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Pim-1: A serine/threonine kinase with a role in cell survival, proliferation, differentiation and tumorigenesis

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Abstract

Pim-1 belongs to a family of serine/threonine protein kinases that are highly conserved through evolution in multicellular organisms. Originally identified from Moloney murine leukemia virus (MuLV)-induced T-cell lymphomas in mice, Pim-1 kinase is involved in the control of cell growth, differentiation and apoptosis. Expression of Pim-1 kinase can be stimulated by a variety of growth factors and regulated at four different levels: transcriptional, post-transcriptional, translational and post-translational. Several signal transduction pathways may be associated with the regulation of Pim-1's expression; accumulating data support that the expression of Pim-1 protein is mediated through activation of JAK/STATs. Recent studies of Pim family kinases indicate that Pim-1 kinase plays important roles outside of the hematopoietic system as well.

Key words: oncogene, Pim-1 kinase, differentiation, apoptosis, tumorigenesis, proliferation, cell survival, signal transduction

Introduction

The *pim-1* gene was originally discovered as a preferential site for proviral integration of the moloney murine leukemia virus (MuLV) in mice (14,96). The integration region was designated *pim-1* for proviral integration site for MuLV. Since the original report of the cloning of murine *pim-1* proto-oncogene (97), Pim-1 cDNAs of human, bovine, rat, frog, and zebrafish have also been cloned (20,64,82,86,111,116). The *pim-1* gene codes for a highly conserved serine/threonine kinase whose expression is not only stimulated by a variety of cytokines, hormones, and mitogens, but is also highly regulated at four different levels; transcriptional, post-transcriptional, translational, and post-translational (see below). Accumulating evidence demonstrates that Pim-1 is associated with multiple cellular functions such as proliferation, differentiation, apoptosis and tumorigenesis. This article will review the present understanding of Pim-1 kinase and focus on the progress of understanding the regulation of its expression and its diverse biological functions.

pim-1 gene, Pim-1 kinase and its substrates

The murine *pim-1* gene was first cloned by proviral tagging from MuLV-infected mice (97). Using the murine *pim-1* cDNA as a probe, the human *pim-1* gene and cDNA were cloned from human cell lines (20,64,65,86,119). Both the human and murine *pim-1* genes are single copy genes and are located on murine chromosome 17 and human chromosome 6p21, respectively (13,33,74,112). The *pim-1* gene has six exons and five introns. The *pim-1* promoter is highly G+C rich and does not contain a TATA or CAAT box, which are characteristics of promoters of housekeeping genes (64,86).

The cDNA sequence predicts that both human and murine *pim-1* encode a 313 amino acid protein with an estimated molecular weight of 34 kDa. *In vitro* translation experiments demonstrated that the murine *pim-1* gene encodes a 44 kDa protein in addition to the predicted 34 kDa protein. The 44 kDa protein contains an amino-terminal extension of the 34 kDa protein and is synthesized by alternative translation initiation from an upstream CUG codon (92). Similar to the human and murine 34 kDa protein, the murine 44 kDa protein also

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exhibits comparable *in vitro* serine/threonine phosphorylating activity (36,80,92). The 44 kDa Pim-1 protein appears to be more stable than the 34 kDa protein although both are short lived (92). The cellular localization of Pim-1 kinase was originally found to be cytoplasmic (92), but more recently it has also been found in the nucleus as we show in Figure 1 (61,110). In sharp contrast to other serine/threonine protein kinases such as mitogen-activated protein kinase (MAPK), protein kinase A (PKA), PKB/Akt, and PKC, Pim-1 phosphotransferase activity does not appear to require activation by upstream kinases. Amino acid sequence comparison of Pim-1 proteins from different species shows very high homology, suggesting evolutionary importance for maintaining the function of Pim-1 kinase (111).

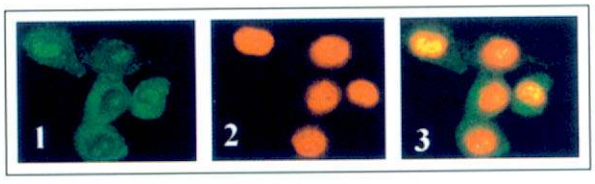

Figure 1. Cellular localization of Pim-1 kinase by confocal microscopy. U937 cells were fixed in methanol and probed with an anti-Pim-1 peptide antibody (fluorescein isothiocyanate (green) panel 1). In panel 2 the nuclei are stained with propidium iodide (red). In panel 3, (green) indicates Pim-1 while propidium iodide (red) indicates nuclear staining and merging of the two results in a yellow color.

Identification of physiological substrates for Pim-1 would facilitate delineation of exact cellular function(s) of Pim-1 kinase. Using synthetic peptides as substrates, we and others have defined the phosphorylation consensus sequence for Pim-1 kinase as Lys/Arg-Lys/Arg-Arg-Lys/Arg-Leu-Ser/Thr-X where X is likely neither a basic nor a large hydrophobic residue (26,81). Employment of this deduced phosphorylation consensus sequence to scan protein data banks reveals a list of putative Pim-1 substrates. The list contains transcription factors, proteins involved in cell growth and proliferation, and others related to apoptosis. Currently, five substrates have been reported to be phosphorylated by Pim-1 kinase. Pim-1 can increase the transcriptional activity of c-Myb by phosphorylating its co-activator, p100 (56). Pim-1 kinase also physically interacts with Cdc25A and increases its phosphatase activity through phosphorylation (68). Heterochromatin protein 1 (HP1) is associated with Pim-1 in HeLa cells. Pim-1 negatively regulates the transcriptional repression activity of HP1 by phosphorylating the serine cluster located at the hinge region of HP1 (46). Pim-1 associated protein-1 (PAP-1) is a novel Pim-1 binding protein whose function is presently unknown. However, PAP-1 was found to be co-localized with Pim-1 in HeLa nuclei and to be phosphorylated by Pim-1 at two serine residues near the C-terminus (61). Recently, we reported that Pim-1 negatively regulates the phosphatase activity of PTP-U2S, which may slow down the differentiation process and subsequent apoptosis of U937 myeloid cells (110). In addition, current studies on Pim-1 from our laboratory indicate that Pim-1 kinase can phosphorylate the cdk inhibitor, $p21^{cip1/waf1}$ (Wang et al., submitted) and the nuclear mitotic apparatus protein (NuMA) (Bhattacharya et al., submitted), which may provide a possible link between Pim-1-mediated cell cycle progression and cell proliferation.

Biological functions of Pim-1 kinase

Pim-1 and tumorigenesis

The involvement of Pim-1 in hematopoietic neoplasia was originally discovered in T cell lymphomas induced by proviral integration of MuLV as shown in Figure 2 (14,96,97). It was subsequently found to be overexpressed in B cell lymphomas (109), erythroleukemias (21) and various human leukemia (3). Ultimately, the oncogenic potential of Pim-1 was demonstrated in experiments involving transgenic mice. Transgenic mice that carry a *pim-1* gene under the transcriptional control of the immunoglobulin enhancer developed T-cell lymphomas, albeit with low incidence (5-10%) and long latency (~7 months) (106). The oncogenic potential of the *pim-1* gene is highly dose-dependent (17,18,19). Homozygous $E\mu$-*pim-1* transgenic mice display a much higher lymphoma incidence (40% with a year) than heterzygous $E\mu$-*pim-1* mice (5-10% with a year) (17,18,19). This dose-effect was also noted for homozygous *pim-1* deficient mice, which are more resistant to MuLV-induced lymphomagenesis than mice with one non-functional *pim-1* allele (105). Significantly, transgenic lines overexpressing the Pim-1 protein at very high levels, by optimization of translation initiation signals (48,49), could not be established due to the rapid tumor onset in the founder animals (7).

The low incidence and long latency of lymphoma development in *pim-1* transgenic mice indicate that overexpression of the *pim-1* gene alone is insufficient for cell transformation. Accordingly, when $E\mu$-*pim-1* mice were exposed to viral, chemical carcinogenic agents, or X-ray radiation, the development of lymphomas was significantly accelerated (9,10,104,106). In most cases, this

correlated with activation of one of the *myc* family oncogenes. In analogy, ~35% of the MuLV-induced tumors in Eμ-*myc* mice carried a proviral insertion near the *pim-1* locus (107), suggesting a synergism between *pim-1* and *myc* genes in the development of lymphomagenesis. This synergism was later verified by experiments involving crosses with various Eμ-*myc* and Eμ-*pim-1* transgenic mice (71,109). All *myc/pim-1* bitransgenic mice showed a dramatic acceleration of lymphomagenesis compared to the single transgenic parent strains, where the *c-myc* gene was more efficient than the N- or *L-myc* genes (71,109). These neoplasms were still of clonal or oligoclonal origin as indicated by presence of distinct rearrangements on TCRβ loci (71,109), suggesting that additional events, such as additional activated oncogenes, were required for the development of a fully malignant tumor. Subsequent experiments have identified other oncogenes such as *gfi-1*, *frat-1* and *runx-2* that strongly cooperate with *pim-1* in T-cell lymphomagenesis (8,45,93,121). *Tiam-1* has also been found to be activated in combination with *pim-1* in lymphomagenesis (31). A similar acceleration of tumor development was also observed in backcrosses of *pim-1* and *bcl-2* transgenic mice (1).

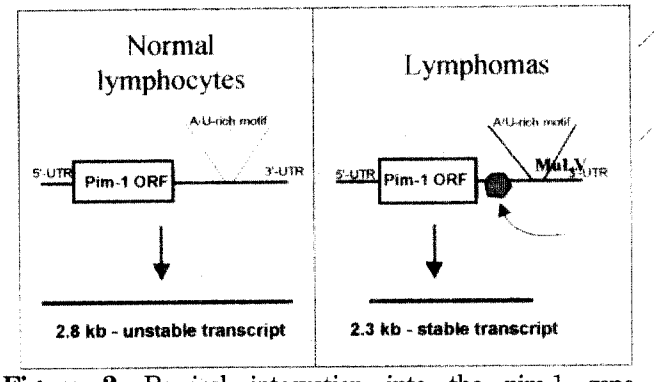

Figure 2. Proviral integration into the pim-1 gene MuLV-induced T-cell lymphomas. Insertion of MuLV provi upstream of the A/U-rich motif in the 3'-UTR of the *pim-1* gene results in short *pim-1* transcripts with higher stability.

At present, the precise mechanism underlying Pim-1 mediated cellular transformation remains obscure. As discussed below, Pim-1 can partially protect cells from apoptosis, and it has been proposed that Pim-1 contributes to transformation by inhibiting apoptosis (70). However, it is unlikely that the inhibition of apoptosis is the sole mechanism, because Pim-1 has been shown to efficiently cooperate with Bcl-2, an anti-apoptotic factor (4) and Gfi-1, an apoptosis inhibitor (30), in lymphomagenesis. If Pim-1 is functioning solely as an apoptosis inhibitor, these combinations should be redundant rather than cooperative. A recent experiment showed that Pim-1 can enhance c-Myb activity by phosphorylating its co-activator p100 (56). C-Myb is a transcriptional factor involved in tumorigenesis (76,113). Like Pim-1, c-Myb is widely expressed during hematopoiesis and is induced by JAK activation in response to a wide range of cytokines (56). Importantly, c-Myb also cooperates with c-Myc in maintenance of tumors (113). This finding raises the possibility that Pim-1 predisposes cells to tumor formation by stimulating c-Myb activity. In addition, Pim-1 kinase phosphorylates Cdc25A and enhances its transformation potential (68). Due to its critical role in cell cycle progression (34,41) and its well documented oncogenic potential in fibroblasts (28) Cdc25A may be another modulator of Pim-1's function in tumorigenesis. However, a more detailed picture waits future research to be carried out.

Pim-1 and cell survival

Apoptosis is widely accepted as a normal physiological process during cell development and differentiation (108). Apoptosis can be induced by a variety of stresses, including deprivation of serum, growth factors or cytokines, heat shock, and anti-cancer reagents (95). Many genes (including *pim-1*) have been shown to be involved in this well-regulated multi-step event. Moroy *et al.* showed that Pim-1 expression rescues both lymph node cells from rapid apoptosis *in vitro* and CD4+/CD8+ double positive thymocytes from dexamethasone-induced apoptosis *in vivo* in Eμ-*pim-1 lpr/lpr* mice (70). In IL-3 dependent FDPC1 cells, enforced expression of Pim-1 kinase acts to inhibit apoptosis primarily by acting as a survival factor (58). Certain apoptotic events as induced by cytokine withdrawal are inhibited by the exogenous expression of Pim-1. This includes the inhibition of apoptosis-associated decay in mitochondrial membrane potential and of the production of reactive oxygen species (60). In proliferating hematopoietic FDC cells, exogenously expressed Pim-1 was observed to efficiently inhibit apoptosis as induced by either Co^{60} or adriamycin treatment. The dose-dependent relationship between levels of Pim-1 expression and ability to inhibit apoptosis was established in several independent clones (83). In addition, Pim-1 kinase can cooperate with c-Myc to prevent apoptosis in BAF/BO3 cells in response to the withdrawal of IL-3 (98).

Although it is widely observed that up-regulation of Pim-1 is associated with cell survival and down-regulation of Pim-1 with apoptosis (53,84,89), there are several

studies indicating that Pim-1 has an apoptotic function. During *in vitro* culturing, bone marrow-derived mast cells from *pim-1*-deficient mice die more slowly upon removal of IL-3 than wild-type mast cells, suggesting that loss of the kinase may actually increase survival (19). Pim-1 kinase has also been shown to induce apoptosis in mouse NS-1-derived cells (102). Studies by Mochizuki *et al.* (68,69) showed that expression of Pim-1 kinase enhanced c-Myc-mediated apoptosis in serum-deprived Rat-1 fibroblasts by the phosphorylation of cell cycle phosphatase cdc25A, a direct transcriptional target for c-Myc (27). Pim-1 kinase enhances the transforming potential as well as the apoptosis-inducing ability of cdc25A.

It is interesting to note that Pim-1 can function as a cell survival factor as well as an apoptosis enhancing factor. Undoubtedly, at least some of these apparent discrepancies result from the different cellular backgrounds in which Pim-1 function was studied. The *pim-1* gene is known to cooperate with a variety of other genes in lymphomagenesis (see above) and it is likely that the spectrum of associated, activated genes in a particular cell determines whether Pim-1 inhibits or promotes apoptosis. The variable effects of Pim-1 expression on inhibiting or enhancing apoptosis are reminiscent of the actions of the *c-myc* gene that has been seen to trigger intracellular signals leading to both transformation and apoptosis (32). Various studies show that Pim-1's function during apoptosis depends on its kinase activity. The kinase-dead Pim-1 mutant exhibits the opposite effects in transfected cells, indicating that Pim-1 kinase promotes or inhibits apoptosis by phosphorylating different substrates. A limited number of substrates have been identified for Pim-1 (see above), but there are more potential substrates that can act as apoptotic or anti- apoptotic factors. It is expected that cellular fate will be determined by the amount and accessibility of those target molecules to Pim-1 kinase in response to different stimuli and cellular conditions.

Pim-1 and differentiation

Pim-1 kinase appears to be involved in the differentiation of a variety of cell types in which it is expressed. In human fetuses, Pim-1 expression is developmentally regulated in sites of hematopoiesis, where it is highly expressed in the fetal liver and spleen but not in the corresponding adult tissues (3). In male mice, *pim-1* message is selectively expressed in haploid post-meiotic early spermatids. This developmentally regulated stage-specific expression of the *pim-1* gene suggests an involvement of the Pim-1 kinase in signal transduction events associated with normal germ cell maturation (100). Analysis of *pim-1* transgenic mice and deficient mice shows that Pim-1 levels determine the size of early B lymphoid compartments in bone marrow. The increase in immature cell number correlates with a loss of more mature cells in transgenic mice, implying that overexpression of Pim-1 may cause a differentiation block (17). In keratinocytes, Pim-1 expression is clearly correlated with increased differentiation, whereas a striking lack of expression is evident in the squamous carcinoma-derived line SCC4, which lacks differentiated features in culture (101). During differentiation stimulated by hydrocortisone or elevated Ca^{2+} concentrations, the expression patterns of Pim-1 and the differentiation marker transglutaminase 1 are identical (101).

During T cell development, high levels of Pim-1 can promote pre-T cell development through β-selection and can relieve the differentiation block imposed by Gfi-1 oncoproteins at the transition from $CD4^-/CD8^-$(double negative) to $CD4^+/CD8^+$(double positive) cells (94). In MuLV-infected Rag-deficient mice, the *pim-1* locus was identified as a major proviral integration site in T-cell lymphomas at all developmental stages, suggesting that Pim-1 can also be involved in compensation of defective-β-selection in Rag-deficient thymocytes. In Eμ-*pim-1* transgenic + *Rag*-deficient mice, Jacobs et al. (38) observed differentiation and slow expansion of large $CD4^-/CD8^-$ (double negative) $CD25^+$ thymocytes into small resting $CD4^+/CD8^+$(double positive) $CD25^-$ pre-T cells in a time-dependent fashion. Recently, we found that Pim-1 is also involved in myeloid cell (U937) differentiation. During phorbol ester-induced U937 cell differentiation, Pim-1 levels increase. However, when the levels of active Pim-1 are manipulated, the rate of differentiation is altered. With overexpression of the dominant negative form of Pim-1, the rate of differentiation increases, while with the overexpression of wild-type Pim-1 the rate of differentiation appears to slow down (110).

Pim-1 and proliferation

As mentioned below, induced expression of Pim-1 kinase is largely restricted to hematopoietic growth factors, including IL-2, IL-3, GM-CSF (15,59). In the human myeloid cell line MO7e, GM-CSF induced Pim-1 protein in a dose-dependent manner, with expression being proportional to the proliferative effect of the cytokine (59). IFN-γ alone did not induce significant proliferation of MO7e cells whereas steel factor (SLF) stimulated proliferation in a dose dependent manner. The combination

of IFN-γ and SLF stimulated a synergistic increase in Pim-1 protein expression which correlated with synergistic effects on cell proliferation because IFN-γ stimulated expression of Pim-1 mRNA and protein in MO7e cells (118). In the IL-3 dependent 32D cell line, erythropoietin induces the expression of Pim-1, which is correlated with the proliferative signal transduced from various mutant erythropoietin receptors (66). In Ba/F3 cells, significant proliferation induced by IL-7 was also accompanied by Pim-1 induction (16). For B6M cells, IL-3 is a survival factor and alone does not stimulate proliferation. Stem cell factor (SCF) can stimulate proliferation of B6M cells, and together IL-3 and SCF synergize to stimulate optimal proliferation. IL-3 but not SCF, leads to activation of STAT5 and subsequently induces Pim-1 expression. These data indicate that this activation of STAT5 and induction of Pim-1 may contribute to the synergistic proliferation observed in response to IL-3 plus SCF (79).

Expression of a *pim-1* transgene in *lpr/lpr* mice results in strong acceleration of lymphoproliferation through inhibition of apoptosis (70). Enforced expression of Pim-1 kinase in IL-3 dependent FDC-D1 cells also leads to IL-3-independent clonogenic proliferation in semisolid medium (58). This result is consistent with the impaired IL-3 response of mast cells from *pim-1* deficient mice. Mast cells with wild type *pim-1* grew well with IL-3, cells heterozygous for the *pim-1* null gene grew at intermediate rates and cells homozygous for the null *pim-1* gene grew poorly suggesting a dosage effect of Pim-1 on IL-3-mediated growth of mast cells (19). Recently, Nosaka et al. (78) showed that with IL-3 dependent Ba/F3 cells, constitutive expression of Pim-1 was sufficient to induce IL-3-independent growth and co-expression of c-Myc enhanced the phenotype.

The *pim-1* and *c-myc* genes cooperate to promote oncogenesis in T and B lymphocytes (6,106). Interestingly, Pim-1 and c-Myc synergistically rescue the defects in STAT3 signalling in BAF-B03 cells although enforced expression of either Pim-1 or c-Myc alone is not sufficient to complement the gp130-mediated STAT3 proliferative signal. Furthermore, expression of a kinase dead Pim-1 mutant partially attenuated the gp130-mediated cell proliferation, further implicating Pim-1's involvement in cell proliferation (98).

Other potential functions of Pim-1

More insight into the functioning of the Pim-1 protein has come from the study of *pim-1*-deficient mice. Despite much evidence that Pim-1 has a well-conserved function, *pim-1* deficient mice showed a surprising absence of phenotypic anomalies apart from erythrocyte microcytosis (55). Only when various hematopoietic cell types of Pim-1 deficient mice were examined with respect to their *in vitro* growth characteristics were differences observed. Mast cells and early pre-B cells from Pim-1 null mice exhibited a reduced response to IL-3 and IL-7 respectively, while the IL-7 response was enhanced in cells from Pim-1 transgenic mice (17,18,19). This was later confirmed by the observation that Pim-1 can reconstitute thymic cellularity in IL-7 and common-γ chain deficient mice, suggesting that Pim-1 functions as an efficient effector of the IL-7 pathway (38).

The minor consequences of gene inactivation compared to the profound effects caused by Pim-1 overexpression suggested the presence of functionally redundant genes. Indeed, two highly homologous family members, Pim-2 and Pim-3, were subsequently isolated (47,105). Despite potential functional redundancy in hematopoietic cell lineages, Pim-1 kinases have distinct functions in brain tissues (2,22). Both Pim-1 and Pim-3 are upregulated in specific regions of the hippocampus and cortex of rats upon synaptic activity, whereas Pim-2 is constitutively expressed there (23,24,47). In addition, induced expression of Pim-1 in the nuclear and dendritic compartments of hippocampal neurons is specifically required for long-term potentiation as shown by lack of such response in Pim-1 knockout mice (47). These data demonstrate that Pim kinases may have important functionally redundant as well as non-redundant roles outside of the hematopoietic system.

Regulation of *pim-1* expression

In general, the expression of genes involved with cell growth is highly controlled because aberrant expression often results in deregulated cell growth and malignant cell transformation. In keeping with its important roles in regulating cell growth, the expression of Pim-1 has been shown to be tightly controlled at multiple levels: transcriptional, post-transcriptional, translational and post-translational.

Transcriptional regulation

pim-1 expression can be induced by various cytokines, mitogens and hormones such as GM-CSF, G-CSF, IL-2, IL-3, IL-5, IL-6, IL-7, IL-9, IL-12, IL-15, erythropoietin, Con A, PMA, interferon-γ, steel cell factor and prolactin (11,12,15,16,43,59,62,65,91,115,117,118). *pim-1* mRNA levels

are rapidly increased with kinetics characteristic of an early response gene (Figure 3). Despite features that characterize its promoter as one belonging to a constitutively expressed housekeeping gene (63), nuclear run-on assays have demonstrated that increases in the steady-state levels of *pim-1* mRNA observed after mitogenic stimulation are, in part, the result of increases in transcriptional activity (87,114). The *pim-1* gene is expressed at high levels in hematopoietic tissue and testes, while other tissues express low or undetectable amounts (3,100). During hematopoiesis, *pim-1* expression is developmentally regulated, it is highly expressed in the fetal liver and spleen but extremely low in the corresponding adult tissues (3). A survey of *pim-1* expression in 38 human cell lines showed the highest expression in myeloid cell lines, intermediate levels in many B cells lines and undetectable levels in T-cell lines (65). In addition, our laboratory showed that *pim-1* expression in PMA plus ionomycin stimulated lymphocytes was inducible only in T cell subpopulations, but not in B cell subpopulations, demonstrating that *pim-1* expression can be regulated in a cell-type specific manner (117).

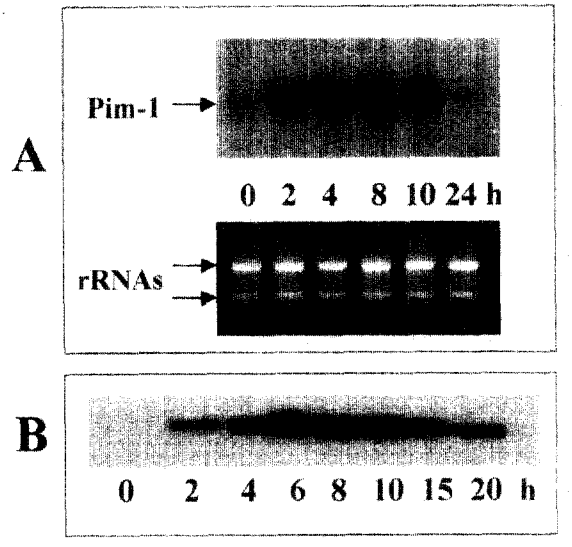

Figure 3. Induced expression of *pim-1* mRNA and Pim-1 protein in PMA-stimulated bovine peripheral blood lymphocytes. A. Time course of *pim-1* mRNA expression. Upper panel, Northern blot of *pim-1* message; lower panel, equal loading of total RNAs. B. Time course of Pim-1 protein expression.

Transcriptional attenuation is also involved in the regulation of *pim-1* expression. The sequence responsible for this effect is located within the first 488 bps of the *pim-1* gene (75). IL-2 stimulation can release transcriptional attenuation of the *pim-1* gene in human thymic blast cells (87). The release of attenuation is rapid, occurring within 1 h of IL-2 treatment and does not depend on new protein synthesis. A possible mechanism for the IL-2-mediated relief of attenuation is through removal of a block to transcriptional elongation, which is due to the presence of a number of dyad symmetry elements capable of forming stem loop structures within the first 488 bps of the *pim-1* gene.

Post-transcriptional regulation

The regulation of mRNA stability is recognized as an important step in the control of certain oncogene and lymphokine genes. Many of these oncogenes and lymphokine genes (including *pim-1*) contain A/U-rich sequences in the 3'-untranslated regions (UTR) of their transcripts (85). These clusters of A/U-rich motifs have been proposed to be a major determinant of mRNA instability (88). Deregulation of oncogene transcripts without A/U-rich motifs is frequently associated with neoplastic transformation (85). Indeed, insertion of MuLV provirus upstream of the A/U-rich sequence in the 3'-UTR of the *pim-1* gene causes increased stability of the *pim-1* transcript and is associated with MuLV-induced T-cell lymphomas (96) (Figure 2). We found that a testes-specific *pim-1* transcript is shorter and more stable than the longer somatic transcript because the shorter transcript arises from the use of an alternate polyadenylation signal, resulting in the removal of the A/U-rich element located in the 3'-UTR of the pim-1 gene (116).

In addition, mitogen stimulation can regulate the stability of *pim-1* mRNA. We demonstrated that ConA or PMA plus ionomycin treatment of lymphocytes resulted in an increase of *pim-1* mRNA levels, which was in part due to an increase in the stability of *pim-1* mRNA (117). We also observed that *pim-1* mRNA stability increased (~2.5 fold) following PMA plus ionomycin treatment of Hut-78 cells (114). Lastly, prolactin, interferon-γ and steel factor have also been found to increase the stability of *pim-1* message (11,118).

Translational regulation

Translational control can be mediated by a variety of elements within the 5'-UTR of mRNAs, including leader length, minicistrons, polyprimidine tracts, secondary structure and the consensus sequences surrounding the initiating AUG codon (5,29,50,51,72). Particularly, it has been shown that extensive secondary structure within the 5'-UTR effectively inhibits translation (99). Expression of a

number of genes encoding growth factor receptors and proto-oncoproteins has been shown to be regulated by elements within the 5'-UTR (52). The 5'-UTR of the *pim-1* mRNA contains sequences that predict translational regulation, including a highly structured 5'-UTR sequence, upstream CUG codons and a poor Kozak consensus sequence. Deletion of the 5'-UTR of *pim-1* increases translation of Pim-1 protein in both *in vitro* and *in vivo* systems. Moreover, increased eIF-4E expression can overcome the inhibitory effect of the 5'-UTR on Pim-1 translation (37). Using a dicistronic construct, we and others have demonstrated that there is, in fact, an internal ribosomal entry site (IRES) in the 5'-UTR of the *pim-1* mRNA (42) (Weaver and Magnuson, in preparation). The capability of *pim-1* to use cap-independent translation via its IRES might be crucial for its multiple cellular functions. IRES usage has already been shown to play a role in control of important cellular processes such as apoptosis, stress responses, cell-cycle progression, and viral infection (35,90,103).

Post-translational regulation

The first indication of post-translational regulation of Pim-1 came from the different turnover rates for murine 34 kDa and 44kDa proteins. Both proteins exhibit comparable kinase activity, but the 44 kDa protein is more stable than the 34 kDa protein. The half-lives of 44 kDa and 34 kDa Pim-1 proteins are ~1 h and 10 min, respectively (92). For human Pim-1 proteins, Western blot analysis typically shows a double band (34 and 35 kDa). Interestingly, the 35 kDa Pim-1 in the normal peripheral blood mononuclear B cells is more stable that the 34 kDa form. In contrast, both forms of Pim-1 proteins from the chronic myelogenous leukemia cells K562 are more stable (half-life of 20 min in K562 versus 5 min in peripheral blood mononuclear cells) (57).

Pim-1 kinase can autophosphorylate on serine and threonine residues, and it has been proposed that the phosphorylation on Ser 190 may modulate the activity of Pim-1. However, work by Palaty et al. (82) showed that by mimicing autophosphorylation on Ser 190 (S190E), *Xenopus laevis* Pim-1 kinase does not lead to a discernable increase in kinase activity. This effect on human Pim-1 has not yet been investigated. Activity and/or stability of many other kinases are regulated by their binding partners. Pim-1 does not have obvious autoregulatory or association domains, but it still may complex with other proteins. A recent report of regulation of Pim-1 by Hsp90 showed that Pim-1 can increase its stability and kinase activity by physically interacting with Hsp90 α and β (67). Our preliminary data reveals that degradation of Pim-1 protein appears to be mediated in part by the ubiquitin-proteasome pathway. Cells treated with MG-132, a proteasome inhibitor, display an increase in ubiquitin-tagged Pim-1 proteins (Petersen and Magnuson, unpublished results). Phosphorylation of Pim-1 at its N-terminus may also regulate its stability. For example, autophosphorylation of the *mos* oncogene encoded serine/threonine protein kinase near its N-terminus at Ser 3 apparently stabilizes the kinase by preventing its ubiquitination and degradation (25,77).

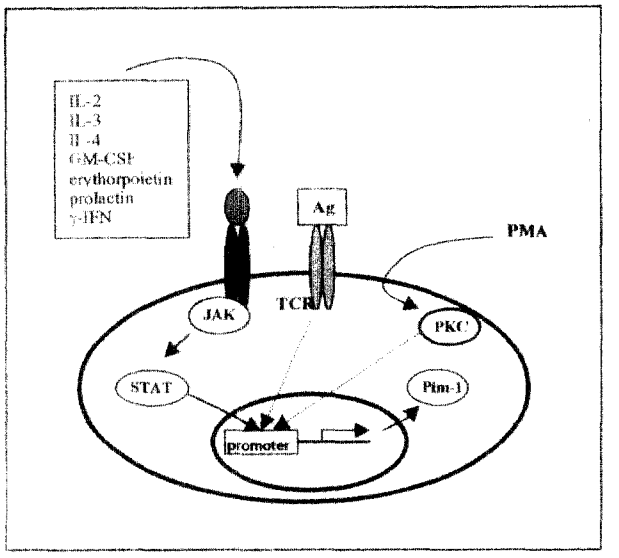

Figure 4. Signaling transduction pathways presently known to be involved in Pim-1 expression. See text for details. TCR = T cell receptor; PKC = protein kinase C; JAK = Janus family tyrosine kinases; STAT = Signal transducers and activators of transcription; Ag = antigen; PMA = phorbol myristate acetate; GM-CSF = granulocyte/ macrophage-colony stimulating factor; IL = interleukin; IFN = interferon.

Signaling pathways involved in Pim-1 expression

As mentioned previously, Pim-1 expression can be induced by various cytokines, mitogens and hormones. Different stimuli can activate distinct signaling pathways, indicating that *pim-1* gene expression may be mediated by different combinations of signaling pathways in different cell types or tissues (Figure 4). We have shown that Pim-1 expression in anti-CD3-mediated T cell activation is associated with PKC activation and is independent of Raf-1 (114). However, in rat pre-T NB2 lymphoma cells, MAPK, PI3-kinase and Jak2 signal pathways appear to be involved in regulating prolactin-induced Pim-1 expression

(54). Interferon-γ stimulated *pim-1* gene transcription in the factor-dependent cell line MO7e occurs via activation of Stat1, which in turn, promotes binding of STAT1 to a GAS-like element within the *pim-1* promoter region (118). By inducible expression of a dominant-negative STAT5 protein in the IL-3-dependent cell line Ba/F3, the *pim-1* gene was shown to be regulated by STAT5-dependent pathways (73). Accumulating data further demonstrate that the JAK2/STAT5 pathway plays an important role in mediating cytokine/growth factor- induced *pim-1* expression because its induced expression has been tightly correlated with the activation of JAK2/STAT5a (16,39,40,43,44,66,78,79). In addition, in anti-CD3-activated human T lymphocytes, interferon-γ stimulates *pim-1* mRNA expression by inducing STAT1, STAT3 and STAT4 binding to the *pim-1* GAS element (62). In this study, interferon-γ priming also enhances IL-2-induced STAT5 binding to the *pim-1* GAS site (62).

Conclusions

In summary, Pim-1 has been shown to be a critical component of major pathways that transmit signals from a variety of growth factors. These signals ultimately determine whether the cell will proliferate, differentiate, or die. Few reported substrates cannot account for the broad spectrum of Pim-1 kinase's functions. The most important unresolved problem is the identification of additional cellular substrates, which Pim-1 phosphorylates in specific physiological environments in order to control differentiation, proliferation, and transformation. The identified phosphorylation consensus sequence for Pim-1 kinase will facilitate this process. The second major issue is gaining a complete understanding of how cells regulate Pim-1 kinase activity. Induced expression of Pim-1 is very rapid and the Pim-1 protein is short-lived. Regulation of stability and degradation of Pim-1 kinase and its cellular localization are crucial for its cellular function, but these aspects remain largely unknown. Finally, the deduced phosphorylation consensus sequence for Pim-1 resembles the phosphorylation site motifs found for several other serine/threonine kinases. For example, the cdk inhibitor p21 has been shown to be a cellular substrate for Akt as well as Pim-1 (120). Elucidation of cross-talk between Pim-1 and other protein kinases through phosphorylation of common targets will likely offer valuable insights into the functions of these involved kinases.

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