

Cloning and Expression of Human Homolog HSMT3 to Yeast SMT3 Suppressor of MIF2 Mutations in a Centromere Protein Gene

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A human HSMT3 cDNA encoding a homolog of the yeast SMT3, a suppressor of MIF2 mutations in a centromere protein gene, was identified and sequenced. The sequence of 95 amino acids deduced from the human HSMT3 cDNA exhibited 51.1% identity and 69.6% similarity to the yeast Smt3p sequence. The HSMT3 transcripts of 1.35Kb were found to be abundantly expressed in various human tissues. © 1996 Academic Press, Inc.

The proper segregation of eukaryotic chromosomes during mitosis and meiosis is mediated by centromere/kinetochore. Centromeres facilitate sister chromatid association. Kinetochores contain spindle attachment sites and molecular motors that move chromosomes on the microtubules during chromosome segregation (1–2). In human, centromere proteins (i.e. CENP-C) were first isolated using autoantibodies from patients of scleroderma, and their sequences have been determined (3). In yeast, *Saccharomyces cerevisiae*, the MIF2 gene has been shown to encode an essential centromere protein which binds A+T-rich CDEII region of centromere DNA (4). The yeast Mif2 protein shares at least two regions of similarity with mammalian centromere protein CENP-C, suggesting an evolutionary conservation of centromere proteins from yeast to mammals (5). Studies using temperature-sensitive mutants showed that the loss of yeast Mif2p function results in chromosome missegregation, mitotic delay, and aberrant microtubule morphologies (6). The yeast SMT3 suppressor was isolated from the suppression of the MIF2 mutations. The SMT3 gene is located on chromosome IV and its nucleotide sequence was recently deposited in the GenBank (accession no. U27233 and U33057).

A porcine cDNA of 230 nucleotides, isolated serendipitously (Cho, unpublished), has a sequence highly similar to 19 human EST sequences. Partial amino acid sequences deduced from this porcine cDNA and the human EST sequences were unexpectedly found to exhibit strong homology with the yeast Smt3 protein sequence. As the first step to identify and elucidate the function, here we report the cloning and expression of a human HSMT3 cDNA using the porcine cDNA as a probe.

MATERIALS AND METHODS

Cloning and sequencing of human HSMT3 cDNA. Human cDNA library in λ ZAPII (Stratagene) was screened using the porcine cDNA probe labeled with digoxigenin system. The positive clones were identified with chemiluminescent detection according to the procedure recommended by the manufacturer (Boehringer Mannheim). The purified cDNA insert was labeled with the Dye Terminator kit (Perkin Elmer), and its nucleotide sequence was determined using an automatic DNA sequencer (Applied Biosystems model 373A). Both strands of the inserted cDNA were completely sequenced.

Northern blot analysis. Human northern blot containing poly(A⁺)-RNAs from adult tissues, including heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas, was obtained from Clontech and hybridized for 16–18h at 42C in formamide, 10× Denhardt's solution, 5× buffer A (0.75M sodium chloride, 50mM sodium phosphate, 5mM EDTA, pH 7.4), 1% SDS, salmon sperm DNA (100ug/ml) with ³²P-labeled cDNA probe. The blot was rinsed twice in 2× NaCl/Cit., 0.05% SDS at room temperature for 10min, and washed twice in 0.1× NaCl/Cit., 0.1% SDS at 50C for 20min. The x-ray film was exposed overnight at –70C.

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CTCCGGCGCTCGCC 14
ATG GCCGACGAAAAGCCCAAGGAAGGAGTCAAGACTGAGAACAACGATCATATTAATTTG 74
M A D E K P K E G V K T E N N D H I N L 20
AAGGTGGCGGGCAGGATGGTTCTGTGGTGCAGTTTAAAGATTAAGAGGCATACACCACCT 134
K V A G Q D G S V V Q F K I K R H T P L 40
AGTAAACTMAATGAAAGCCTATGTGAACGACAGGGATGTCAATGAGGCAGATCAGATTC 194
S K L M K A Y C E R K Q G L S M R Q I R F 60
CGATTTGACGGGGCAACCAATCAATGAAACAGACACACCTGCACAGTTGGAATGGAGGAT 254
R F D G Q P I N E T D T P A Q L E M E D 80
GAAGATACAATGATGTGTTCACACAGCAGCGGAGGTGTCTACTGAAAAGGGAACCTG 314
E D T I D V F Q Q Q T G G V Y * 95

CTTCTTTACTCCAGAACTCTGTTCCTTTAAAGACCAAGATTACATTCTCAATTAGAAAAC 374
GCAATTTGGTTCCACCACATCCTGACTACTACCGTATAGTTTTCTCTATCTTTTCATTTTC 434
CCCCTTCCCCATTCCTTTATTTGTACATAAAGTAACTGGTATATGTGCACACAGCATATTCG 494
ATTTTTTTTTTTTTAACTAAACAGCCAATGGTATGTPTTTGATTGACATCAAGTGGAGAC 554
GGGATGGGAAAAATACTGATTCGTGAAAATACCCCTTTCTCCATTAGTGGCATGCTC 614
ATTCAGCTCTTATCTTTATATTTCCAGTAAAGTTATTTTGCTCTCACGTGTTTTAACAAAAA 674
AAAAAACACAACATAAAAAATCCTTGCATACCTTGTTCAAATGGAGAATTTAATGTTTTT 734
TCATTTATCATTGTAAAACCAAGGACAATTTTATAACTTTTTTGTACGTAGCTGTTACAT 794
GTAGGCAATCTGCTTTAAGTAGGGATAAATTACTCTAAAACAAAAAGAATCCTAGAT 854
AGTTTTCCCTTCAAGTCAAGCGTCTTGTGTGTTAAATAAACTTCTTGTTTAAAAATGAAAA 914
AAAAAAA 922
    
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FIG. 1. The nucleotide and deduced amino acid sequences of human HSMT3 cDNA. The stop codon TGA is indicated by an asterisk and the polyadenylation signal of AATAAA is underlined.

RESULTS

Human HSMT3 cDNA and Amino Acid Sequences

A human HSMT3 cDNA clone was isolated and its sequence of 922 nucleotides was determined (GenBank accession no. L76416). This cDNA contains a protein-coding sequence of 285 nucleotides, 5' (14 nucleotides) and 3' (611 nucleotides) noncoding regions, and poly(A) tail of 12 nucleotides (Fig. 1). The deduced sequence of 95 amino acids from the human HSMT3 cDNA was found to exhibit 51.1% identity and 69.6% similarity with yeast Smt3 protein (Genbank U27233 and U33057) (Fig. 2). This result indicates the strong evolutionary conservation between human HSMT3 and yeast Smt3 proteins.

Northern Blot Analysis

The Northern blot analysis of human poly (A)-RNAs indicated that the HSMT3 transcripts of 1.35Kb were abundantly expressed in the various tissues, including heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas (Fig. 3). The 19 human EST sequences exhibiting strong homology with the HSMT3 cDNA sequence were obtained from brain, liver, spleen, white blood cells, endothelial cells, and testis. These results are consistent with the ubiquitous expression of the HSMT3 gene in dividing tissues.

Human	MAD-----E-KP--KEGVKTENNNDHINLKVAGQDGSVVQFKIKRHTPLSKLM	44
Yeast	MSDSEVNQEAKEPEVKPEVKE--THINLKV-SDGSSEIFFKIKKTTPLRRLM	49
Human	KAYCERQGLSMRQIRFRFDGQPINETDTPAQLEMEDEDITIDVFFQQQTGG-VY	95
Yeast	EAFAKRQKGEMDSLRFLYDGI RIQADQTPEDLDMEDNDIEAHREQIGGATY	101

FIG. 2. Comparison of amino acid sequences between human HSMT3 and yeast Smt3 proteins.

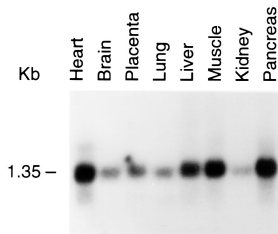


FIG. 3. Northern blot analysis of human HSMT3 transcripts from various tissues.

DISCUSSION

The size of the human HSMT3 transcript was found to be 1.35Kb using Northern blot analysis, while this HSMT3 cDNA clone contains only 922 nucleotides. The remaining sequence may be present in the 5' non-coding region. It may be noted that a porcine PSMT3 cDNA clone was found to contain a 5' non-coding region of 166 nucleotides (Cho, unpublished).

The deduced amino acid sequence of the human HSMT3 cDNA exhibits 51.1% identity and 69.6% similarity with that of yeast Smt3 protein. The strong evolutionary conservation between human HSMT3 and yeast Smt3 proteins indicates its functional significance. However, the exact function of these proteins remain to be elucidated.

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REFERENCES

1. Pluta, A. F., Mackay, A. M., Ainsztein, A. M., Goldberg, I. G., and Earnshaw, W. C. (1995) *Science* **270**, 1591–1594.
2. Bloom, K. (1993) *Cell* **73**, 621–624.
3. Saitoh, H., Tomkiel, J., Cooke, C. A., Rattie, H. III, Maurer, M., Rothfield, N. F., and Earnshaw, W. C. (1992) *Cell* **70**, 115–125.
4. Meluh, P. B., and Koshland, D. (1995) *Mol. Biol. Cell* **6**, 794–807.
5. Brown, M. T. (1995) *GENE* **160**, 111–116.
6. Brown, M. T., Goetsch, L., and Hartwell, L. H. (1993) *J. Cell Biol.* **9**, 2544–2550.