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Cloning and Characterization of the Human PIM-1 Gene: A Putative Oncogene Related to the Protein Kinases

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The mouse PIM-1 gene has been implicated in the evolution of retrovirus-associated mouse lymphomas. We have initiated a study of the human PIM-1 gene because of its potential importance as a human oncogene. We have isolated genomic and cDNA clones for this gene and characterized this locus in detail. The predicted PIM-1 protein is 313 amino acids in length. It has homology to a number of the protein kinases but does not have a transmembrane region. The amino acid corresponding to tyrosine-416 of pp60v-src is a tyrosine (position 198), which is consistent with the hypothesis that PIM-1 is a tyrosine kinase rather than a serine-threonine kinase. The PIM-1 gene was found to have six exons and five introns derived from 5 kb of genomic DNA. The site of transcription initiation was localized by S1 nuclease protection studies which indicated that the mature PIM-1 mRNA was approximately 2.7 kb in length. The promotor of this gene had no TATA or CAAT box but did have multiple GC boxes (CCGCCC) that might bind the Sp1 protein. The PIM-1 gene was expressed in myeloid and B lymphoid cell lines, but not in T lymphoid and nonhemopoietic lines. This initial characterization of PIM-1 will allow us to define its role in normal and malignant hematolymphoid differentiation.

Key words: human PIM-1 gene, protein kinase, oncogene, hematolymphoid genes

Tumor-associated retroviruses in animals frequently activate cellular proto-oncogenes by integrating adjacent to them. Examples of activation by retroviral insertion include c-myc by avian leukosis virus, erb-B by avian leukosis virus, c-myc by Abelson and Moloney retrovirus, and int-1 and int-2 by mouse mammary tumor virus [1–5]. The PIM-1 gene has recently been added to this list [6]. Murine leukemia retroviruses frequently integrate in the PIM-1 locus in mouse T cell lymphomas [7]. The increased levels of PIM-1 mRNA that result apparently have a role in the evolution of these lymphomas. It is interesting that abnormalities of *c-myc* and PIM-1 are often found in the same tumor, suggesting that these genes might cooperate in oncogenesis.

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Because of our interest in the genes associated with human leukemia and lymphoma, we began to investigate the human PIM-1 gene. We have previously reported the cloning of a genomic fragment for the human PIM-1 gene and the use of this clone to map the human PIM-1 gene to chromosome band 6p21 [8]. In this report we describe the isolation of cDNA clones for this gene, the complete characterization of the cDNA and genomic clones, and our studies of the expression of the PIM-1 gene.

MATERIALS AND METHODS

Isolation of RNA and Northern Blotting

The following cell lines were used for RNA isolation: B lineage—380, 697, Alli, Daudi, BL2, ST486, JD38, Manca, CA46, PA682, SB, LY67, LY91, GM607, GM1056, GM2471, GM2669, GM1500, RPMI 8226; T lineage—Jurkat, HUT78, Molt4, SUPT1, CEM, JM; myeloid lineage—K562, ML3, KG1, HL-60, WEH1-3B (the only mouse line studied); nonhemopoietic—BR3, BR5, Colo320, 2102E, JAR, WM793, A424, TERA1 and WM373 [9–12].

RNA was isolated from cell lines by previously published methods. For Northern blots RNA was fractionated in formaldehyde gels and transferred to nitrocellulose as published [13].

cDNA Libraries

Two K562 cDNA libraries were screened. The first has been previously described [14]. The second library was constructed in lambda gt10 by using published methods [15,16].

Nucleic Acid Sequencing

All sequencing was performed in the M13 system by using the chain termination technique [17]. Data represent information from both strands.

RESULTS

Cloning

Our group has previously reported the cloning of a 13-kb genomic fragment containing the human PIM-1 gene from the 380 cell line [8]. With the aid of a probe from this fragment, the K562 cDNA libraries were screened. Approximately, 0.02% of the clones were positive. Upon detailed restriction mapping, two of these clones were found to cover approximately 2.6 kb of the PIM-1 transcriptional unit. The cDNA clones and appropriate regions from the genomic clone were sequenced. Our goal was to define the nature of the PIM-1 protein, promotor region, and intron/exon structure.

Deduced PIM-1 Protein Structure

The K562 cDNA clones covered 2514 bp of the mature PIM-1 transcript as noted in Figure 1. The major protein-coding region defined a protein of 313 amino acids. This protein-coding region initiates translation at the first ATG codon in the cDNA clones. Because of our concern about possible additional upstream translation initiation sites we defined the site of transcription initiation by S1 mapping and

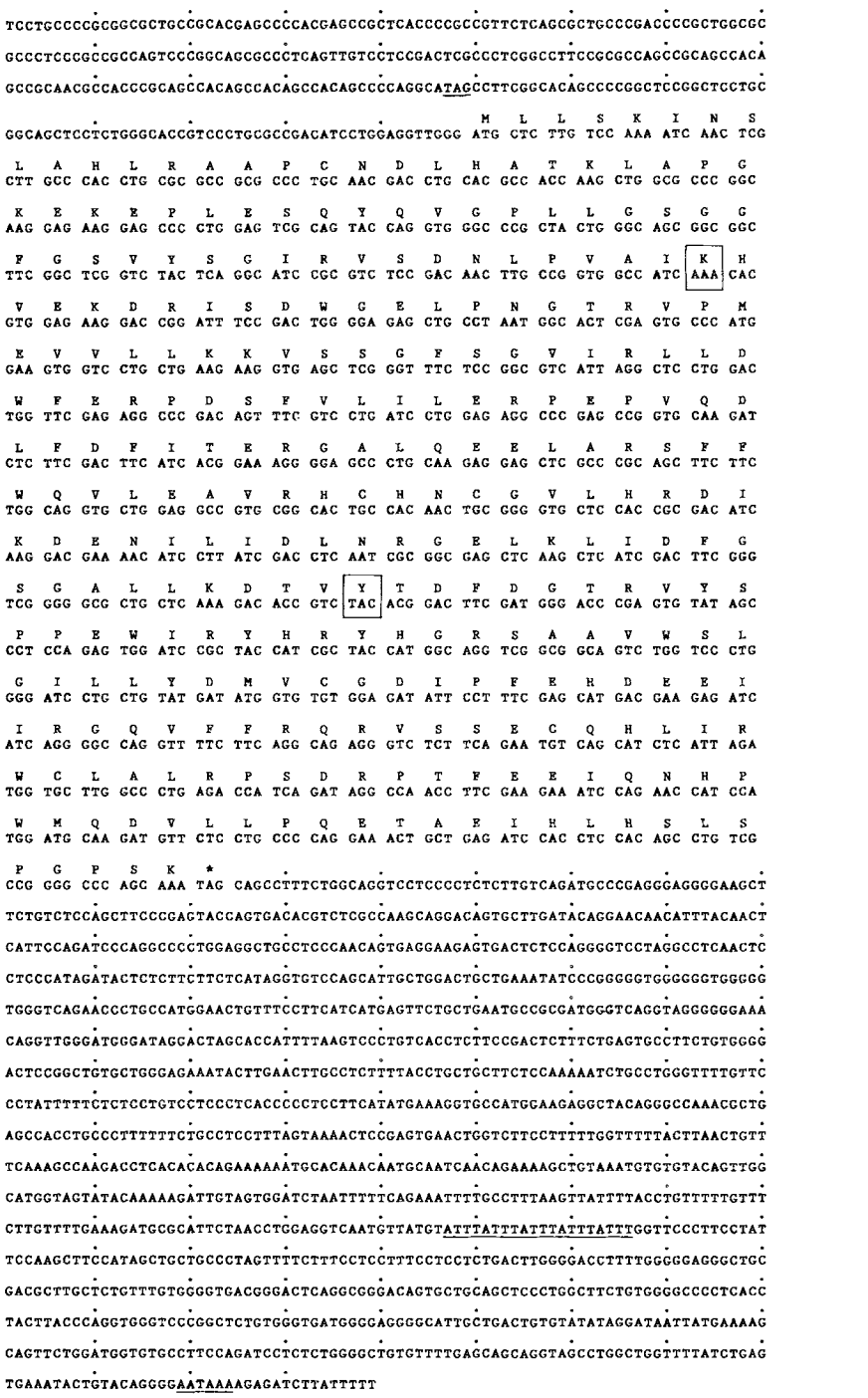
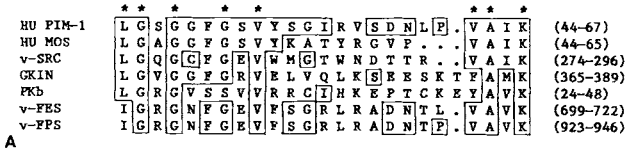


Fig. 1. Amino acid sequence. The amino acid sequence of the PIM-1 protein is deduced from the cDNA clones. The single-letter amino acid code indicates the region of the predicted protein of 313 amino acids. Boxes identify Lys 67 and Tyr 198. The poly A signal is underlined. The cDNA clones contained a 289-bp 5′ untranslated region rich in G and C with a single termination codon (underlined). A 3′ AT-rich repeat is also indicated.

genomic sequencing. We found no alternative translation initiation sites upstream in the transcribed region of the PIM-1 gene. An analysis of the deduced PIM-1 amino acid sequence did not reveal a transmembrane region. The human PIM-1 protein was found to be highly homologous to the mouse PIM-1 protein; 94% of the sequence was identical [18].

A search of the Bionet protein sequence data base revealed that PIM-1 was homologous to a number of the protein kinases, including c-mos from rat, mouse, and man, bovine cGMP-dependent protein kinase, phosphorylase kinase b, gamma subunit, v-ros, v-erb-B, v-kit, v-fes, and v-fps [19–29]. This homology to the protein kinases was most obvious in several regions within the catalytic domain. These regions are indicated in Figure 2. The region from amino acid 44 to 67 is the region of ATP binding [30]. Specifically, the motif LGXGXXG is highly conserved among protein kinases. It is suspected that this sequence aligns with the ribose ring of deoxynucleotide triphosphates [30]. Lysine-67 of PIM-1 is homolgous to lysine-71 of cAMP-dependent protein kinase and lysine-295 of pp60v-src, both of which can be modified by 5′-p-fluorosulphonylbenzoyladenosine (FBSA), the ATP analog [31,32].

The region from amino acid 165 to 204 is also highly conserved among protein kinases (Fig. 2). Specifically, Tyr-198 appears to be homologous to Tyr-416 of pp 60 v-src. It is this tyrosine in the v-src protein that can be autophosphorylated. A tyrosine in a homologous location is present in all tyrosine kinases described to date [30]. The homology that PIM-1 shows to the tyrosine-kinase family in this region would be



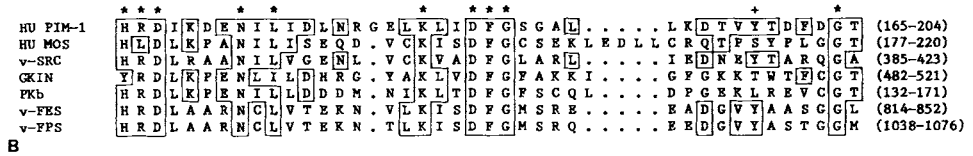


Fig. 2. Homology of PIM-1 with other protein kinases. **A:** The region of PIM-1 from amino acid 44 to 67 is compared to homologous regions of human mos, v-src, G kinase, phosphorylase kinase B-γ subunit, v-fes, and v-fps. This region is the ATP binding region. Asterisks denote highly conserved amino acid positions. Homologous bases are boxed and the amino acid positions used for comparison are noted in parentheses. The lysine at position 67 is highly conserved among all protein kinases. **B:** The central part of the kinase region of PIM-1 is compared to homologous regions of other protein kinases. Homologous bases are boxed and asterisks identify highly conserved amino acids as in Figure 2A. The tyrosine at position 198 (+) is homologous to that of v-src position 416. This tyrosine is conserved in all tyrosine kinases. Note that mos, which is a serine-threonine kinase, does have a tyrosine in this region but without adjacent homologous bases. All alignments are similar to those of Hunter and Cooper [30]. (For sequences, see [21–24, 26, 48–50].)

consistent with the hypothesis that PIM-1 is a tyrosine kinase and Tyr-198 can be autophosphorylated. Further experiments will clarify this issue.

The carboxy-terminal part of the PIM-1 protein has several regions which are homologous to the protein kinases. However, the carboxy-terminus of PIM-1 does not have a tyrosine as do some of the other tyrosine kinases [33,34]. It has been suggested that phosphorylation of a tyrosine in this region negatively regulates these kinases. Furthermore, elimination of the terminal tyrosine by truncation of the protein is directly related to oncogenicity in a number of tyrosine kinases. Therefore, if it can be shown that PIM-1 codes for a tyrosine kinase, the lack of a carboxy terminal tyrosine in the PIM-1 sequence might have important implications for the possible mechanisms of activation and regulation of this proto-oncogene.

Although there are regions of PIM-1 that are highly homologous to the other protein kinases, the overall homology throughout the entire sequence is low. A comparison of the PIM-1 sequence with that of several other protein kinases revealed less than 20% identical amino acids over the full length of the protein. This suggests that PIM-1 has some unique functional role among the protein kinases.

Transcriptional Unit

A schematic of the PIM-1 transcriptional unit is indicated in Figure 3. We defined the site of transcription initiation by S1 nuclease analysis by using mRNA from K562 and GM607. There are six exons and five introns. There are two polyadenylation signals at the 3′ end of the gene, but only one polyA signal is present in our cDNA clones. Apparently the second polyA signal is most frequently used. We predict that the processed mRNA is approximately 2.7 kb in length.

The striking feature about the PIM-1 promotor is the high GC content (greater than 75% from nucleotide -1 to -300). The promotor region does not contain a TATA or CAAT box. However there are seven GC boxes which may be capable of binding the Sp-1 protein [35]. Additionally, the sequence (ATGCAGAT) is present starting at position -248. This sequence is very similar to an octamer found in immunoglobulin enhancer and promotor regions (ATGCAAAT) [36]. This octamer sequence may be important for the regulation of PIM-1 expression.

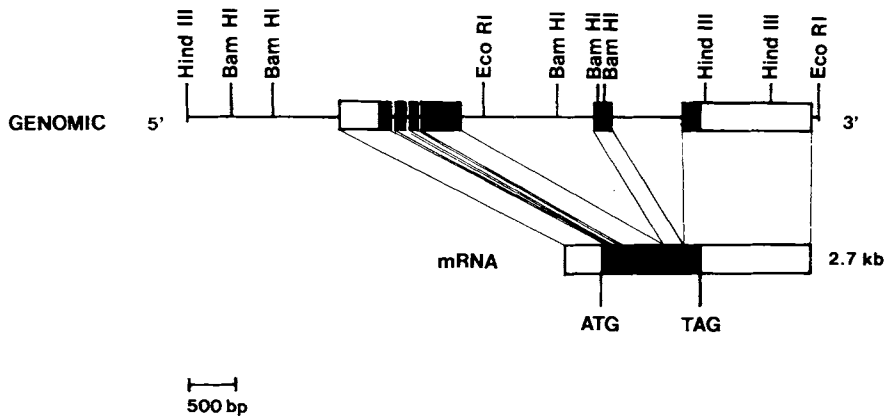


Fig. 3. PIM-1 gene structure. This figure shows the intron/exon structure of PM-1. The mature transcript arises from 5.0 kb of genomic DNA. Boxes indicate the genomic regions represented in the mature transcript and shaded regions represent the protein-coding regions.

Expression in Human Cell Lines

We analyzed over 38 human cell lines for expression of the PIM-1 transcript by Northern blotting. These included four myeloid lines, 19 B lymphocytic lines, seven T lymphocytic lines, and a number of nonhemopoietic lines. Two of the myeloid lines, K562 and KG-1, had the highest levels of PIM-1 mRNA (see Fig. 3). Among the other myeloid lines, ML3 had moderate levels and HL-60 had undetectable levels. Fourteen of 19 B lymphocytic lines were positive. Surprisingly, none of the seven T cell lines and only one of nine nonhemopoietic lines expressed PIM-1. Therefore the PIM-1 gene is expressed selectively in myeloid and B lymphoid lines, perhaps in a manner dictated by the stage of differentiation of these lineages.

We additionally compared the mouse and human PIM-1 mRNA to confirm that the size of the transcript was conserved. The transcript from WEHI-3B, a mouse myeloid line, was the same size as the human PIM-1 transcript (Fig. 4).

DISCUSSION

In this report we describe the cDNA cloning of the human PIM-1 gene and the characterization of the gene locus. The human PIM-1 gene was found to code for a 313 amino acid protein in the K562 cell line. This protein showed significant homology to a number of the protein kinases. This protein has a tyrosine at position 198 which may be homolgous to Tyr-416 of pp60 v-src. This finding is consistent with the hypothesis that PIM-1 is a tyrosine protein kinase rather than a serine-threonine kinase. No transmembrane region was identified.

The mature PIM-1 mRNA was found to be approximately 2.7 kb in length. The gene consisted of six exons and five introns. The promotor region was unusual in that no TATA or CAAT box was identified. The promotor is therefore similar to a small number of genes that include hydroxy-methyl glutaryl coenzyme A reductase (HMG CoA reductase), adenosine deaminase (ADA), hypoxanthine phosphoribosyl transferase (HPRT), adenosine phosphoribosyl transferase (APRT), 3-phosphoglycerate kinase (PGK), and dihydrofolate reductase (DHRF) [37–42]. The reason that this family of genes has this unusual promotor structure will be an area of future investigation.

The expression of the PIM-1 gene is unusual because it is primarily restricted to myeloid and B lymphoid cell lineages. In the mouse it has been shown that normal T cells and T cell tumors express pim-1 [7,43]. Our data show that human T cell lines do not express PIM-1 in appreciable amounts. This discrepancy in lineage expression is surprising for a gene that is so highly conserved. Further study of this issue is in progress.

One of our primary interests in the human PIM-1 gene locus relates to its disruption by chromosomal abnormalities or viral insertions. Chromosomal abnormalities of chromosome 6p have been reported in myeloid and undifferentiated leukemia, T cell lymphoma, and malignant melanoma [44–47]. Certainly, a number of tumor-associated viruses (HIV, HTLVI, HBLV, etc) might integrate in this region, as occurs in retroviral infection in mouse leukemogenesis. In our studies of a small number of human cancer samples to date, we have been unable to find abnormalities of this locus by Southern blotting. Nonetheless, we suspect that such abnormalities exist and plan to study a larger number of patients with probes that span the entire PIM-1 gene locus and, if necessary, to employ reversed-field gel electrophoresis to identify cancer samples with abnormalities of this gene.

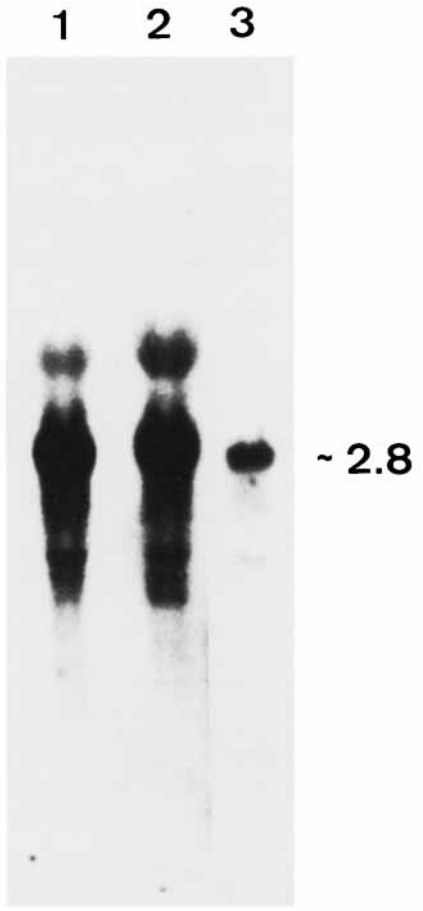


Fig. 4. Expression of the PIM-1 gene. Expression of the PIM-1 gene was analyzed by Northern blotting. A band of approximately 2.8 kb was found in K562 (**lane 1**), KG-1 (**lane 2**), and WEHI-3B (a mouse myeloid line, **lane 3**).

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