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Characterization of the Human Full-Length *PTK7* cDNA Encoding a Receptor Protein Tyrosine Kinase-Like Molecule Closely Related to Chick KLG[1]

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A 220-bp fragment of *PTK7* cDNA was previously cloned from normal human melanocyte RNAs by means of the reverse transcription-polymerase chain reaction [Lee, S.-T., Strunk, K.M., and Spritz, R.A. (1993) *Oncogene* 8, 3403-3410]. We now report the cloning of the human full-length *PTK7* cDNA and its characterization. The 1,070-amino acid PTK7 polypeptide deduced from the cDNA sequence constitutes receptor protein tyrosine kinase (RPTK), but has several unusual residues in some of the highly conserved tyrosine kinase motifs. *PTK7* mRNA was expressed at the highest level in a human erythroleukemia cell line among tested samples, and at relatively high levels in liver, lung, pancreas, kidney, placenta, and melanocytes. Human PTK7 is 72% identical to chick KLG, suggesting that PTK7 is homologous or possibly orthologous to chick KLG, and that these represent a new subfamily of RPTKs.

Key words: full-length cDNA, mRNA expression, PTK7, RPTK-like molecule, transmembrane receptor.

Receptor protein tyrosine kinases (RPTKs), a class of cell-surface receptors, that transduce extracellular signals across the cell membrane, play important roles in regulating cell proliferation, migration, and differentiation. Many RPTKs bind secreted, soluble polypeptide ligands known as growth factors, but some RPTKs are also activated by membrane-bound proteins or extracellular matrix proteins (for reviews, see Refs. *1* and *2*).

The catalytic domains of both receptor and non-receptor protein tyrosine kinases have been highly conserved throughout evolution, and 11 highly conserved tyrosine kinase subdomains have been recognized (*3*). Some members of the RPTK family, often referred to as RPTK-like or RPTK-related molecules, have unusual amino acid residues in some of the highly conserved motifs known to be essential for kinase activity. These motifs include the GXGXXG motif within subdomain I, which acts as a clamp anchoring the non-transferable phosphates of ATP (*4*), and the DFG triplet in subdomain VII, which chelates the Mg^{2+} ion that bridges the β- and γ-phosphates of ATP, thereby helping to orient the γ-phosphate for transfer (*4*). Examples of such RPTK-like molecules include *Drosophila* Dtrk (*5*) and chick KLG (*6*), in which the DFG triplet is modified, and human Ror1, Ror2 (*7*), and RYK (murine homologues RYK/MRK/Vik/Nbtk-1/Nyk-r) (*8-14*), in which both the DFG triplet and the GXGXXG motif are modified.

A 220-bp fragment of *PTK7* cDNA corresponding to tyrosine kinase subdomains VIb to IX was first identified during an extensive survey of tyrosine kinase mRNAs expressed in normal human melanocytes (*15*). In the deduced PTK7 amino acid sequence the DFG triplet in subdomain VII was replaced by the sequence, ALG. Here we report the cloning of full-length *PTK7* cDNA, its complete nucleotide sequence, and its expression in various tissues. We compare the deduced amino acid sequence of PTK7 with peptide sequence databases, and we discuss possible functional roles of PTK7.

To obtain full-length *PTK7* cDNA, we first screened a λgt10 human SV-80 transformed fibroblast cDNA library (*16*) using the 220-bp fragment of the *PTK7* cDNA (*15*) as a probe and subsequently the 5′-end fragment of the isolated λ *PTK7* cDNA clones. Four overlapping λ clones which encompass the full-length *PTK7* cDNA were isolated by screening approximately 6×10^5 phages. Inserts from the λ clones were subcloned into pUC19 and restriction-mapped, and restriction fragments of the inserts were subcloned into M13mp18 and M13mp19, and both strands sequenced (*17*).

The full-length *PTK7* cDNA extends for 4,187 nucleotides (Fig. 1). The cDNA contains a long open reading frame of 3,213 bp (nucleotides 150-3362) flanked by a 149-bp 5′-untranslated sequence and an 825-bp 3′-untranslated sequence containing an apparent polyadenylation signal (nucleotides 4169-4174). The deduced PTK7 polypeptide consists of 1,070 amino acid residues, with a calculated molecular mass of 118 kDa (Fig. 1). The nucleotide sequence surrounding the first methionine codon is

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Abbreviations: bp, base pair(s); FGF, fibroblast growth factor; kb, kilobase; NGF, nerve growth factor; RPTK, receptor protein tyrosine kinase.

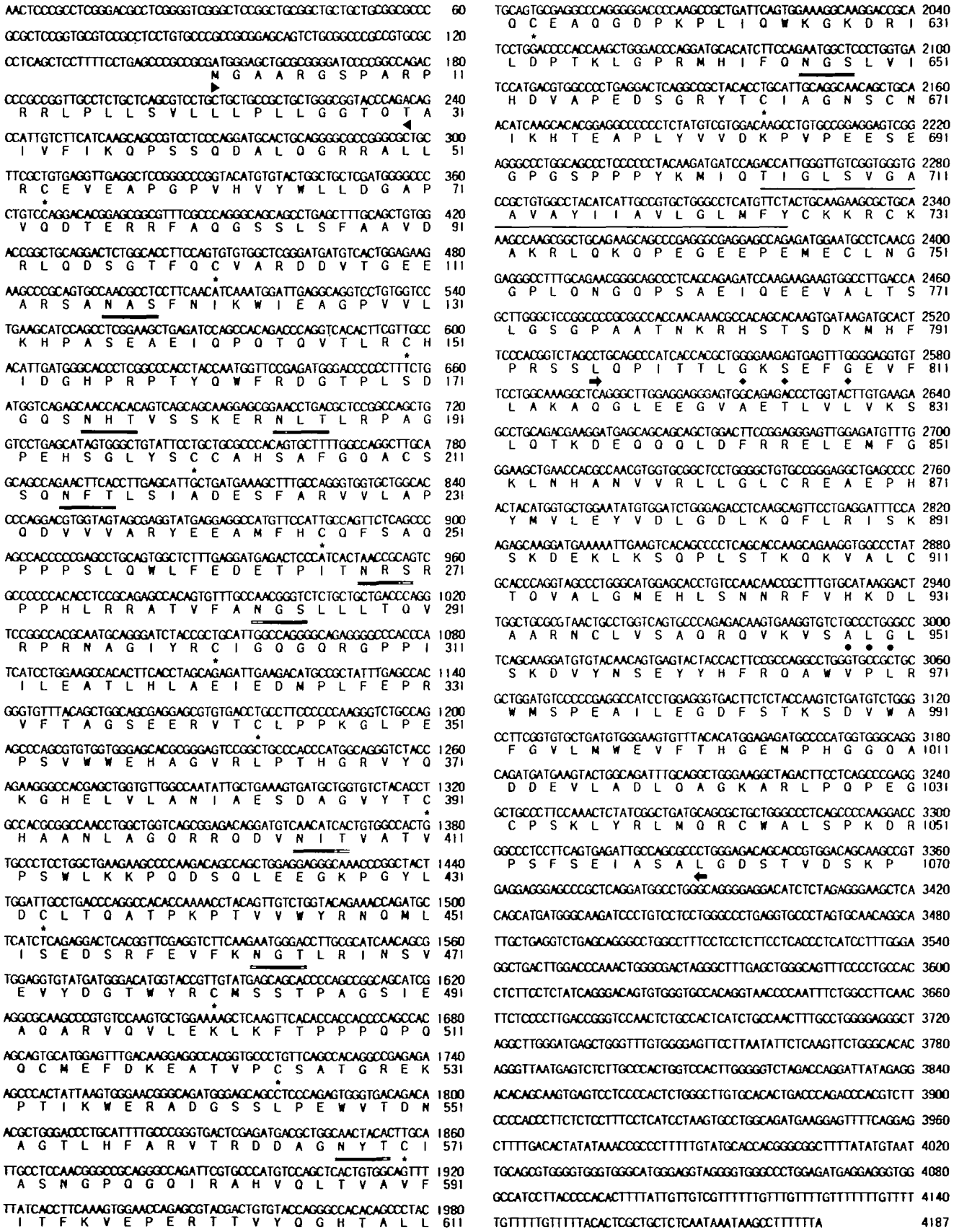

Fig. 1

characteristic of translation initiation contexts found in mammals (*18*). The initiating methionine codon was followed by an amino-terminal signal peptide, an extracellular domain, a transmembrane domain, and an intracellular domain. The extracellular domain (amino acids 31-703) contains seven immunoglobulin-like loops (*19*) and ten putative *N*-glycosylation sites (NXS/T, X can be any amino acid except proline). The intracellular domain (amino acids 726-1070) contains typical structural features of a catalytic domain of tyrosine kinase (referred to as "the catalytic domain" below; amino acids 796-1061) (*3*). However, 7 of the 40 consensus residues of typical tyrosine kinases (*3*) are altered in PTK7 (Fig. 2). In particular, the second glycine residue of the GXGXXG motif in subdomain I (amino acids 803-808) was substituted by serine. In addition, the aspartate and phenylalanine residues of the DFG triplet (amino acids 948-950) in subdomain VII were replaced by alanine and leucine, respectively.

The PTK7 amino acid sequence was compared with the polypeptide sequence databases by BLAST analysis (National Center for Biotechnology Information, USA), and the sequences showing high homology were aligned with PTK7 by means of PROSIS software (Hitachi, USA). The comparison demonstrated that PTK7 exhibits maximum homology with chick KLG (*6*); 72.0% identity over the entire polypeptide (Fig. 2). The catalytic domain (83.8% identity over the 266-amino acid overlap) is more conserved than the extracellular domain (66.8% identity over the 674-amino acid overlap) in the two proteins. Seven putative immunoglobulin-like loops, and 7 of the 10 possible *N*-glycosylation sites in the extracellular domain of PTK7 are also found at homologous positions of chick KLG. In the catalytic domain of chick KLG, 5 of the 40 tyrosine kinase consensus residues are altered; the DFG triplet in subdomain VII is replaced by ALS, similarly to the ALG sequence in PTK7, but the GXGXXG motif in subdomain I is conserved. Although chick KLG is more conserved in structural features characteristic of active tyrosine kinases than PTK7, no kinase activity was detected for chick KLG (*6*). PTK7 is thus also likely to lack tyrosine kinase activity.

In addition to chick KLG, proteins showing limited homology with PTK7 include (i) members of the fibroblast growth factor (FGF) receptor family: FGF receptor 3 (identical amino acids, human 28.1% and mouse 27.5%) (*20, 21*) and FGF receptor 2 (chick 26.3%, mouse 25.9%, and human 25.8%) (*22-24*); (ii) members of the nerve growth factor (NGF) receptor family: NGF receptor/TrkA (human 27.0% and black rat 25.6%) (*25, 26*), TrkB (mouse 23.9% and rat 23.5%) (*27, 28*), TrkC (human 25.3% and chick 23.3%) (*29, 30*), and *Drosophila* Dtrk (26.1%), which is a neural cell adhesion molecule highly related to mammalian NGF receptors (*5*). The catalytic domain of PTK7 (amino acids 796-1061) showed general homology with many tyrosine kinases, the highest with *Drosophila* Dtrk (identical amino acids 40.9%), except chick KLG. Interestingly, the extracellular domain of PTK7 (amino acids 31-703), which determines the ligand specificity, showed higher homology with those of cell adhesion molecules of

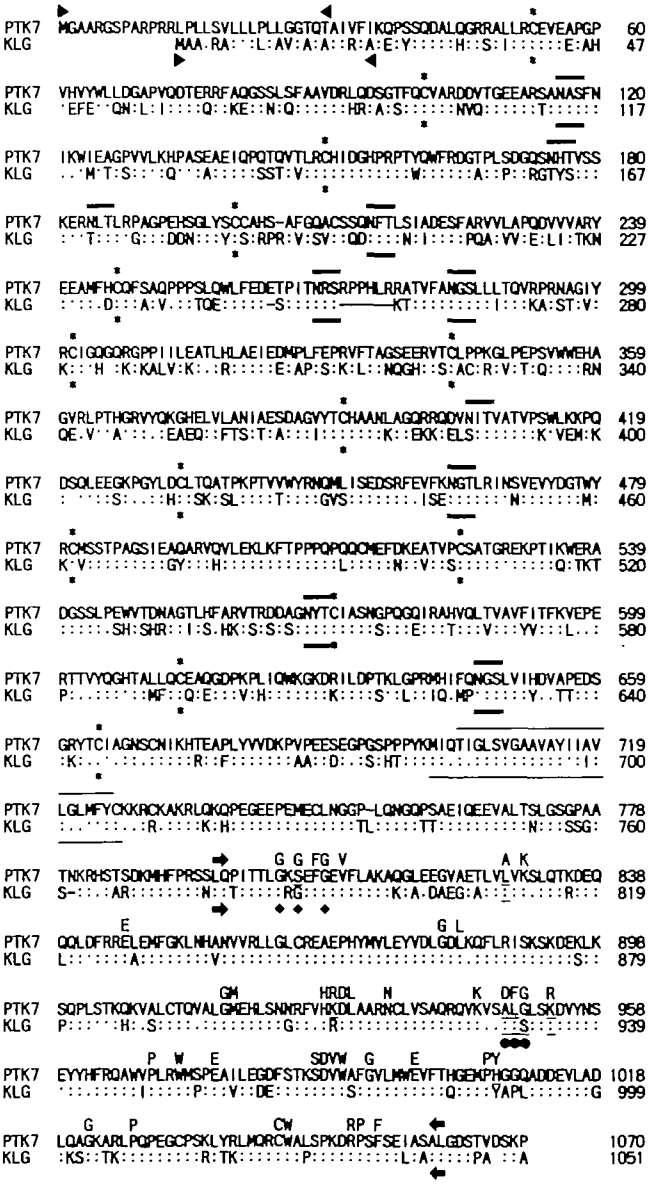

Fig. 1. **Nucleotide sequence of the human *PTK7* cDNA and its deduced amino acid sequence.** Amino acids are shown in a single-letter code below the nucleotide sequence. Arrowheads indicate the beginning and end of the signal peptide determined according to von Heijne (*34*); asterisks, cysteine residues involved in the formation of 7 putative immunoglobulin-like loops, identified according to the criteria of Williams and Barclay (*19*); double solid lines, possible *N*-glycosylation sites; solid line, transmembrane domain, determined according to Klein *et al.* (*35*); arrows, the beginning and end of the catalytic domain; closed diamonds, positions of glycine residues in the GXGXXG motif; closed circles, position of the DFG triplet; and dotted line, polyadenylation signal.

Fig. 2. **Evolutionarily conserved residues in the catalytic domain of PTK7, and alignment of the amino acid sequences of PTK7 and KLG.** The most highly conserved 40 amino acid residues identified by Hanks and Quinn (*3*) in the catalytic domains of known tyrosine kinases are shown over the amino acid sequence of PTK7, and the amino acid residues in PTK7 and KLG that are different from these highly conserved amino acid residues are underlined. Colons denote amino acid residues identical between human PTK7 and chick KLG, and dashes represent gaps introduced to achieve maximal alignment. Other symbols are the same as in Fig. 1. In the chick KLG amino acid sequence, the positions of the signal peptide and the transmembrane domain are indicated according to Chou and Hayman (*6*).

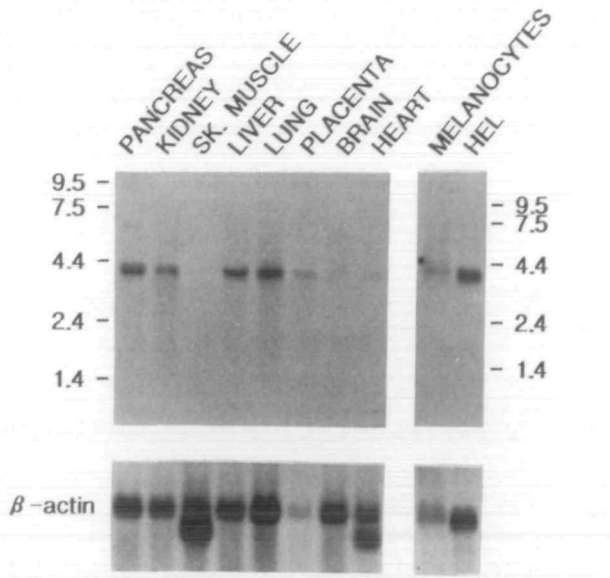

Fig 3 **Northern blot hybridization analysis of *PTK7* mRNAs.** Northern blots containing 2 μg of poly(A)$^+$ RNA isolated from the indicated human tissues (MTN Blot; Clontech, USA) and from two cell lines, normal human melanocytes and HEL erythroleukemia cells, were hybridized as described (*36*) The final washing of the blots was performed at 60°C in 0.1×SSPE, 0 5% SDS. Hybridized blots were autoradiographed for 5 days for PTK7 and for 4 h for β-actin Hybridization signals were quantitated by scanning densitometry and normalized as to those of β-actin mRNA Top panel, *PTK7* cDNA probe, and bottom panel, β-actin cDNA probe The sizes of RNA markers are indicated.

the immunoglobulin superfamily, such as *Drosophila* Dtrk (26.9%), neural cell adhesion molecule L1 (human 26.2% and mouse 25.2%) (*31, 32*), and chick axonin 1 (25.5%) (*33*), than to RPTKs for growth factors *per se*.

We examined the expression of *PTK7* mRNA in various tissues and cell lines by northern blot analysis using a ^{32}P-radiolabeled 3.2-kb *PTK7* cDNA (nucleotides 1010-4187) as a probe (Fig. 3). As expected, the transcript size was 4.2 kb. The levels of the *PTK7* mRNA were relatively low when compared with levels of β-actin mRNA as a reference. In tissues, *PTK7* mRNA was expressed at relatively high levels in liver, lung, pancreas, kidney, and placenta, at relatively low levels in brain and heart, and at barely detectable levels in skeletal muscle. In cell lines, *PTK7* mRNA was detected at the highest level in a human HEL erythroleukemia cell line and at a relatively high level in normal cultured melanocytes.

Together, our results suggest that PTK7 is a member of the RPTK family, but that it most likely lacks the catalytic activity of tyrosine kinase. Such proteins are generally called "RPTK-like" proteins. Among known RPTK-like proteins, PTK7 is most closely related to chick KLG, whose function is not known. Human PTK7 and chick KLG show 72% amino acid sequence identity. Considering that orthologues of human and chick RPTKs exhibit an average 82.5±10.4% (mean±standard deviation) amino acid sequence identity (n=6), PTK7 and KLG are likely to be human and chick orthologues, together representing a new subfamily of RPTK-like proteins. However, *PTK7* mRNA was detected at a relatively high level in human liver, in contrast to KLG, which is not expressed in liver (*6*). Therefore, we cannot exclude the possibility that PTK7 and KLG, although very similar, are not orthologous.

At present, we do not know the biological function of PTK7, which most likely lacks tyrosine kinase catalytic activity. It is intriguing that the extracellular domain of PTK7 is more closely related to those of various cell adhesion molecules than to those of RPTKs for growth factors. In addition, both the extracellular and catalytic domains of PTK7 are most closely related to those of *Drosophila* Dtrk, a neural cell adhesion molecule (*5*), except chick KLG. Accordingly, we speculate that PTK7 may function as a cell adhesion molecule.

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