Regional localization of rat and mouse protein-tyrosine phosphatase PTPα/LRP gene (Ptpra) by fluorescence in situ hybridization

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ABSTRACT

Protein tyrosine phosphatases (PTPases) negatively regulate the effect(s) of protein tyrosine kinases and are implicated in the regulation of a variety of biological events including cell activation, differentiation, and neoplastic transformation. To gain insight into the role(s) of the PTPases, we mapped the gene encoding for the widely expressed receptor-like protein tyrosine phosphatase PTPα/LRP (locus symbol Ptpra) to rat chromosome 3q36 and mouse chromosome 2G by fluorescence in situ hybridization method. These results indicate that there is a conserved syntenic group between human 20p13, rat 3q36, and mouse 2G.

1. INTRODUCTION

Reversible phosphorylation of tyrosine residues of proteins is regulated by both protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPases). Both classes of enzyme are thought to be involved in integrating external stimuli into the cells, control of cell growth and differentiation, and malignant transformation. Several protein tyrosine phosphatases have been identified and molecularly cloned, and appeared to be conserved during the course of evolution. Both transmembrane as well as cytoplasmically localized PTPases have been identified.

The widely expressed receptor-like protein tyrosine phosphatase a (also known as LCA-related phosphatase, LRP) is a transmembrane PTPase with two cytoplasmic PTP domains and a short extracellular domain (Sap et al., 1990). PTPα/LRP has been cloned from human (Jirik et al., 1990; Kalpan et al., 1990), murine

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(Matthews et al., 1990; Sap et al., 1990), and rat (Moriyama et al., 1992) cDNA libraries. Northern blot analysis revealed a ubiquitous pattern of expression of PTPα/LRP mRNA. The tissue distribution pattern of PTPα/LRP mRNA in rat is similar to that in mouse, with brain showing the highest levels followed by heart, kidney, and lung (Matthews et al., 1990; Moriyama et al., 1992).

PTPα/LRP (locus symbol PTPRA, previously PTTPA and PTPL2) has been mapped to human chromosome 20 (Jirik et al., 1990) and more specifically to 20pter-q12 (Kalpan et al., 1990) and 20p13 (Jirik et al., 1992). The murine PTPα/LRP was mapped to mouse chromosome 2, closely linked to the murine Il-1α and Bmp-2α (Sap et al., 1990). No other species was reported on chromosomal location of PTPα/LRP.

The regional mapping of gene encoding PTPα/LRP on rat and mouse chromosomes is of considerable interest in their genomic organization during mammalian evolutionary history, as well as its usefulness in comparative analysis of consistent cytogenetic abnormalities observed in human, rat, and mouse neoplastic cells. In the present study we assigned the gene encoding PTPα/LRP to rat and mouse chromosomes.

2. MATERIALS AND METHODS

The probe used in this study was the EcoRI fragment (1772 bp) of the coding region of the mouse PTPα/LRP cDNA (Matthews et al., 1990; Sap et al., 1990). It was isolated by the screening of cDNA library prepared from mouse NIH-3T3 fibroblast using a mouse LAR cDNA fragment (unpublished data) as a probe. The identity of the fragment with the EcoRI fragment of mouse PTPα/LRP cDNA was established by DNA sequencing. The procedure used for fluorescence in situ hybridization (FISH) method was previously described (Saadat et al., 1995). In brief, the cDNA probe was labeled with biotin-16-dUTP (Boehringer Mannheim) using nick-translation reaction. Because this fragment was conserved between rat and mouse (Matthews et al., 1990; Moriyama et al., 1992; Sap et al., 1990), it was used as probe for gene assignment in both species. The probe was hybridized separately to metaphase chromosomes which were prepared from primary cultures of skin and lung tissues of WKAH rat and DRC mouse, respectively. Chromosomal DNA was denatured by treatment in 70% formamide/2×SSC (1×SSC; 150 mM sodium chloride, 15 mM sodium citrate, pH 7.0) at 70°C for 2 min, and followed by dehydration in ethanol. The hybridization mixture was 40 ml/slide of 50% formamide, 4×SSC (6×SSC for rat slides), 10% dextran sulphate, and 1% bovine serum albumin containing 600–900 ng denatured labelled cDNA probe. Hybridization of probe to denatured chromosome preparation was carried out overnight at 37°C in a humidified chamber. Amplification and fluorescence detection were carried out by use of fluorescein isothiocyanate-conjugated avidin (avidin-FITC) and biotinylated anti-avidin D as described
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previously (Saadat et al., 1995). Slides were counterstained with propidium iodide and evaluated by fluorescence microscopy. Metaphase chromosomes were identified by Q-bands after staining with quinacrine and Hoechst 33258. The chromosome terminology of ISCN (1978) was applied; i.e. human, rat, and mouse chromosomes by HSA, RNO, and MMU, respectively.

3. RESULTS AND DISCUSSION

Hybridization with the mouse cDNA probe yielded a clear specific hybridization signals on rat chromosomes. Examination of 60 rat metaphases indicated that double-spot signals on both sister chromatids specifically observed in 46 (about 76%) of metaphases to the region q36 of rat chromosome 3, thus concluded that the rat Ptpra located on RNO3q36 (Fig. 1 a-c). For localization of mouse Ptpra, a total of 45 mouse metaphases were examined, in which 38 (about 85%) had double-spot signals on both sister chromatids of chromosome 2 at region G (Fig. 1d-f), confirming the previous assignment (Sap et al., 1990). Thus, the mouse Ptpra was regionally mapped to MMU2G. It should be mentioned that no specific signals were observed on any other rat or mouse chromosome or their chromosomal segment.

It is pointed out that there is similarities in the chromosomal banding patterns of RNO3 when compared to MMU2 (Nesbitt, 1974; Yoshida, 1978). In spite of the fact that this similarity extends to homology on the gene level, the conserved segment among these two species is not defined, because the regional assignment is not made on the RNO3 (Levan et al., 1991). Our results demonstrate, however, that the PTPα/LRP gene is a member of a conserved syntenic group between RNO3q36 and MMU2G. PTPα/LRP gene was mapped on HSA20p13 (Jirik et al., 1992), taken together, there is a conserved segment between HSA20p13, MMU2G, and RNO3q36.

Although Il-1α, Bmp-2 and Ptpra were closed linked on the mouse chromosome 2, each locus of the Il-1α (human symbol, IL1A) and Bmp-2 (human symbol, BMP2) was separated on human chromosomes (IL1A on human 2q12-q21 and BMP2 on human 20p12, respectively). Therefore, the mouse chromosomal regions around the Ptpra and Bmp-2a genes are syntenic to human chromosomes 2 and 20, respectively.

Although PTPases have been considered as anti-oncogens, recent reports indicate that PTPα/LRP plays a crucial role on positive regulation in neuronal differentiation, cell transformation and tumorigenesis. Ecotopic expression of functional PTPα in P19 cells lead it to neuronal differentiation after retinoic acid treatment through the activation of pp60c-src, an oncogene product playing a crucial role in P19 cell differentiation, due to direct dephosphorylation on regulatory Tyr527 residue by PTPα (den Hertog et al., 1993). Furthermore, PTPα-overexpressed embryonal fibroblasts were led to the activation of pp60c-src
Fig. 1. Chromosomal localization of the rat (a-c) and mouse (d-f) Ptpra by FISH. The chromosomes were stained with propidium iodide (a and d) and Q-banded (b and e). Arrows indicate the positions of hybridization signals.
through dephosphorylation on Tyr527 with concomitant cell transformation and tumorigenesis (Fallen et al., 1983). These facts suggest that overexpression of PTPα/LRP affects the normal cell growth and differentiation in cooperation with oncogenes.

It is of particular interest that the gene encoding PTPα/LRP located near the fragile sites on human, rat, and mouse chromosomes (Djalali et al., 1987; Hecht et al., 1988; Robinson et al., 1987). Chromosomal abnormalities of these regions have been described in various types of leukemia and solid tumors, including fibrosarcoma, renal cell carcinoma, hepatoma, and transformed tracheal epithelial cells (Herens et al., 1992; Chen et al., 1993; Endo et al., 1990; Maeda et al., 1980; Hecht, 1988). However, the corresponding breakpoints and fragile sites on each chromosome have not been characterized at the molecular level. Further studies are necessary to clarify what mutation event(s) or genetic change(s) in the gene for the PTPα/LRP together with genes at juxtapeditions in the affected abnormal chromosomes is essential for neoplastic transformation.

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REFERENCES


