

Molecular cloning of a human polypeptide related to yeast sds22, a regulator of protein phosphatase-1

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Abstract sds22 is a regulatory polypeptide of protein phosphatase-1 that is required for the completion of mitosis in both fission and budding yeast. We report here the cDNA cloning of a human polypeptide that is 46% identical to yeast sds22. The human homolog of sds22 consists of 360 residues, has a calculated molecular mass of 41.6 kDa and shows a tandem array of 11 leucine-rich repeat structures of 22 residues. Northern analysis revealed a major transcript of 1.39 kb in all 8 investigated human tissues. sds22 was detected by western analysis in both the cytoplasm and the nucleus of rat liver cells as a polypeptide of 44 kDa.

Key words: Cell cycle; Dephosphorylation; Mitosis; Protein phosphatase

1. Introduction

The serine/threonine protein phosphatases of type-1 (PP-1) comprise an abundant and widely distributed group of enzymes that all consist of an isoform of the same catalytic subunit (PP-1_C) and one or more regulatory subunits [1–3]. The noncatalytic subunits of PP-1 not only determine the activity and substrate specificity of the holoenzymes, but also target the phosphatase to a particular cellular location. The primary structure of several mammalian regulatory polypeptides of PP-1 is already known; they include cytoplasmic (inhibitor-1/DARPP-32, inhibitor-2) and nuclear (NIPP-1) inhibitory polypeptides, a glycogen-binding subunit (G-subunit) and two regulatory subunits of the myosin-associated phosphatase of smooth muscle [1–6].

sds22 was originally identified in *Schizosaccharomyces pombe* as a suppressor of defects in mitosis that result from a particular mutation in a PP-1_C isoform [7]. A further functional analysis revealed that sds22 is essential for chromosome dysjunction during the metaphase/anaphase transition. sds22 does not itself display a protein phosphatase activity, but may facilitate PP-1 mediated dephosphorylation by interaction with PP-1_C [7,8]. The central part of sds22 consists of 11 leucine-rich repeats that are essential for the interaction with PP-1_C, while

the N-terminal and C-terminal domains of sds22 are important for its stability and subcellular localization, respectively. Recent studies have led to the identification of an sds22 homolog in *Saccharomyces cerevisiae* that also appears to be essential for cell division and also forms a complex with PP-1_C [9,10]. A protein homologous to sds22 is also encoded by the *Caenorhabditis elegans* genome [11].

We report here the molecular cloning of a human protein that is highly homologous to sds22 from yeast. It is also shown that the human sds22 homolog is expressed in a variety of tissues and is present in both the cytoplasm and the nucleus.

2. Experimental

2.1. Nucleotide sequence and 5'-RACE analysis

Most of the nucleotide sequence encoding human brain sds22 was obtained by analysis of a cDNA clone that was deposited as an expressed sequence tag (see Results and Discussion). The first 47 nucleotides were obtained by 5'-RACE-PCR analysis using a human brain 5'-RACE-readyTM cDNA kit from Clontech. A primary PCR reaction (35 cycles) was conducted using an anchor-specific primer and an antisense primer (GATGTTTCTCAGCAGAT) corresponding to nucleotides 470–486 in Fig. 1. In a secondary PCR amplification (35 cycles) the anchor-specific primer was used together with a nested antisense primer (ACCTCAAATCCTTCAAT) corresponding to nucleotides 286–302 in Fig. 1. The products of the second PCR amplification were subcloned in the pGEM₇ vector (Promega), following the instructions of the manufacturer, and sequenced using T7 and Sp6 as primers.

Nucleotide sequencing was performed using the dideoxynucleotide chain termination method [12] in an A.L.F. sequenator (Pharmacia LKB) with the Pharmacia autoread sequencing kit. Both vector-specific and cDNA-specific oligonucleotide primers were used. Sequencing was performed on both strands.

2.2. Northern analysis

A Northern blot with poly(A)⁺ RNA from eight different human tissues (2 µg each) was purchased from Clontech. The membranes were developed with a probe encompassing nucleotides 48–1299 of the full-length sds22 cDNA (Fig. 1) and ³²P-labeled to a specific radioactivity of 2 × 10⁹ cpm/µg by random priming (Gibco BRL). The blots were prehybridized (3 h) and hybridized (overnight) at 42°C in the presence of 35% formamide, 5 × SSPE, 10 × Denhardt's, 2% SDS and 100 µg/ml denatured DNA from salmon sperm [13]. During hybridization the probe concentration amounted to 1 ng/ml. Subsequently, the membranes were washed twice for 10 min at room temperature in 2 × SSC plus 0.05% SDS, followed by a 40-min wash at 50°C in 0.1 × SSC plus 0.1% SDS [13]. Filters were autoradiographed by exposure at –80°C with intensifying screens for 72 h.

2.3. Immunological techniques

A synthetic peptide, with a sequence corresponding to the 14 C-terminal residues of human sds22 plus an additional N-terminal cysteine, was coupled to keyhole limpet hemocyanin and to bovine serum albumin, using the Pierce immunogen conjugation kit. Rabbit polyclonal antibodies to the hemocyanin-coupled peptides were affinity-purified on albumin-coupled peptide that was linked to CNBr-activated

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The nucleotide sequence reported in this paper has been deposited in the EMBL/GenBank database under the accession number Z50749.

Abbreviations: LRR; leucine-rich repeat; PP-1, protein phosphatase-1; PP-1_C; catalytic subunit of PP-1; RACE; rapid amplification of cDNA-ends.

As has previously also been demonstrated for the yeast homologs [7–10], a large fraction of human sds22 (residues 70–311) consists of 11 leucine-rich tandem repeat structures (Fig. 2), also known as LRR motifs that may participate in protein–protein interactions [7–10,16]. In contrast to yeast sds22, however, all repeats in the human polypeptide are exactly 22 residues long. Analysis of the deduced primary structure also revealed that human sds22 is relatively rich in leucine (16.1%), glutamic acid (12.8%) and asparagine (8.3%), but con-

tains only 5 proline residues (1.4%). It is an acidic polypeptide ($pI = 4.7$), although the charge is not distributed evenly in the three regions that correspond to functional domains in the fission yeast homolog (see Introduction). Indeed, while the N-terminus (residues 1–69) is acidic ($pI = 4.0$), the LRR domain (residues 70–311) is somewhat less acidic ($pI = 5.1$), and the C-terminus (residues 312–360) is very basic ($pI = 10.2$). It is also worth noting that human sds22 contains putative phosphorylation sites for protein kinases A and C, casein kinase-2 and tyrosine protein kinases (not shown), suggesting that the polypeptide, like the fission yeast homolog [8], is a phosphoprotein.

An alignment of human sds22 with the homologs from *Schizosaccharomyces pombe*, *Saccharomyces cerevisiae* and *Caenorhabditis elegans* revealed an extensive homology between all polypeptides (not shown). Using the 'bestfit' program of the GCG software [17], the identity between human sds22 and the yeast or *C. elegans* homologs in the overlapping region amounted to 46%. The homology extends to the entire polypeptide chain but is particularly strong in the LRR domain. Unique for human sds22 is an extension of 32–38 residues at the N-terminus, which accounts for the larger mass of this homolog.

3.2. Tissue distribution and subcellular localization

Northern blot analysis showed one major mRNA species of 1.39 kb in all 8 investigated human tissues (Fig. 3), indicating a ubiquitous expression of sds22. At equal mRNA loading, the 1.39 kb transcript was particularly abundant in skeletal muscle, heart and brain. Compared to the size of this major mRNA species, the cDNA encoding sds22 was about 0.1 kb smaller (Fig. 1), which is likely to be explained by the missing poly(A)⁺-

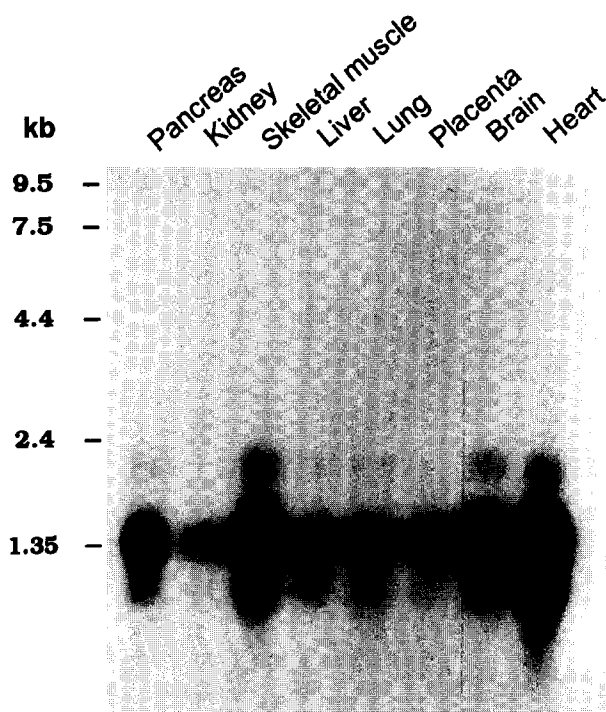


Fig. 3. Northern blot analysis in various human tissues. Each lane contained 2 μ g poly(A)⁺ RNA isolated from the indicated tissues. The blots were hybridized with a probe corresponding to bp 48–1299 of the full-length sequence of sds22 shown in Fig. 1.

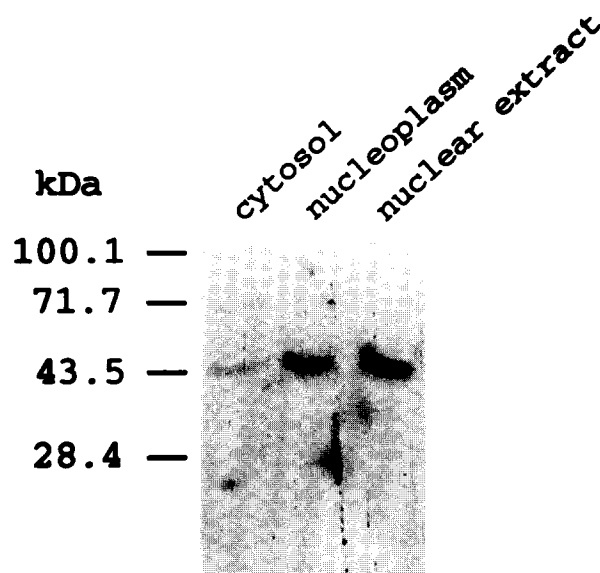


Fig. 4. Subcellular localization of sds22. A cytosolic fraction, the nucleoplasm and a nuclear 0.3 M NaCl extract were prepared from rat liver as indicated in section 2. About 15 μ g protein of each fraction was subjected to 10% Tricine-SDS-PAGE, transferred to a membrane, and probed with antibodies against the C-terminus of sds22.

tail (Fig. 1). In addition to the major 1.39 mRNA transcript, two minor transcripts of 2.0 kb and 1.1 kb were observed (Fig. 3).

Antibodies raised against a synthetic peptide with a sequence encompassing the 14 C-terminal residues of human sds22 were used for western blotting of rat liver fractions (Fig. 4). The antibodies recognized a polypeptide of 44.4 ± 0.2 kDa (mean $\pm$ S.E.M; $n = 4$) in the cytosolic fraction, the nucleoplasm and the nuclear salt extract. However, at equal protein loading, the signal was much more intense in the nuclear fractions, indicating that sds22 is enriched in the nucleus, in agreement with its subcellular distribution in yeast [7,8].

3.3. Conclusions

Given the extensive overall homology (46%) with yeast sds22, we propose that the novel sequence reported here represents the human homolog of sds22. The high degree of phylogenetic conservation suggests that sds22 fulfills an essential role, which agrees with its proposed role in mitosis (see section 1). The present data constitute the basis for a functional analysis of mammalian sds22. It will be of particular interest to see whether the human protein, like the yeast homologs, is associated with PP-1_C. Preliminary evidence indicates that in a hepatic nuclear extract nearly all the type-I catalytic subunit is associated with either NIPP-1 or an unidentified inhibitory polypeptide of 111 kDa [14]. However, it cannot be excluded that sds22 is only associated with a minor fraction of nuclear PP-1_C or that the association is lost during tissue fractionation. Alternatively, sds22 may not be a true regulatory subunit of PP-1 and may only be temporarily associated with the catalytic subunit, for example at the end of mitosis. Since hepatocytes are nearly all in G₀ phase of the cell cycle, such an association would have been missed.

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