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Characterization of SHP-1 protein tyrosine phosphatase transcripts, protein isoforms and phosphatase activity in epithelial cancer cells☆

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ABSTRACT

We identified 7 SHP-1 (*PTPN6*) transcripts using epithelial cancer-derived cell lines. Four were shown to utilize the epithelial promoter 1 to transcribe a full-length, a partial (exon 3) or complete (exons 3 & 4) deletion of the N-SH2 domain, and also a non-coding transcript having a stop codon caused by a frame shift due to intron 2 retention. Three additional transcripts were shown to utilize the hematopoietic promoter 2 to transcribe a full-length, a partial (exon 3) deletion of the N-SH2 domain and a non-coding transcript with intron 2 retention. We show that endogenous proteins corresponding to the open-reading-frame (ORF) transcripts are produced. Using GST-fusion proteins we show that each product of the ORF SHP-1 transcripts has phosphatase activity and isoforms with an N-SH2 deletion have increased phosphatase activity and novel protein–protein interactions. This study is the first to document utilization of promoter 2 by SHP-1 transcripts and a noncoding transcript in human epithelial cells.

1. Introduction

The intricate balance of phosphotyrosine residues on proteins is controlled by the opposing actions of protein tyrosine kinases (PTK) and protein tyrosine phosphatases (PTP) [1–5]. Perturbation of PTP signaling can lead to abnormal accumulations of phosphorylated proteins which can disrupt normal cell proliferation, differentiation, growth, and adhesion.

SHP-1 (also designated as SHPTP-1, SHP, HCP and PTP1C, PTPN6) is a 68 kDa cytosolic PTP predominantly expressed in hematopoietic and epithelial cells [6–8]. The human SHP-1 gene is located on chromosome 12p13 [9,10] and consists of 17 exons and 16 introns, spanning 17 kb of DNA (6). SHP-1 contains two tandem src-homology 2 (SH2) domains, three potential SH3-binding motifs (PxxP) for binding, a catalytic phosphatase domain and a C-terminal tail with two sites for tyrosine phosphorylation [11–15]. In physiological conditions SHP-1 engages in auto-inhibition due to intramolecular interaction between its N-terminus SH2 (N-SH2) domain and its C-terminal tail [5,16]. N-terminal deletion of SHP-1 can result in excessive phosphatase activity [17], which can disrupt normal signaling pathways and may contribute to tumorigenesis [18].

Two promoters have been reported for SHP-1: promoter 1 (P1) or epithelial promoter (EP) which directs the transcription of exon 1 encoding the amino acid sequence, Methionine–Leucine–Serine–Arginine–Glycine (MLSRG) and promoter 2 (P2) or hematopoietic promoter (HP) which initiates the transcription of exon 2 encoding the amino sequence, Methionine–Valine–Arginine (MVR) at the N-terminus [6]. P1 has been suggested to be predominantly expressed by epithelial cells whereas P2 is active in cells of the hematopoietic lineage [6]. The exclusivity of these promoters, however has recently been questioned [8].

Loss of SHP-1 leads to extensive hematopoietic disruptions in mice including aberrant expansion and tissue accumulation of myeloid/monocytic cells that results in death in motheaten (me/me) mice that do not express SHP-1 at about 2-3 weeks or in motheaten-viable (mev/mev) mice that express aberrant SHP-1, at 9-12 weeks [17–19]. An absence of SHP-1 expression has been observed in the development of several human cancers including B and T cell lymphoma and T-cell chronic lymphocyte leukemia (TCLL) and chronic myelogenous leukemia (CML) [18,20,21]. In addition, growth of CML or malignant hematopoietic cell lines can be suppressed with introduction of wild-type SHP-1 [18,20–25]; these findings implicate SHP-1 as a potential tumor suppressor and as a critical regulator of a broad range of hematopoietic cell functions [20–25]. The role of SHP-1 in epithelial cells, however, and its

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implication in epithelial tumorigenesis still remains unclear. Enhanced expression of SHP-1 has been documented in several epithelial cancers, including prostate [26], breast [27] and ovarian cancers [7,8]. The differences in SHP-1 expression may be an outcome of the cell type versus its role in cancer progression and development [25-27]. However, recent work has shown that SHP-1 is a negative regulator of proliferation in malignant breast adenocarcinoma using its N-terminal domain [28]. In prostate cancer, proper binding between SHP-1 and somatostatin is required to negatively regulate cancer growth [26].

In this study, we examined SHP-1 transcripts in different human colon, breast and other epithelial cancer-derived cells to obtain a better understanding of the possible role of SHP-1 in these cancers. We show for the first time P1 and P2 utilization in human epithelial cells. In addition to the three transcripts previously reported [6]; we identified an additional 4 transcripts, 2 having open reading frames and 2 noncoding transcripts having a frame shift due to retention of an intron, that was previously reported for hematopoietic SHP-1 [18]. This study is also the first to show endogenous protein expression representing the variant SHP-1 mRNA as well as protein binding patterns of variant SHP-1 proteins encoded by alternatively-spliced transcripts. SHP-1 alternative splicing and production of protein isoforms may have functional consequences and subsequent biological impact in human epithelial cells.

2. Results and discussion

2.1. Identification of multiple SHP-1 transcripts in epithelial cancer cells

We have re-examined the SHP-1 transcripts present in epithelial cancer-derived cell lines. Previously, it was thought that the selective expression of SHP-1 is governed by two specific promoter regions: promoter 1 (P1) or epithelial promoter (EP) which is located upstream from exon 1 (exon 1a in ref. [29]) or by promoter 2 (P2) also known as hematopoietic promoter (HP) situated upstream from exon 2 (exon 1b in ref. [29]) (Fig. 1). In contrast to a previous report (Banville et al. [6]) that only promoter 1 is used in epithelial cancer cells, in our study we identified SHP-1 mRNA transcripts containing exon 1 and exon 2, indicating that both promoters are utilized in epithelial cells (Fig. 2A). Furthermore, we have characterized 4 additional, novel SHP-1 transcripts (Figs. 1, 2B and C) which add to the previous studies conducted by Banville et al. [6] who showed 3 SHP-1 transcripts using A431 cancer-derived cells. In our studies, we used multiple cancer-derived cell lines, including from breast and colon cancers, and forward primers specifically designed for P1 or P2 promoter use. Under the control of P1, we identified a full-length SHP-1 transcript and transcripts lacking either exon 3 or both exons 3 and 4, which correspond to those found by Banville et al. [6] (Fig. 2B). In addition to A431, we identified novel SHP-1 transcripts in epithelial cancer cell lines not previously examined, including colon cancer cell line Colo 205, benign breast-derived cell line MCF-10A, and the breast cancer cell lines MCF-7, MB231, and SK-BR-3 (Fig. 2). Our results also indicated that multiple transcripts were differentially expressed, depending on the cancer cell used (Fig. 2B). Under the control of P2, we found SHP-1 transcripts corresponding to wild-type and exon 3 deleted SHP-1 mRNA (Fig. 2). We did not find a transcript lacking both exons 3 and 4 under the control of the P2 promoter. Again, as with P1 promoter, we found differential expression of transcripts under the control of P2 in different cancer-derived cells (Fig. 2). These are the first SHP-1 transcripts and SHP-1 variants identified that specifically use promoter 2 in human epithelial cells. Additionally, we have identified two novel SHP-1 transcripts, one using the epithelial promoter and one using the hematopoietic promoter, that retain intron 2 (Fig. 2; largest bp product; 685 bp using P1 and 710 bp using P2), resulting in a non-coding transcript, similar to those we found earlier in KiT 225 leukemia-derived and the HuT 78 lymphoma-

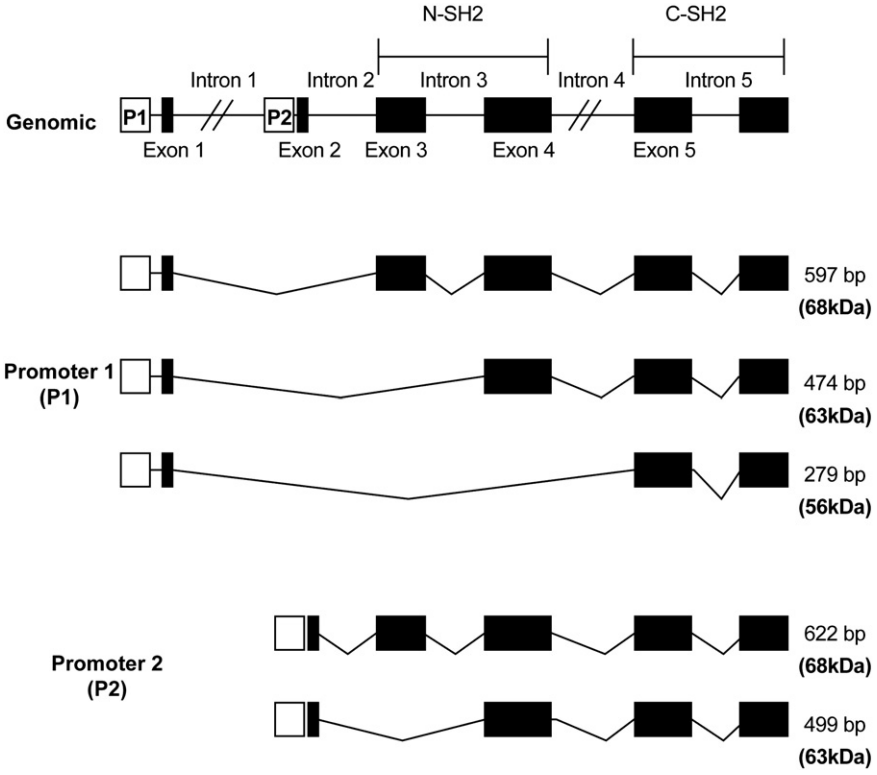

Fig. 1. Schematic diagram of N-terminal region of genomic SHP-1. Shows the positions of introns and exons 1 thru 6 and representations of the multiple SHP-1 mRNA transcripts detected by RT-PCR in various human epithelial cancer cell lines. The sizes of the corresponding PCR products in base pairs (bp) are indicated. These transcripts have open reading frames and translate a full-length 68 kDa protein and truncated proteins of 63 kDa and 56 kDa, with partial and complete deletion of the SHP-1 N-SH2 domain, respectively.