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FLT4, a Novel Class III Receptor Tyrosine Kinase in Chromosome 5q33-qter

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Abstract

The receptors for at least two hematopoietic growth factors, namely the stem cell factor and colony-stimulating factor 1, belong to class III receptor tyrosine kinases. Here we describe cloning of a partial complementary DNA for FLT4, an additional member of this gene family from human leukemia cells. The FLT4 tyrosine kinase domain is 79% homologous with the previously cloned FLT1 (M. Shibuya et al., Oncogene, 5: 519-524, 1990) tyrosine kinase and maps to the chromosomal region 5q33-qter. We have found FLT4 expression in human placenta, lung, heart, and kidney, whereas the pancreas and brain appeared to contain very little if any FLT4 RNA. The results suggest that FLT4 functions in multiple adult tissues.

Introduction

We have been interested in RTKs expressed in megakaryoblastic leukemia cell lines because the factors regulating the proliferation and differentiation of megakaryoblasts are poorly known and knowledge of their receptors could eventually lead to their identification (1). Two known hematopoietic growth factors, the stem cell factor and colony-stimulating factor 1, are known to exert their effects on target cells through binding and activating specific protooncogene-encoded receptor tyrosine kinases, named c-kit and c-fms, respectively (see Refs. 2 and 3). These receptors, together with the platelet-derived growth factor receptors α and β, belong to class III RTKs (4, 5). Members of this class are characterized by extracellular domains composed of five so-called immunoglobulin-like loops and an intracellular tyrosine kinase domain interrupted by a kinase insert sequence (6). As our approach to studies of the relevant RTKs, we have used polymerase chain reaction cloning of novel tyrosine kinases expressed by leukemia cells with megakaryoblastic differentiation potential. Earlier we reported the cloning of several RTKs from the K562 leukemia cell line which retains the potential for erythroid/megakaryoblastic differentiation (1, 7). However, none of the genes we cloned previously was specific for the megakaryoblastic cell lineage. We have therefore searched for additional RTKs in the HEL erythroleukemia cell line, which also has a dual erythroid/megakaryoblastic phenotype and is inducible to further expression of megakaryoblastic markers by treatment with the tumor promoter 12-O-tetradecanoylphorbol-13-acetate (8). Here we report the identification, chromosomal mapping, and partial characterization of a novel member of RTK class III from this cell line.

Results and Discussion

Polymerase chain reaction amplification of tyrosine kinase-related sequences from the HEL cell cDNA library resulted in the identification of clone OTK-1, which did not correspond to any reported sequence in the available databases. The subdomain VIII (14) WMAPE amino acid sequence motif deduced from the amplified nucleotide sequence of OTK-1 contained the methionine residue indicative of transmembrane tyrosine kinases. The HEL cell cDNA library was therefore screened with the probe, positive clones were isolated, and the region of cDNA corresponding to the putative tyrosine kinase and transmembrane domains was sequenced. The deduced amino acid sequence of the open reading frame located with sequences of class III RTKs (Fig. 1). Furthermore, the FLT1 and FLT3/FLK2 RTK cDNAs (14–17), encoded by loci in human chromosome band 13q12 (18, 19), appeared as its closest homologues. The FLT1 cDNA was originally cloned by Shibuya et al. (15) as a c-fms-related RTK from a human placental cDNA library. The FLT3 and FLK2 mouse cDNAs were cloned independently by two groups (16, 19), but sequence comparisons showed that they represent the same gene, the human homologue of which was isolated and chromosomally mapped. As also the name FLT2, referring to the FGR1 (fg) gene, has appeared in the literature (albeit transiently; see Rosnet et al., 1991 (19), we have named our novel tyrosine kinase FLT4 to avoid any confusion.

As can be seen from Fig. 1, the deduced FLT4 polypeptide has a putative transmembrane region followed by a sequence homologous to the tyrosine kinase domains of other members of this receptor family. This domain is divided into two distinct

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2 The abbreviations used are: RTK, receptor tyrosine kinase; cDNA, complementary DNA.

3 O. Aprelikova et al., unpublished data.
NOVEL RECEPTOR TYROSINE KINASE

FLT4

Fig. 1. Deduced amino acid sequence of the cytoplasmic domain of FLT4 and its comparison with the corresponding FLT1 and FLT3/FLK2 receptor tyrosine kinase sequences (IS. 16, 19) (only differing residues are marked). The amino acid sequences were aligned and a few gaps (dashes) were introduced for optimal homology. The putative transmembrane domains have been underlined and the characteristic residues for binding of ATP and autophosphorylation as well as the kinase insert are marked with boldface letters.

regions, TK1 and TK2, by a 65-amino acid kinase insert sequence (boldface letters in Fig. 1). The putative ATP binding site in the TK1 domain and autophosphorylation site (Y) in the TK2 domain are also marked with boldface letters in Fig. 1. The TK1 and TK2 domains of FLT4 are 78 and 80% homologous with the corresponding domains of FLT1 and 59 and 55% homologous with the corresponding domains of FLT3/FLK2.

The degree of homology is less with other members of RTK class III.

It was of great interest to determine the chromosomal localization of FLT4 because some clustering of class III receptor genes has been found to take place (19). Thus rodent-human cell hybrids were analyzed, indicating linkage of FLT4 to human chromosome 5 (data not shown). Then regional assignment on Chromosome 5 was determined using hybrids carrying partial chromosome 5s as shown in Fig. 2. The presence of human chromosome 5q33-qter in the hybrids correlated with the presence of FLT4 sequences. The regional mapping results indicated that the FLT4 locus is telomeric to the CSFlR/platelet-derived

Fig. 2. Localization of the FLT4 gene in the region 5q33 →qter. The portions of chromosome 5 retained in the different hybrid cell lines indicated on the top abscissa are sketched to the right of the chromosome 5 ideogram (12, 13, 20). These hybrids were tested for presence of the FLT4 locus by filter hybridization and the results are shown below the sketches. The region of chromosome 5 common to FLT4-positive hybrids and absent from the FLT4-negative hybrids is 5q33.1-qter.

Fig. 3. FLT4 mRNA expression in adult human tissues. Two pg of polyadenylated RNA from the indicated tissues (Multiple Tissue Northern Blot; Clontech Inc.) were analyzed by Northern blotting and hybridization with FLT4 cDNA probe. The estimated sizes of the transcripts are shown on the left in kilobases (kb). Control hybridizations with probes for constitutively expressed genes showed an even loading of the lanes (data not shown).
growth factor receptor β (PDGFRB) locus as well as to the β-adrenergic receptor (ADRBβ) locus since these loci are all present in the hybrid GB13, which was negative for FLT4.

Hybridization of polyadenylated RNA from various human tissues with the FLT4 cDNA fragment showed mRNA bands of 5.8- and 4.5-kilobase mobility and a weakly labeled band of 6.2 kilobases in placenta, lung, heart, and kidney. faint mRNA bands were seen in the liver and skeletal muscle, whereas the pancreas and brain appeared to contain very little if any FLT4 RNA (Fig. 3). Although high amounts of FLT1 and FLT3 mRNAs have also been reported in the placenta, differences in the expression patterns of FLT4 and the previously cloned FLT1 and FLT3/FLK2 genes are exemplified by the absence of FLT4 mRNA signals in the brain sample, where abundant FLT3 mRNA and some FLT1 mRNA have been reported (15, 17). Also, no FLT3 mRNA has been seen in the heart, where FLT4 expression was present. Interestingly, FLT3/FLK2 mRNA has also been detected in hematopoietic progenitor-enriched cell populations from fetal mouse liver (16).

The FLT4 sequence is interesting, because it is homologous to two hematopoietic receptor tyrosine kinases, because the FLT4 mRNA is expressed in a restricted set of leukemia cells, and because the FLT4 gene maps to the distal long arm of chromosome 5, near a region where a variety of growth factors and growth factor receptors have previously been located (20). We are currently analyzing possible lesions of this locus in hematopoietic dyscrasias and malignancies with deletions and translocations of this region of chromosome 5.

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References


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