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Characterization and comparison of the intronic promoter of murine osteoclastic protein-tyrosine phosphatase, PTP-oc, with the human PTP-oc promoter

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Abstract

This study characterized the murine osteoclastic protein-tyrosine phosphatase (PTP-oc) promoter located within intron 12 of the Glepp1 gene. A 2-kb DNA fragment containing the putative intronic promoter showed strong promoter activity in pre-osteoclastic RAW264.7 and U937 cells, but not in non-osteoclastic cells. Deletion analyses identified a proximal region with elements required for basal activity, and upstream repressor and enhancer elements. The cell-type-specificity of the promoter was conferred by upstream domains. At least nine conserved response elements, with potential transcription factor binding sites, were identified in both human and murine promoters. EMSA and ChIP indicate the presence of occupied binding sites for Pit-1a, Ikaros-1/2, and D1DR transcription factors in the murine promoter. Site-directed mutagenesis of response elements resulted in down- or up-regulation of promoter activity: some of the effects were different between the murine and human promoter, suggesting that there may be inter-species differences in the regulation of the PTP-oc promoter.

Keywords: Promoter; Protein-tyrosine phosphatase; Osteoclasts; Site-directed mutagenesis; Deletion analysis; Chromatin immunoprecipitation

Our laboratory has previously cloned an osteoclastic protein-tyrosine phosphatase, termed PTP-oc, from a rabbit osteoclast cDNA library [1]. PTP-oc is a structurally unique transmembrane PTP[1]: it has a very short (eight amino acid residues) extracellular domain, lacks a signal peptide proximal to its N terminus; compared to most transmembrane PTPs, it is relatively small with 405 amino acid residues [2]; and it contains only a single PTP catalytic domain [1]. With the exception of the 28 amino acid residues insert near its N terminus, PTP-oc shows complete sequence identity with the transmembrane and intracellular domains of a renal receptor-like PTP, termed glomerular epithelial protein 1 (Glepp1), which is also known as PTP-U2, PTPRO, PTP-ϕ, CRYP2, or PTP-BK [2]. PTP-oc and Glepp1 are derived from the same gene on human chromosome 12p12-p13 [3] or on mouse chromosome 6 [4]. While Glepp1 is expressed in kidney, brain, spleen, and hematopoietic cells [1], PTP-oc is expressed predominantly in hematopoietic cells (i.e., B lymphocytes and cells of monocyte–macrophage lineage, including osteoclast precursor cells, and mature osteoclasts) [1].

Glepp1 and PTP-oc not only display tissue-specific expression, but also show tissue-specific functions. Studies with Glepp1 knockout mice (by targeted disruption of exon 1 of Glepp1) reveal that knocking out Glepp1 expression

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[1] *Abbreviations used:* PTP, protein-tyrosine phosphatase; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; BAC, bacterial artificial chromosome.

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adversely affected key kidney functions [5]. Recent studies from our laboratory suggest that PTP-oc may function as a positive regulator of osteoclasts in part by activating c-Src through PTP-oc-mediated dephosphorylation of its tyrosine-527 residue (PY-527). Accordingly, suppression of PTP-oc expression by an antisense oligodeoxynucleotide in primary rabbit osteoclasts [6] or by PTP-oc siRNA in murine RAW264.7 cells [7] reduced their bone resorption activity, accompanied by an increase in c-Src PY-527 level. Osteoclast-like cells derived from human monocytic U937 cells overexpressing wild-type PTP-oc produced larger and deeper resorption pits than those derived from control U937 cells; whereas osteoclast-like cells derived from cells expressing the phosphatase-deficient PTP-oc mutant yielded significantly smaller and shallower pits than control osteoclast-like cells [8]. Some resorption activators increased PTP-oc expression and reduced the c-Src PY527 level with a corresponding activation of c-Src PTK activity in primary rabbit osteoclasts [9].

Although it has been assumed that PTP-oc was a truncated splicing variant of Glepp1 [10], we found that the expression of PTP-oc was driven by an intronic cell-type-specific promoter [11]. The most compelling evidence for this conclusion is that a 1.3-kb human DNA fragment immediately upstream of the PTP-oc transcription start site (within intron 12 of human Glepp1) exhibits strong promoter activity, and has a number of signature promoter motifs and all of the typical characteristics of a tissue-specific promoter. Furthermore, the sequence surrounding to the PTP-oc mRNA transcription start site also does not contain sequence homology compatible with a splicing acceptor site [11]. Moreover, consistent with the cell-type-specificity of PTP-oc expression, the human intronic PTP-oc promoter was functional in cells of the osteoclast lineage, but not in osteoblasts, kidney cells, or fibroblasts [11]. Evidence for a PTP-oc (PTPROt) intronic promoter within intron 12 of the human Glepp1 (PTPRO) gene was also shown by Jacob and Motiwala [12].

The concept that two physiologically important enzymes (Glepp1 in the kidney and PTP-oc in the osteoclast) are derived from the same gene but are driven by different cell-type-specific promoters is intriguing. Understanding the nature of the intronic promoter of PTP-oc could yield important insights into the tissue-specific regulation of PTP-oc. Moreover, a direct approach to confirm the physiological function of PTP-oc is to evaluate the effect of knocking out PTP-oc gene expression on osteoclast functions. Because Glepp1 and PTP-oc share parts of the same gene structure, the commonly used approaches for producing knockout mice (i.e., interruption or deletion of an exon) would abolish both Glepp1 and PTP-oc expression. On the other hand, it might be possible to create PTP-oc knockout mice without affecting Glepp1 expression by targeted inactivation or deletion of the PTP-oc proximal promoter. This novel approach would require a good understanding of the nature of the putative PTP-oc intronic proximal promoter in the mouse. Accordingly, the primary objective of this study was to characterize the murine PTP-oc proximal promoter, and the secondary goal was to compare the murine PTP-oc proximal promoter with the corresponding human intronic promoter.

Materials and methods

Materials

Tissue culture plasticware was obtained from Falcon (Oxnard, CA). Dulbecco's modified Eagle's medium (DMEM) was from Mediatech, Inc. (Herndon, VA). Fetal bovine serum (FBS) was from Life Technologies (Grand Island, NY). Trypsin and the crude collagenase were products of Irvine Scientific (Santa Ana, CA). A 188-kb murine genomic BAC clone RPCI23-125A24 containing the entire murine PTP-oc (mPTP-oc) gene and the intronic promoter sequence was purchased from the BACPAC Resource Center (Oakland, CA). Fuji X-ray film (Fuji Co., Ltd., Tokyo, Japan) was obtained through local suppliers. Anti-c-Src, anti-actin, anti-Ikaros-1,2 (anti-Ik-1,2), anti-D1DR, and anti-Pit-1a antibodies were products of UpState Biotechnology (Lake Placid, NY), Santa Cruz Biotechnology (Santa Cruz, CA), or BD Transduction Laboratories (San Diego, CA). Oligonucleotide primers for each test PTP-oc promoter construct were designed with the software program at the IDT DNA Technique Website (www.idtdna.com/SciTools/SciTools.aspx) and were synthesized by IDT (Coralville, IA). Other chemicals were molecular biology grade and were from Fisher or Sigma.

Cell cultures

A subclonal line of murine monocytic pre-osteoclastic RAW264.7 cells was obtained from Dr. A. Ian Cassady of the University of Queensland (Brisbane, Australia). Human TE85 osteosarcoma cells (a cell line showing osteoblastic characteristics) were originally obtained from Dr. J. Fogh of the Sloan-Kettering Institute (New York, NY). Human skin fibroblasts (HSF) were isolated from foreskin samples by collagenase digestion. Human U937 promyelomonocytic leukemic cells (ATCC CRL-1593.2), human embryonic kidney HEK293 cells (ATCC CRL-1573), human hepatocellular carcinoma HepG2 cells (ATCC HB-8065) were purchased from American Type Culture Collection (Manassas, VA). With the exception of U937 cells, each cell type was maintained in DMEM supplemented with 10% FBS. U937 cells were maintained in RPMI 1640 medium with 10% FBS. All cells were grown in humidified incubators containing 5% CO_2 and 95% air. Media were changed every 2–3 days.

PTP-oc promoter-luciferase reporter plasmids

The 1.3-kb human PTP-oc proximal promoter-luciferase reporter plasmid (pGL3-h^{p}PTP-oc) was prepared as previously described [10]. To construct the murine PTP-oc promoter-luciferase reporter plasmid (pGL3-m^{p}PTP-oc), a 2-kb (−1886/+116) DNA fragment corresponding to the mPTP-oc proximal promoter was generated by PCR using the high fidelity native Pfu DNA polymerase (Stratagene, La Jolla, CA) with genomic DNA from the RPCI23-125A24 murine BAC clone as template and the following primer set: 5′-AAA ACT CGA GAG ACT AAC CTC AGG CT-3′ (forward primer) and 5′-AAA AAA GCT TAC TCA CAG GCA GGT AG-3′ (reverse primer). The PCR was subjected to a hot start for 3 min at 94 °C prior to the addition of the Pfu DNA polymerase, followed by 35 amplification cycles: denaturing for 30 s at 94 °C, annealing for 1 min at 59 °C, and extending for 2 min at 72 °C per cycle. The PCR product was purified, sequenced, and cloned into the promoterless, luciferase-based pGL3-basic plasmid (Promega, Madison, WI) at the XhoI and HindIII restriction sites to produce the 2-kb pGL3-m^{p}PTP-oc reporter construct.

A total of 12 deletion constructs were produced by PCR using the same condition as described above with the same reverse primer and forward primers progressively closer to the transcription initiation site as shown in Table 1. Each PCR product was gel-purified, subcloned into the