chemosensitizes pancreatic cancer cells to gemcitabine. *Cancer Biol Ther* 2013; **14**(6): 492-501. [PMID: 23760491 DOI: 10.4161/cbt.24343]
- 7 **Konietzko U**, Kauselmann G, Scafidi J, Staubli U, Mikkers H, Berns A, Schweizer M, Waltereit R, Kuhl D. Pim kinase expression is induced by LTP stimulation and required for the consolidation of enduring LTP. *Embo J* 1999; **18**(12): 3359-3369. [PMID: 10369676 DOI: 10.1093/emboj/18.12.3359]
- 8 **Mikkers H**, Allen J, Knipscheer P, Romeijn L, Hart A, Vink E, Berns A. High-throughput retroviral tagging to identify components of specific signaling pathways in cancer. *Nat Genet* 2002; **32**(1): 153-159. [PMID: 12185366 DOI: 10.1038/ng950]
- 9 **Cuypers HT**, Selten G, Quint W, Zijlstra M, Maandag ER, Boelens W, van

- Wezenbeek P, Melief C, Berns A. Murine leukemia virus-induced T-cell lymphomagenesis: integration of proviruses in a distinct chromosomal region. *Cell* 1984; **37**(1): 141-150. [PMID: 6327049]
- 10 **van Lohuizen M**, Verbeek S, Krimpenfort P, Domen J, Saris C, Radaszkiewicz T, Berns A. Predisposition to lymphomagenesis in pim-1 transgenic mice: cooperation with c-myc and N-myc in murine leukemia virus-induced tumors. *Cell* 1989; **56**(4): 673-682. [PMID: 2537153]
- 11 **Breuer ML**, Cuypers HT, Berns A. Evidence for the involvement of pim-2, a new common proviral insertion site, in progression of lymphomas. *Embo J* 1989; **8**(3): 743-748. [PMID: 2721500]
- 12 **Feldman JD**, Vician L, Crispino M, Tocco G, Marcheselli VL, Bazan NG, Baudry M, Herschman HR. KID-1, a protein kinase induced by depolarization in brain. *J Biol Chem* 1998; **273**(26): 16535-16543. [PMID: 9632723]
- 13 **Fujii C**, Nakamoto Y, Lu P, Tsuneyama K, Popivanova BK, Kaneko S, Mukaida N. Aberrant expression of serine/threonine kinase Pim-3 in hepatocellular carcinoma development and its role in the proliferation of human hepatoma cell lines. *Int J Cancer* 2005; **114**(2): 209-218. [PMID: 15540201 DOI: 10.1002/ijc.20719]
- 14 **Mukaida N**, Wang YY, Li YY. Roles of Pim-3, a novel survival kinase, in tumorigenesis. *Cancer Sci* 2011; **102**(8): 1437-1442. [PMID: 21518143 DOI: 10.1111/j.1349-7006.2011.01966.x]
- 15 **Nawijn MC**, Alendar A, Berns A. For better or for worse: the role of Pim oncogenes in tumorigenesis. *Nat Rev Cancer* 2011; **11**(1): 23-34. [PMID: 21150935 DOI: 10.1038/nrc2986]
- 16 **Brault L**, Gasser C, Bracher F, Huber K, Knapp S, Schwaller J. PIM serine/threonine kinases in the pathogenesis and therapy of hematologic malignancies and solid cancers. *Haematologica* 2010; **95**(6): 1004-1015. [PMID: 20145274 DOI: 10.3324/haematol.2009.017079]
- 17 **Blanco-Aparicio C**, Carnero A. Pim kinases in cancer: diagnostic, prognostic and treatment opportunities. *Biochem Pharmacol* 2013; **85**(5): 629-643. [PMID: 23041228]

- DOI: 10.1016/j.bcp.2012.09.018]
- 18 **Qian KC**, Wang L, Hickey ER, Studts J, Barringer K, Peng C, Kronkaitis A, Li J, White A, Mische S, Farmer B. Structural basis of constitutive activity and a unique nucleotide binding mode of human Pim-1 kinase. *J Biol Chem* 2005; **280**(7): 6130-6137. [PMID: 15525646 DOI: 10.1074/jbc.M409123200]
 - 19 **Bullock AN**, Debreczeni J, Amos AL, Knapp S, Turk BE. Structure and substrate specificity of the Pim-1 kinase. *J Biol Chem* 2005; **280**(50): 41675-41682. [PMID: 16227208 DOI: 10.1074/jbc.M510711200]
 - 20 **Bullock AN**, Russo S, Amos A, Pagano N, Bregman H, Debreczeni JE, Lee WH, von Delft F, Meggers E, Knapp S. Crystal structure of the PIM2 kinase in complex with an organoruthenium inhibitor. *PLoS One* 2009; **4**(10): e7112. [PMID: 19841674 DOI: 10.1371/journal.pone.0007112]
 - 21 **Kumar A**, Mandiyan V, Suzuki Y, Zhang C, Rice J, Tsai J, Artis DR, Ibrahim P, Bremer R. Crystal structures of proto-oncogene kinase Pim1: a target of aberrant somatic hypermutations in diffuse large cell lymphoma. *J Mol Biol* 2005; **348**(1): 183-193. [PMID: 15808862 DOI: 10.1016/j.jmb.2005.02.039]
 - 22 **Jacobs MD**, Black J, Futer O, Swenson L, Hare B, Fleming M, Saxena K. Pim-1 ligand-bound structures reveal the mechanism of serine/threonine kinase inhibition by LY294002. *J Biol Chem* 2005; **280**(14): 13728-13734. [PMID: 15657054 DOI: 10.1074/jbc.M413155200]
 - 23 **Zhang P**, Wang H, Min X, Wang Y, Tang J, Cheng J, Li D, Chen X, Cheng F, Wang N, Yang H. Pim-3 is expressed in endothelial cells and promotes vascular tube formation. *J Cell Physiol* 2009; **220**(1): 82-90. [PMID: 19229879 DOI: 10.1002/jcp.21733]
 - 24 **Lu A**, Tang Y, Ran R, Clark JF, Aronow BJ, Sharp FR. Genomics of the periinfarction cortex after focal cerebral ischemia. *J Cereb Blood Flow Metab* 2003; **23**(7): 786-810. [PMID: 12843783 DOI: 10.1097/01.WCB.0000062340.80057.06]
 - 25 **Liu D**, He M, Yi B, Guo WH, Que AL, Zhang JX. Pim-3 protects against cardiomyocyte apoptosis in anoxia/reoxygenation injury via p38-mediated signal

- pathway. *Int J Biochem Cell Biol* 2009; **41**(11): 2315-2322. [PMID: 19505587 DOI: 10.1016/j.biocel.2009.05.021]
- 26 **Eichmann A**, Yuan L, Breant C, Alitalo K, Koskinen PJ. Developmental expression of pim kinases suggests functions also outside of the hematopoietic system. *Oncogene* 2000; **19**(9): 1215-1224. [PMID: 10713710 DOI: 10.1038/sj.onc.1203355]
- 27 **Deneen B**, Welford SM, Ho T, Hernandez F, Kurland I, Denny CT. PIM3 proto-oncogene kinase is a common transcriptional target of divergent EWS/ETS oncoproteins. *Mol Cell Biol* 2003; **23**(11): 3897-3908. [PMID: 12748291]
- 28 **Yang XY**, Ren CP, Wang L, Li H, Jiang CJ, Zhang HB, Zhao M, Yao KT. Identification of differentially expressed genes in metastatic and non-metastatic nasopharyngeal carcinoma cells by suppression subtractive hybridization. *Cell Oncol* 2005; **27**(4): 215-223. [PMID: 16308470]
- 29 **Popivanova BK**, Li YY, Zheng H, Omura K, Fujii C, Tsuneyama K, Mukaida N. Proto-oncogene, Pim-3 with serine/threonine kinase activity, is aberrantly expressed in human colon cancer cells and can prevent Bad-mediated apoptosis. *Cancer Sci* 2007; **98**(3): 321-328. [PMID: 17270021 DOI: 10.1111/j.1349-7006.2007.00390.x]
- 30 **Zheng HC**, Tsuneyama K, Takahashi H, Miwa S, Sugiyama T, Popivanova BK, Fujii C, Nomoto K, Mukaida N, Takano Y. Aberrant Pim-3 expression is involved in gastric adenoma-adenocarcinoma sequence and cancer progression. *J Cancer Res Clin Oncol* 2008; **134**(4): 481-488. [PMID: 17876606 DOI: 10.1007/s00432-007-0310-1]
- 31 **Arvand A**, Denny CT. Biology of EWS/ETS fusions in Ewing's family tumors. *Oncogene* 2001; **20**(40): 5747-5754. [PMID: 11607824 DOI: 10.1038/sj.onc.1204598]
- 32 **Li YY**, Wu Y, Tsuneyama K, Baba T, Mukaida N. Essential contribution of Ets-1 to constitutive Pim-3 expression in human pancreatic cancer cells. *Cancer Sci* 2009; **100**(3): 396-404. [PMID: 19154409 DOI: 10.1111/j.1349-7006.2008.01059.x]
- 33 **Aksoy I**, Sakabedoyan C, Bourillot PY, Malashicheva AB, Mancip J, Knoblauch K, Afanassieff M, Savatier P. Self-renewal of murine embryonic stem cells is supported by the serine/threonine kinases Pim-1 and Pim-3. *Stem Cells* 2007; **25**(12):

- 2996-3004. [PMID: 17717068 DOI: 10.1634/stemcells.2007-0066]
- 34 **Zhu N**, Ramirez LM, Lee RL, Magnuson NS, Bishop GA, Gold MR. CD40 signaling in B cells regulates the expression of the Pim-1 kinase via the NF-kappa B pathway. *J Immunol* 2002; **168**(2): 744-754. [PMID: 11777968]
- 35 **Ito H**, Duxbury M, Benoit E, Clancy TE, Zinner MJ, Ashley SW, Whang EE. Prostaglandin E2 enhances pancreatic cancer invasiveness through an Ets-1-dependent induction of matrix metalloproteinase-2. *Cancer Res* 2004; **64**(20): 7439-7446. [PMID: 15492268 DOI: 10.1158/0008-5472.CAN-04-1177]
- 36 **Safe S**, Abdelrahim M. Sp transcription factor family and its role in cancer. *Eur J Cancer* 2005; **41**(16): 2438-2448. [PMID: 16209919 DOI: 10.1016/j.ejca.2005.08.006]
- 37 **Sharrocks AD**. The ETS-domain transcription factor family. *Nat Rev Mol Cell Biol* 2001; **2**(11): 827-837. [PMID: 11715049 DOI: 10.1038/35099076]
- 38 **Hoover DS**, Wingett DG, Zhang J, Reeves R, Magnuson NS. Pim-1 protein expression is regulated by its 5'-untranslated region and translation initiation factor eIF-4E. *Cell Growth Differ* 1997; **8**(12): 1371-1380. [PMID: 9419425]
- 39 **Culjkovic B**, Topisirovic I, Skrabanek L, Ruiz-Gutierrez M, Borden KL. eIF4E is a central node of an RNA regulon that governs cellular proliferation. *J Cell Biol* 2006; **175**(3): 415-426. [PMID: 17074885 DOI: 10.1083/jcb.200607020]
- 40 **Ma J**, Arnold HK, Lilly MB, Sears RC, Kraft AS. Negative regulation of Pim-1 protein kinase levels by the B56beta subunit of PP2A. *Oncogene* 2007; **26**(35): 5145-5153. [PMID: 17297438 DOI: 10.1038/sj.onc.1210323]
- 41 **Losman JA**, Chen XP, Vuong BQ, Fay S, Rothman PB. Protein phosphatase 2A regulates the stability of Pim protein kinases. *J Biol Chem* 2003; **278**(7): 4800-4805. [PMID: 12473674 DOI: 10.1074/jbc.M208246200]
- 42 **Thomas M**, Lange-Grunweller K, Weirauch U, Gutsch D, Aigner A, Grunweller A, Hartmann RK. The proto-oncogene Pim-1 is a target of miR-33a. *Oncogene* 2012; **31**(7): 918-928. [PMID: 21743487 DOI: 10.1038/onc.2011.278]
- 43 **Kim KT**, Carroll AP, Mashkani B, Cairns MJ, Small D, Scott RJ. MicroRNA-16 is down-regulated in mutated FLT3 expressing murine myeloid FDC-P1 cells and

- interacts with Pim-1. *PLoS One* 2012; **7**(9): e44546. [PMID: 22970245 DOI: 10.1371/journal.pone.0044546]
- 44 **Katare R**, Caporali A, Zentilin L, Avolio E, Sala-Newby G, Oikawa A, Cesselli D, Beltrami AP, Giacca M, Emanuelli C, Madeddu P. Intravenous gene therapy with PIM-1 via a cardiotropic viral vector halts the progression of diabetic cardiomyopathy through promotion of prosurvival signaling. *Circ Res* 2011; **108**(10): 1238-1251. [PMID: 21474815 DOI: 10.1161/CIRCRESAHA.110.239111]
- 45 **Eiring AM**, Harb JG, Neviani P, Garton C, Oaks JJ, Spizzo R, Liu S, Schwind S, Santhanam R, Hickey CJ, Becker H, Chandler JC, Andino R, Cortes J, Hokland P, Huettner CS, Bhatia R, Roy DC, Liebhaber SA, Caligiuri MA, Marcucci G, Garzon R, Croce CM, Calin GA, Perrotti D. miR-328 functions as an RNA decoy to modulate hnRNP E2 regulation of mRNA translation in leukemic blasts. *Cell* 2010; **140**(5): 652-665. [PMID: 20211135 DOI: 10.1016/j.cell.2010.01.007]
- 46 **Huang X**, Ding L, Bennewith KL, Tong RT, Welford SM, Ang KK, Story M, Le QT, Giaccia AJ. Hypoxia-inducible mir-210 regulates normoxic gene expression involved in tumor initiation. *Mol Cell* 2009; **35**(6): 856-867. [PMID: 19782034 DOI: 10.1016/j.molcel.2009.09.006]
- 47 **Zhang F**, Liu B, Wang Z, Yu XJ, Ni QX, Yang WT, Mukaida N, Li YY. A novel regulatory mechanism of pim-3 kinase stability and its involvement in pancreatic cancer progression. *Mol Cancer Res* 2013; **11**(12): 1508-1520. [PMID: 24165482 DOI: 10.1158/1541-7786.MCR-13-0389]
- 48 **Yang E**, Zha J, Jockel J, Boise LH, Thompson CB, Korsmeyer SJ. Bad, a heterodimeric partner for Bcl-XL and Bcl-2, displaces Bax and promotes cell death. *Cell* 1995; **80**(2): 285-291. [PMID: 7834748]
- 49 **Zha J**, Harada H, Yang E, Jockel J, Korsmeyer SJ. Serine phosphorylation of death agonist BAD in response to survival factor results in binding to 14-3-3 not BCL-X(L). *Cell* 1996; **87**(4): 619-628. [PMID: 8929531]
- 50 **Aho TL**, Sandholm J, Peltola KJ, Mankonen HP, Lilly M, Koskinen PJ. Pim-1 kinase promotes inactivation of the pro-apoptotic Bad protein by phosphorylating it on the

- Ser112 gatekeeper site. *Febs Lett* 2004; **571**(1-3): 43-49. [PMID: 15280015 DOI: 10.1016/j.febslet.2004.06.050]
- 51 **Yan B**, Zemskova M, Holder S, Chin V, Kraft A, Koskinen PJ, Lilly M. The PIM-2 kinase phosphorylates BAD on serine 112 and reverses BAD-induced cell death. *J Biol Chem* 2003; **278**(46): 45358-45367. [PMID: 12954615 DOI: 10.1074/jbc.M307933200]
- 52 **Liu LM**, Zhang JX, Wang XP, Guo HX, Deng H, Luo J. Pim-3 protects against hepatic failure in D-galactosamine (D-GalN)-sensitized rats. *Eur J Clin Invest* 2010; **40**(2): 127-138. [PMID: 20039932 DOI: 10.1111/j.1365-2362.2009.02235.x]
- 53 **Breggia AC**, Wojchowski DM, Himmelfarb J. JAK2/Y343/STAT5 signaling axis is required for erythropoietin-mediated protection against ischemic injury in primary renal tubular epithelial cells. *Am J Physiol Renal Physiol* 2008; **295**(6): F1689-F1695. [PMID: 18815218 DOI: 10.1152/ajprenal.90333.2008]
- 54 **Chang M**, Kanwar N, Feng E, Siu A, Liu X, Ma D, Jongstra J. PIM kinase inhibitors downregulate STAT3(Tyr705) phosphorylation. *Mol Cancer Ther* 2010; **9**(9): 2478-2487. [PMID: 20667852 DOI: 10.1158/1535-7163.MCT-10-0321]
- 55 **Zhang Y**, Wang Z, Li X, Magnuson NS. Pim kinase-dependent inhibition of c-Myc degradation. *Oncogene* 2008; **27**(35): 4809-4819. [PMID: 18438430 DOI: 10.1038/onc.2008.123]
- 56 **Winn LM**, Lei W, Ness SA. Pim-1 phosphorylates the DNA binding domain of c-Myb. *Cell Cycle* 2003; **2**(3): 258-262. [PMID: 12734436]
- 57 **Chen XP**, Losman JA, Cowan S, Donahue E, Fay S, Vuong BQ, Nawijn MC, Capece D, Cohan VL, Rothman P. Pim serine/threonine kinases regulate the stability of Socs-1 protein. *Proc Natl Acad Sci U S A* 2002; **99**(4): 2175-2180. [PMID: 11854514 DOI: 10.1073/pnas.042035699]
- 58 **Peltola KJ**, Pauku K, Aho TL, Ruuska M, Silvennoinen O, Koskinen PJ. Pim-1 kinase inhibits STAT5-dependent transcription via its interactions with SOCS1 and SOCS3. *Blood* 2004; **103**(10): 3744-3750. [PMID: 14764533 DOI: 10.1182/blood-2003-09-3126]

- 59 **Gu JJ**, Wang Z, Reeves R, Magnuson NS. PIM1 phosphorylates and negatively regulates ASK1-mediated apoptosis. *Oncogene* 2009; **28**(48): 4261-4271. [PMID: 19749799 DOI: 10.1038/onc.2009.276]
- 60 **Peng C**, Knebel A, Morrice NA, Li X, Barringer K, Li J, Jakes S, Werneburg B, Wang L. Pim kinase substrate identification and specificity. *J Biochem* 2007; **141**(3): 353-362. [PMID: 17234686 DOI: 10.1093/jb/mvm040]
- 61 **Mochizuki T**, Kitanaka C, Noguchi K, Muramatsu T, Asai A, Kuchino Y. Physical and functional interactions between Pim-1 kinase and Cdc25A phosphatase. Implications for the Pim-1-mediated activation of the c-Myc signaling pathway. *J Biol Chem* 1999; **274**(26): 18659-18666. [PMID: 10373478]
- 62 **Bachmann M**, Hennemann H, Xing PX, Hoffmann I, Moroy T. The oncogenic serine/threonine kinase Pim-1 phosphorylates and inhibits the activity of Cdc25C-associated kinase 1 (C-TAK1): a novel role for Pim-1 at the G2/M cell cycle checkpoint. *J Biol Chem* 2004; **279**(46): 48319-48328. [PMID: 15319445 DOI: 10.1074/jbc.M404440200]
- 63 **Wang Z**, Bhattacharya N, Mixter PF, Wei W, Sedivy J, Magnuson NS. Phosphorylation of the cell cycle inhibitor p21Cip1/WAF1 by Pim-1 kinase. *Biochim Biophys Acta* 2002; **1593**(1): 45-55. [PMID: 12431783]
- 64 **Zhang Y**, Wang Z, Magnuson NS. Pim-1 kinase-dependent phosphorylation of p21Cip1/WAF1 regulates its stability and cellular localization in H1299 cells. *Mol Cancer Res* 2007; **5**(9): 909-922. [PMID: 17855660 DOI: 10.1158/1541-7786.MCR-06-0388]
- 65 **Morishita D**, Katayama R, Sekimizu K, Tsuruo T, Fujita N. Pim kinases promote cell cycle progression by phosphorylating and down-regulating p27Kip1 at the transcriptional and posttranscriptional levels. *Cancer Res* 2008; **68**(13): 5076-5085. [PMID: 18593906 DOI: 10.1158/0008-5472.CAN-08-0634]
- 66 **Wang YY**, Taniguchi T, Baba T, Li YY, Ishibashi H, Mukaida N. Identification of a phenanthrene derivative as a potent anticancer drug with Pim kinase inhibitory activity. *Cancer Sci* 2012; **103**(1): 107-115. [PMID: 21981263 DOI:

- 10.1111/j.1349-7006.2011.02117.x]
- 67 **Wu Y**, Wang YY, Nakamoto Y, Li YY, Baba T, Kaneko S, Fujii C, Mukaida N. Accelerated hepatocellular carcinoma development in mice expressing the Pim-3 transgene selectively in the liver. *Oncogene* 2010; **29**(15): 2228-2237. [PMID: 20101231 DOI: 10.1038/onc.2009.504]
- 68 **Mikkers H**, Nawijn M, Allen J, Brouwers C, Verhoeven E, Jonkers J, Berns A. Mice deficient for all PIM kinases display reduced body size and impaired responses to hematopoietic growth factors. *Mol Cell Biol* 2004; **24**(13): 6104-6115. [PMID: 15199164 DOI: 10.1128/MCB.24.13.6104-6115.2004]
- 69 **Beharry Z**, Mahajan S, Zemskova M, Lin YW, Tholanikunnel BG, Xia Z, Smith CD, Kraft AS. The Pim protein kinases regulate energy metabolism and cell growth. *Proc Natl Acad Sci U S A* 2011; **108**(2): 528-533. [PMID: 21187426 DOI: 10.1073/pnas.1013214108]
- 70 **Zippo A**, De Robertis A, Serafini R, Oliviero S. PIM1-dependent phosphorylation of histone H3 at serine 10 is required for MYC-dependent transcriptional activation and oncogenic transformation. *Nat Cell Biol* 2007; **9**(8): 932-944. [PMID: 17643117 DOI: 10.1038/ncb1618]
- 71 **Vlacich G**, Nawijn MC, Webb GC, Steiner DF. Pim3 negatively regulates glucose-stimulated insulin secretion. *Islets* 2010; **2**(5): 308-317. [PMID: 21099329]
- 72 **Amaravadi R**, Thompson CB. The survival kinases Akt and Pim as potential pharmacological targets. *J Clin Invest* 2005; **115**(10): 2618-2624. [PMID: 16200194 DOI: 10.1172/JCI26273]
- 73 **Lindsley CW**. The Akt/PKB family of protein kinases: a review of small molecule inhibitors and progress towards target validation: a 2009 update. *Curr Top Med Chem* 2010; **10**(4): 458-477. [PMID: 20180757]
- 74 **Cheng F**, Weidner-Glunde M, Varjosalo M, Rainio EM, Lehtonen A, Schulz TF, Koskinen PJ, Taipale J, Ojala PM. KSHV reactivation from latency requires Pim-1 and Pim-3 kinases to inactivate the latency-associated nuclear antigen LANA. *PLoS Pathog* 2009; **5**(3): e1000324. [PMID: 19266083 DOI: 10.1371/journal.ppat.1000324]

- 75 **Major MB**, Roberts BS, Berndt JD, Marine S, Anastas J, Chung N, Ferrer M, Yi X, Stoick-Cooper CL, von Haller PD, Kategaya L, Chien A, Angers S, MacCoss M, Cleary MA, Arthur WT, Moon RT. New regulators of Wnt/beta-catenin signaling revealed by integrative molecular screening. *Sci Signal* 2008; **1**(45): a12. [PMID: 19001663 DOI: 10.1126/scisignal.2000037]
- 76 **Verbeek S**, van Lohuizen M, van der Valk M, Domen J, Kraal G, Berns A. Mice bearing the E mu-myc and E mu-pim-1 transgenes develop pre-B-cell leukemia prenatally. *Mol Cell Biol* 1991; **11**(2): 1176-1179. [PMID: 1990273]
- 77 **Yang H**, Wang Y, Qian H, Zhang P, Huang C. Pim protein kinase-3 is regulated by TNF-alpha and promotes endothelial cell sprouting. *Mol Cells* 2011; **32**(3): 235-241. [PMID: 21870113 DOI: 10.1007/s10059-011-1026-z]
- 78 **Red-Horse K**, Crawford Y, Shojaei F, Ferrara N. Endothelium-microenvironment interactions in the developing embryo and in the adult. *Dev Cell* 2007; **12**(2): 181-194. [PMID: 17276337 DOI: 10.1016/j.devcel.2007.01.013]
- 79 **Ferrara N**, Kerbel RS. Angiogenesis as a therapeutic target. *Nature* 2005; **438**(7070): 967-974. [PMID: 16355214 DOI: 10.1038/nature04483]
- 80 **Niedergethmann M**, Hildenbrand R, Wostbrock B, Hartel M, Sturm JW, Richter A, Post S. High expression of vascular endothelial growth factor predicts early recurrence and poor prognosis after curative resection for ductal adenocarcinoma of the pancreas. *Pancreas* 2002; **25**(2): 122-129. [PMID: 12142733]
- 81 **Wang C**, Li HY, Liu B, Huang S, Wu L, Li YY. Pim-3 promotes the growth of human pancreatic cancer in the orthotopic nude mouse model through vascular endothelium growth factor. *J Surg Res* 2013. [PMID: 23845873 DOI: 10.1016/j.jss.2013.06.004]
- 82 **Nagy JA**, Chang SH, Shih SC, Dvorak AM, Dvorak HF. Heterogeneity of the tumor vasculature. *Semin Thromb Hemost* 2010; **36**(3): 321-331. [PMID: 20490982 DOI: 10.1055/s-0030-1253454]
- 83 **Shah N**, Pang B, Yeoh KG, Thorn S, Chen CS, Lilly MB, Salto-Tellez M. Potential roles for the PIM1 kinase in human cancer - a molecular and therapeutic appraisal.

- Eur J Cancer 2008; **44**(15): 2144-2151. [PMID: 18715779 DOI: 10.1016/j.ejca.2008.06.044]
- 84 **Bachmann M**, Moroy T. The serine/threonine kinase Pim-1. *Int J Biochem Cell Biol* 2005; **37**(4): 726-730. [PMID: 15694833 DOI: 10.1016/j.biocel.2004.11.005]
- 85 **Weirauch U**, Beckmann N, Thomas M, Grunweller A, Huber K, Bracher F, Hartmann RK, Aigner A. Functional role and therapeutic potential of the pim-1 kinase in colon carcinoma. *Neoplasia* 2013; **15**(7): 783-794. [PMID: 23814490]
- 86 **Guo S**, Mao X, Chen J, Huang B, Jin C, Xu Z, Qiu S. Overexpression of Pim-1 in bladder cancer. *J Exp Clin Cancer Res* 2010; **29**: 161. [PMID: 21143989 DOI: 10.1186/1756-9966-29-161]
- 87 **Nga ME**, Swe NN, Chen KT, Shen L, Lilly MB, Chan SP, Salto-Tellez M, Das K. PIM-1 kinase expression in adipocytic neoplasms: diagnostic and biological implications. *Int J Exp Pathol* 2010; **91**(1): 34-43. [PMID: 19878356 DOI: 10.1111/j.1365-2613.2009.00673.x]
- 88 **Hu XF**, Li J, Vandervalk S, Wang Z, Magnuson NS, Xing PX. PIM-1-specific mAb suppresses human and mouse tumor growth by decreasing PIM-1 levels, reducing Akt phosphorylation, and activating apoptosis. *J Clin Invest* 2009; **119**(2): 362-375. [PMID: 19147983 DOI: 10.1172/JCI33216]
- 89 **Morishita D**, Takami M, Yoshikawa S, Katayama R, Sato S, Kukimoto-Niino M, Umehara T, Shirouzu M, Sekimizu K, Yokoyama S, Fujita N. Cell-permeable carboxyl-terminal p27(Kip1) peptide exhibits anti-tumor activity by inhibiting Pim-1 kinase. *J Biol Chem* 2011; **286**(4): 2681-2688. [PMID: 21062737 DOI: 10.1074/jbc.M109.092452]
- 90 **Holder S**, Zemsanova M, Zhang C, Tabrizi M, Bremer R, Neidigh JW, Lilly MB. Characterization of a potent and selective small-molecule inhibitor of the PIM1 kinase. *Mol Cancer Ther* 2007; **6**(1): 163-172. [PMID: 17218638 DOI: 10.1158/1535-7163.MCT-06-0397]
- 91 **Chen LS**, Redkar S, Bearss D, Wierda WG, Gandhi V. Pim kinase inhibitor, SGI-1776, induces apoptosis in chronic lymphocytic leukemia cells. *Blood* 2009;

- 114**(19): 4150-4157. [PMID: 19734450 DOI: 10.1182/blood-2009-03-212852]
- 92 **Pogacic V**, Bullock AN, Fedorov O, Filippakopoulos P, Gasser C, Biondi A, Meyer-Monard S, Knapp S, Schwaller J. Structural analysis identifies imidazo[1,2-b]pyridazines as PIM kinase inhibitors with in vitro antileukemic activity. *Cancer Res* 2007; **67**(14): 6916-6924. [PMID: 17638903 DOI: 10.1158/0008-5472.CAN-07-0320]
- 93 **Dakin LA**, Block MH, Chen H, Code E, Dowling JE, Feng X, Ferguson AD, Green I, Hird AW, Howard T, Keeton EK, Lamb ML, Lyne PD, Pollard H, Read J, Wu AJ, Zhang T, Zheng X. Discovery of novel benzylidene-1,3-thiazolidine-2,4-diones as potent and selective inhibitors of the PIM-1, PIM-2, and PIM-3 protein kinases. *Bioorg Med Chem Lett* 2012; **22**(14): 4599-4604. [PMID: 22727640 DOI: 10.1016/j.bmcl.2012.05.098]
- 94 **Xia Z**, Knaak C, Ma J, Beharry ZM, McInnes C, Wang W, Kraft AS, Smith CD. Synthesis and evaluation of novel inhibitors of Pim-1 and Pim-2 protein kinases. *J Med Chem* 2009; **52**(1): 74-86. [PMID: 19072652 DOI: 10.1021/jm800937p]
- 95 **Lin YW**, Beharry ZM, Hill EG, Song JH, Wang W, Xia Z, Zhang Z, Aplan PD, Aster JC, Smith CD, Kraft AS. A small molecule inhibitor of Pim protein kinases blocks the growth of precursor T-cell lymphoblastic leukemia/lymphoma. *Blood* 2010; **115**(4): 824-833. [PMID: 19965690 DOI: 10.1182/blood-2009-07-233445]
- 96 **Nishiguchi GA**, Atallah G, Bellamacina C, Burger MT, Ding Y, Feucht PH, Garcia PD, Han W, Klivansky L, Lindvall M. Discovery of novel 3,5-disubstituted indole derivatives as potent inhibitors of Pim-1, Pim-2, and Pim-3 protein kinases. *Bioorg Med Chem Lett* 2011; **21**(21): 6366-6369. [PMID: 21945284 DOI: 10.1016/j.bmcl.2011.08.105]
- 97 **Gavara L**, Saugues E, Alves G, Debiton E, Anizon F, Moreau P. Synthesis and biological activities of pyrazolo[3,4-g]quinoxaline derivatives. *Eur J Med Chem* 2010; **45**(11): 5520-5526. [PMID: 20864224 DOI: 10.1016/j.ejmech.2010.08.067]
- 98 **Suchaud V**, Gavara L, Saugues E, Nauton L, Theyry V, Anizon F, Moreau P. Identification of 1,6-dihydropyrazolo[4,3-c]carbazoles and

- 3,6-dihydropyrazolo[3,4-c]carbazoles as new Pim kinase inhibitors. *Bioorg Med Chem* 2013; **21**(14): 4102-4111. [PMID: 23735828 DOI: 10.1016/j.bmc.2013.05.011]
- 99 **Akue-Gedu R**, Letribot B, Saugues E, Debiton E, Anizon F, Moreau P. Kinase inhibitory potencies and in vitro antiproliferative activities of N-10 substituted pyrrolo[2,3-a]carbazole derivatives. *Bioorg Med Chem Lett* 2012; **22**(11): 3807-3809. [PMID: 22543026 DOI: 10.1016/j.bmcl.2012.03.098]
- 100 **Gavara L**, Suchaud V, Nauton L, They V, Anizon F, Moreau P. Identification of pyrrolo[2,3-g]indazoles as new Pim kinase inhibitors. *Bioorg Med Chem Lett* 2013; **23**(8): 2298-2301. [PMID: 23499503 DOI: 10.1016/j.bmcl.2013.02.074]
- 101 **Letribot B**, Akue-Gedu R, Santio NM, El-Ghozzi M, Avignant D, Cisnetti F, Koskinen PJ, Gautier A, Anizon F, Moreau P. Use of copper(I) catalyzed azide alkyne cycloaddition (CuAAC) for the preparation of conjugated pyrrolo[2,3-a]carbazole Pim kinase inhibitors. *Eur J Med Chem* 2012; **50**: 304-310. [PMID: 22386260 DOI: 10.1016/j.ejmech.2012.02.009]
- 102 **Li YY**, Wang YY, Taniguchi T, Kawakami T, Baba T, Ishibashi H, Mukaida N. Identification of stemonamide synthetic intermediates as a novel potent anticancer drug with an apoptosis-inducing ability. *Int J Cancer* 2010; **127**(2): 474-484. [PMID: 19921695 DOI: 10.1002/ijc.25048]
- 103 **Wang Z**, Li XM, Shang K, Zhang P, Wang CF, Xin YH, Zhou L, Li YY. T-18, a stemonamide synthetic intermediate inhibits Pim kinase activity and induces cell apoptosis, acting as a potent anticancer drug. *Oncol Rep* 2013; **29**(3): 1245-1251. [PMID: 23314349 DOI: 10.3892/or.2013.2233]
- 104 **Akue-Gedu R**, Rossignol E, Azzaro S, Knapp S, Filippakopoulos P, Bullock AN, Bain J, Cohen P, Prudhomme M, Anizon F, Moreau P. Synthesis, kinase inhibitory potencies, and in vitro antiproliferative evaluation of new Pim kinase inhibitors. *J Med Chem* 2009; **52**(20): 6369-6381. [PMID: 19788246 DOI: 10.1021/jm901018f]
- 105 **Rhodes N**, Heerding DA, Duckett DR, Eberwein DJ, Knick VB, Lansing TJ, McConnell RT, Gilmer TM, Zhang SY, Robell K, Kahana JA, Geske RS, Kleymenova EV, Choudhry AE, Lai Z, Leber JD, Minthorn EA, Strum SL, Wood ER,

- Huang PS, Copeland RA, Kumar R. Characterization of an Akt kinase inhibitor with potent pharmacodynamic and antitumor activity. *Cancer Res* 2008; **68**(7): 2366-2374. [PMID: 18381444 DOI: 10.1158/0008-5472.CAN-07-5783]
- 106 **Chen WS**, Xu PZ, Gottlob K, Chen ML, Sokol K, Shiyanova T, Roninson I, Weng W, Suzuki R, Tobe K, Kadowaki T, Hay N. Growth retardation and increased apoptosis in mice with homozygous disruption of the Akt1 gene. *Genes Dev* 2001; **15**(17): 2203-2208. [PMID: 11544177 DOI: 10.1101/gad.913901]
- 107 **Easton RM**, Cho H, Roovers K, Shineman DW, Mizrahi M, Forman MS, Lee VM, Szabolcs M, de Jong R, Oltersdorf T, Ludwig T, Efstratiadis A, Birnbaum MJ. Role for Akt3/protein kinase Bgamma in attainment of normal brain size. *Mol Cell Biol* 2005; **25**(5): 1869-1878. [PMID: 15713641 DOI: 10.1128/MCB.25.5.1869-1878.2005]
- 108 **Woulfe D**, Jiang H, Morgans A, Monks R, Birnbaum M, Brass LF. Defects in secretion, aggregation, and thrombus formation in platelets from mice lacking Akt2. *J Clin Invest* 2004; **113**(3): 441-450. [PMID: 14755341 DOI: 10.1172/JCI20267]

Table 1. Increased expression patterns for Pim kinases in various types of malignancies

Tumor subtype	Pim-1	Pim-2	Pim-3
Solid tumor			
Pancreatic cancer	+	ND	+
Colon carcinoma	+	ND	+
Gastric cancer	+	ND	+
Hepatocellular carcinoma	+	+	+
Prostate adenocarcinoma	+	+	+
Bladder carcinoma	+	ND	ND
Squamous cell carcinoma of head and neck	+	ND	ND
Nasopharyngeal carcinoma	ND	ND	+
Oral squamous cell carcinoma	+	ND	ND
Liposarcoma	+	ND	ND
Ewing's sarcoma	ND	ND	+
Hematological malignancies			
Acute myeloid leukemia	ND	+	ND
B Cell chronic lymphocytic leukemia	ND	+	ND
Primary mediastinal large B cell lymphoma	+	ND	ND
Mantle cell lymphoma	+	+	ND
Diffuse large B cell lymphoma	+	+	ND
Burkitt's lymphoma	+	ND	ND

ND: not determined.

Figure Legends:

Figure 1. Amino acid alignment of human Pim family proteins ^[13]. The amino acid sequences of human Pim family kinases are aligned and common residues shared with Pim-3 are highlighted. The box indicates the hinge region. Residues marked with white and red color are important for ATP binding and substrate selectivity, respectively.

Figure 2. Presumed biological functions of Pim-3. Pim-3 can interact with various target molecules, and thereby regulates various biological pathways including apoptosis, cell cycle, protein synthesis, and transcription.

Figure 3. Presumed roles of Pim-3 in pancreatic carcinogenesis. Pim-3 expression is regulated at transcriptional and post-transcriptional levels, by transcription factors such as Ets-1 and post-translational controllers such as TCTP respectively. Pim-3 kinase activation contributes to pancreatic carcinogenesis by inducing cell survival, cell cycle progression, gene transcription, protein synthesis in tumor cells, and angiogenesis.

Figure 1 Li et al

		10	20	30	40	50	
Pim-1	1	MLLSKINSLA	HL-RAAPCND	LHATKLAPGK	-EKEPLESOY	QVGPLLGS GG	50
Pim-2	1	MLTKPLQ---	----GPPAP	PGTPTPPPGG	KDREAFEAEY	RLGPLL GKGG	50
Pim-3	1	MLLSKFGSLA	HLCGPGGV DH	LPVKILQPAK	ADKESFEKAY	QVGAVLGS GG	50
		60	70	80	90	100	
Pim-1	51	FGSVYSGIRV	SDNLPVAIKH	VEKDRISDWG	ELPNGTRVPM	EVVLLKKVSS	100
Pim-2	51	FGTVFAGHRL	TDRLQVAIKV	IPRNRVLGWS	PLSDSVTCPL	EVAL LWKVGA	100
Pim-3	51	FGTVYAGSRI	ADGLPVAVKH	VVKERVTEWG	SL-GGATVPL	EVVLLRKVGA	100
		110	120	130	140	150	
Pim-1	101	--GFSGVIRL	LDWFERPDSF	VLIIERPEPV	QDLFD FITER	GALQEELARS	150
Pim-2	101	GGGHPGVIRL	LDWFETQEGF	MLVIERPLPA	QDLFDYITEK	GPLGEGPSRC	150
Pim-3	101	AGGARGVIRL	LDWFERPDGF	LLVIERPEPA	QDLFD FITER	GALDEPLARR	150
		160	170	180	190	200	
Pim-1	151	FFWQVLEAVR	HCHNCGVLHR	DIK DENILID	LNRGELKLID	FGSGALLKDT	200
Pim-2	151	FFGQVVAIQ	HCHSRGVVHR	DIK DENILID	LRRGCAKLID	FGSGALLHDE	200
Pim-3	151	FFAQVLA AVR	HCHSCGVVHR	DIK DENLLVD	LRS GELKLID	FGSGALLKDT	200
		210	220	230	240	250	
Pim-1	201	VYTDFDGTRV	YSPPEWIRYH	RYHGRSAAVW	SLGILLYDMV	CGDIPFEHDE	250
Pim-2	201	PYTDFDGTRV	YSPPEWISRH	QYHALPATVW	SLGILLYDMV	CGDIPFERDQ	250
Pim-3	201	VYTDFDGTRV	YSPPEWIRYH	RYHGRSATVW	SLGVLLYDMV	CGDIPFEQDE	250
		260	270	280	290	300	
Pim-1	251	EIIRGQVFFR	QRVSSECQHL	IRWCLALRPS	DRPTFEEIQN	HPWMQDVLL-	300
Pim-2	251	EILEAELHFP	AHVSPDCCAL	IRRCLAPKPS	SRPSLEEILL	DPWMQTPAED	300
Pim-3	251	EILRGRL LFR	RRVSPECQQL	IRWCLSLRPS	ERPSLDQIAA	HPWMLGADGG	300
		310	320	330	340		
Pim-1	301	P-----QET	AEIHL-----	----HSLSPG	P-----	SK	
Pim-2	301	VTPQPLQRRP	CPFGLVLATL	SLAWPGLAPN	GQKSHPMAMS	QG	
Pim-3	301	A-----PES	CDLRL-----	----CTLDPD	DVASTTSSE	SL	

Figure 2. Li et al.

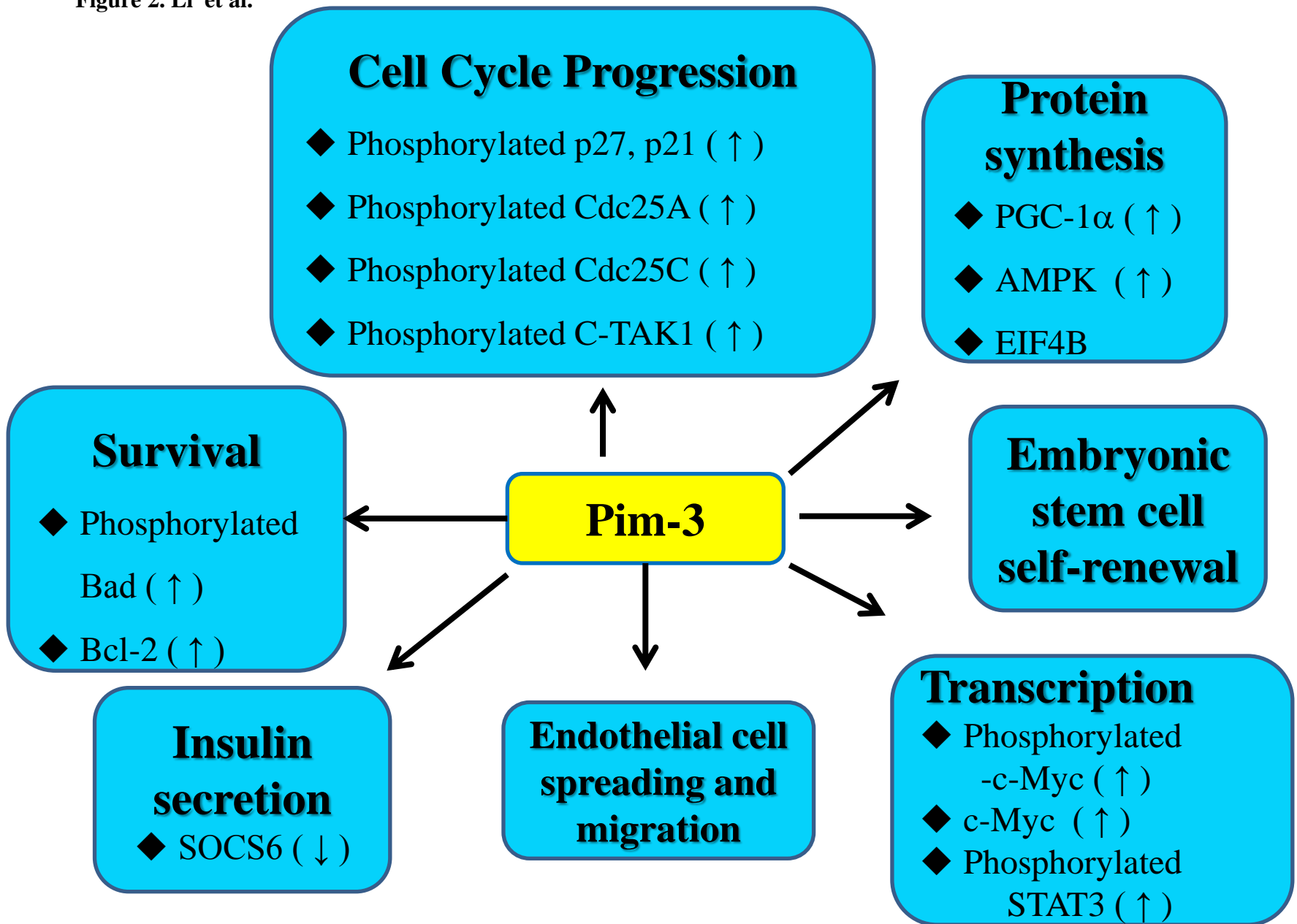


Figure 3. Li et al.

