Retroviral insertional mutagenesis and characterization of the frequently activated PIM kinases
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Chapter II

An introduction to the *Pim* family of proto-oncogenes
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General Pim features

The PIM proto-oncogenes belong to a distinct family of serine/threonine kinases (Figure 1). The first member of this family, Pim1, was identified as a target for proviral activation in Moloney murine leukemia virus (M-MuLV) induced T cell lymphomas (Cuypers et al., 1984). In the mouse, Pim1

**Figure 1.** Phylogenetic tree of mammalian serine/threonine kinases.
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encodes two proteins, a 33 kD and a 44 kD protein, which is initiated from an upstream CTG codon (Saris et al., 1991). In human, PIM1 encodes a 34 kD protein (Domen et al., 1987; Meeker et al., 1987; Zakut-Houri et al., 1987). The half-life of mouse and human PIM1 mRNA as well as the protein is very short (Saris et al., 1991). Pim1 mRNA is labile due to the presence of five copies of an ATTATA destabilization motif in the 3'UTR (Saris et al., 1991). The Pim family of kinases contains besides Pim1 two very homologous Pim2 and Pim3 genes (Allen and Berns, 1996; Baytel et al., 1998; Feldman et al., 1998; van der Lugt et al., 1995). PIM3 shares 71% and PIM2 61% of the amino acids with PIM1 (Figure 2). Pim2 has similar properties as Pim1; the mRNA and protein is labile, and its transcription can be initiated from an upstream CTG codon (van der Lugt et al., 1995).

![Figure 2. Alignment of the mouse PIM1, PIM2 and PIM3 proteins.](image)

The Pim genes are rather ubiquitously expressed and the encoded proteins reside in both the nucleus and cytoplasm of the cell. The highest Pim1 mRNA

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levels are found in thymus and testis (Selten et al., 1985), *Pim2* in brain and thymus (Allen et al., 1997), and *Pim3* in mammary gland (this thesis) and kidney (Feldman et al., 1998). During mouse development either *Pim1* and *Pim3* or *Pim2* and *Pim3* are co-expressed (Eichmann et al., 2000). The expression of *Pim1*, *Pim2*, and likely also *Pim3*, is regulated by a range of cytokines and growth factors: IL-2 (Allen et al., 1997; Dautry et al., 1988), IL-3 (Allen et al., 1997; Domen et al., 1993b; Lilly et al., 1992), GM-CSF and G-CSF (Lilly et al., 1992), IL-4 (Allen et al., 1997), IL-5 (Temple et al., 2001), IL-6 (Lilly et al., 1992), IL-7 (Allen et al., 1997; Domen et al., 1993a), IL-9 (Allen et al., 1997), IL-12, IL-15, IFNo (Matikainen et al., 1999), IFNy (Allen et al., 1997; Yip-Schneider et al., 1995), erythropoietin (Miura et al., 1994), (Nagata and Todokoro, 1995), prolactin (Borg et al., 1999; Buckley et al., 1995), ConA (Allen et al., 1997), and LPS (Allen et al., 1997). The majority of these factors transduce their signal through the JAK/STAT pathway. Together with the presence of Stat-binding elements in the promoter of mouse *Pim1*, this implies an important role for the JAK/STAT cascade in regulating expression of the *Pim* genes. Although this notion is generally accepted, the direct involvement of STAT and expression of *Pim* has only been demonstrated for STAT3 in relation to IL-6 (Shirogane et al., 1999). The strong and early induction of the *Pim* genes in response to distinctive cytokines would predict an important function for PIMs in cytokine signaling. Cytokine signaling induces either proliferation or differentiation and blocks apoptosis. Hirano and colleagues demonstrated that simultaneous expression of *Pim1* and c-Myc abrogates the requirement for STAT3 to mediate cell cycle progression and prevent apoptosis. In addition, ectopic expression of *Pim1* overrules the cytokine-dependent survival of a murine pre-B cell line, possibly via a BCL2-dependent pathway, illustrating the crucial role for PIM in cytokine signaling (Lilly et al., 1999).

**Pim** and tumorigenesis

The role of *Pim1* in mouse lymphomagenesis has been well documented. *Pim1* transgenic mice, expressing *Pim1* under the control of the immunoglobulin Eμ enhancer, are highly predisposed to lymphomas (van Lohuizen et al., 1989). Carcinogenic agents such as ENU (Breuer et al., 1989; Breuer et al., 1991), heterocyclic amines (Sorensen, 1996, 1997) and leukemia viruses (van Lohuizen, 1989; Allen, 1998), but also X-ray (van der Houven van Oordt et al., 1998) and exposure to electro-magnetic fields cause an accelerated onset of tumor formation in these mice (McCormick et al., 1998; Repacholi et al., 1997). An even more dramatic acceleration of tumor formation is seen in *Pim1* and c-Myc, N-Myc or L-Myc double transgenic mice that die as a result of pre-B cell leukemia (Moroy et al., 1991; Verbeeck et al., 1991). This observation illustrates that PIM1 is a very efficient collaborator of MYC in lymphomagenesis. Similar experiments with *Pim2* transgenic mice have shown that *Pim2* is an equally potent oncogene as *Pim1* (Allen et al., 1997). In human tumors, levels of *PIM1* are high in some acute myeloid and lymphoid leukemias (Amson et al., 1989; Nagarajan et al., 1986); Allen, personal communication), but the enhanced expression is not the result of translocations or amplifications involving the *PIM1* locus. In addition,
expression of PIM1 appears to be a marker for prostate carcinomas as metastatic tumors lose expression of PIM1, which is relatively high in the primary tumors (Dhanasekaran et al., 2001).

**PIM signaling targets**

In contrast to the role of PIM1 in mouse lymphomagenesis, the signaling pathways in which PIM1 plays a decisive role remain largely unknown. The generation of *Pim1*-deficient mice, which are apparently normal (Laird et al., 1993; te Riele et al., 1990), initially, only underscored the role of PIM1 in interleukin signaling ((Domen et al., 1993a; Domen et al., 1993b). *Pim1*-deficient bone marrow cells exhibit a reduced proliferation in response to IL-3 or IL-7. It was shown by the group of Kuhl that besides the role PIM1 in the hematopoietic system, PIM1 might act in the establishment of long-term memory, since *Pim1*-deficient hippocampal granule cell neurons lack consolidation of enduring long-term potentiation (LTP) (Konietzko et al., 1999).

A decennia after its cloning, the first substrate of PIM1, protein P100, was identified (Leverson et al., 1998). P100 can bind to the transcription factor c-MYB and upon phosphorylation of P100 by PIM1, the c-MYB mediated transactivation is enhanced. Shortly after P100, two other substrates of PIM1, were discovered, the cell cycle phosphatase CDC25a (Mochizuki et al., 1999) and a heterochromatin binding protein HPy (Koike et al., 2000). The PIM1-mediated phosphorylation of CDC25a and HPy alters these coactivator proteins' phosphatase and repressor activity, respectively in vitro. Other PIM targets that were reported on are SOCS (Chen et al., 2002; Losman et al., 1999), NFATc (Rainio et al., 2002), TFAF2/SNX6 (Ishibashi et al., 2001) and PAP1 (Maita et al., 2000). Phosphorylation of SOCS1 stabilizes the protein resulting in suppression of cytokine signaling. TFAF2/SNX6 is supposed to be translocated into the nucleus upon phosphorylation by PIM, whereas phosphorylation of the transcription factor NFATc has been reported to increase its transactivation potential. Future experiments might teach us whether these proteins indeed are the PIM1 targets that provide the link between PIM and lymphomagenesis.

**References**


