Journal of Biomedicine and Biotechnology • 2004:3 (2004) 130–136 • S1110724304307084 • http://jbb.hindawi.com

RESEARCH ARTICLE

A Gene Encoding Sialic-Acid-Specific 9-O-Acetylesterase Found in Human Adult Testis

Hu Zhu,[1,2] Hsiao Chang Chan,[2] Zuoming Zhou,[1] Jianming Li,[1] Hui Zhu,[1]
Lanlan Yin,[1] Ming Xu,[1] Lijun Cheng,[1] and Jiahao Sha[1]*

[1]*Key Laboratory of Reproductive Medicine, Nanjing Medical University, Nanjing 210029, China*
[2]*Epithelial Cell Biology Research Center, Department of Physiology, The Chinese University of Hong Kong, Hong Kong*

Received 19 July 2003; accepted 17 November 2003

Using differential display RT-PCR, we identified a gene of 2750 bp from human adult testis, named *H-Lse*, which encoded a putative protein of 523 amino acids and molecular weight of 58 kd with structural characteristics similar to that of mouse lysosome sialic-acid-specific 9-O-acetylesterase. Northern blot analysis showed a widespread distribution of H-Lse in various human tissues with high expression in the testis, prostate, and colon. In situ hybridization results showed that while H-Lse was not detected in embryonic testis, positive signals were found in spermatocytes but not spermatogonia in adult testis of human. The subcellular localization of H-Lse was visualized by green fluorescent protein (GFP) fused to the amino terminus of H-Lse, showing compartmentalization of H-Lse in large dense-core vesicles, presumably lysosomes, in the cytoplasm. The developmentally regulated and spermatogenic stage-specific expression of H-Lse suggests its possible involvement in the development of the testis and/or differentiation of germ cells.

INTRODUCTION

Sialic acids are a diverse family of acidic nine-carbon sugars that are frequently found as terminal units of oligosaccharide chains on different glycoconjugates in higher invertebrates and vertebrates [1, 2]. As a part of determinants in many glycoproteins [3, 4], sialic acids play an important role in intercellular and/or intermolecular recognition [5]. The 9-O-acetylation and de-O-acetylation are the most common modifications of sialic acids found in mammalian cell surface sialoglycoconjugates, which can alter its size, hydrophobicity, net charge, and antigenicity [2, 6, 7]. These modifications can regulate a variety of biological phenomena, including endogenous lectin recognition, tumor antigenicity, virus binding, and complement activation [8, 9].

Enzymes specifically capable of removing O-acetyl esters from the 9-position of sialic acids are sialic-acid-specific 9-O-acetylesterase. The enzymes in mammals have two forms, one is cytosolic sialic-acid-specific 9-O-acetylesterase (Cse) in the cytosolic fraction and another is lysosome sialic-acid-specific 9-O-acetylesterase (Lse) in the lysosomal/endosomal compartment [10]. Lse is likely to participate in the terminal lysosomal degradation of 9-O-acetylated sialoglycoconjugates, while Cse is likely to salvage any 9-O-acetylated molecules that escape the initial action of the Lse enzyme. The process of de-O-acetylation of sialic acid, which is catalyzed by sialic-acid-specific 9-O-acetylesterase, has been implicated in organogenesis and cellular differentiation [2, 5].

Spermatogenesis is a complicated process of germ cell differentiation in adult testis, which is established during testicular development. There are five types of germ cells, each at a specific developmental stage, found in the seminiferous tubules: spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and sperms. They can be divided into three groups according to their DNA content: 4N DNA content cells (4C cells), 2N DNA content cells (2C cells), and 1N DNA content cells (1C cells). The separation of these cells enables researchers to investigate the molecular mechanisms underlying testicular development and/or spermatogenesis. In the present study, we separated the 2C and 4C cells of seminiferous tubules in human adult testis by flow cytometry, and identified human H-Lse by differential display RT-PCR. The expression pattern of H-Lse was found to be developmentally regulated and stage-specific, indicating its possible role in testicular development and/or germ cell differentiation.

MATERIALS AND METHODS

Preparation of disaggregated seminiferous tubules cells for the SORT

Human testes were obtained from Donation Center of Nanjing Medical University with consent of relatives. The seminiferous tubules were collected in DMEM/F12, which contained collagenase, and washed to remove the Leydig cells as well as interstitial cells. Trypsin treatment and a brief treatment with DNase I were used to release

the spermatogenic cells from seminiferous tubules. The suspension of cells was filtered with nylon mesh.

Disaggregated spermatogenic cells were suspended at 1×10^6 cells/mL in 0.5 M sodium citrate solution (PH 2.35) with fresh 0.1% DEPC overnight at room temperature and at 4°C for two days; they were centrifuged and resuspended in 0.5 M sodium citrate solution (PH 4.5) with fresh 0.1% DEPC for at least 1 day. The day before use, the cells were centrifuged and resuspended in PBS with 10 mM HEPES (PH 7.0), 0.1% BSA, and fresh 0.1% DEPC. Then the cells were spun down and resuspended in PBS with 100 μg/mL PI (propidium iodide) and fresh 0.1% DEPC. The cells were stained overnight at 4°C [11].

Flow sorting of 2C cells and 4C cells

The flow cytometry (FCM) used in this research was FACSVantage SE (Becton Dickinson, Calif) equipped with argon laser (power: 200 mW, wavelength: 488 nm); a 585 nm/42 nm filter set was used before the FL2 detector. Cellquest (Becton Dickinson) was used for sorting and the sorting mode was Normal-R. Drops per sort were 3 and drop delay was 13.6. The density of cells for sorting was about 1×10^6 cells/mL.

RNA isolation and differential display PCR

Isolation of total RNA from 2C cells and 4C cells was performed with Trizol Reagent (Gibco BRL, Ontario). One hundred nanograms of total RNA was used for differential display RT-PCR [12]. The first chain cDNA was synthesized by using T12G, T12C, and T12A oligo (dT) primers, and then was used as template in PCR. PCR was performed as follows: 94°C, 1 minute; 37°C, 1 minute; 72°C, 2 minutes for 40 cycles. Ten microlitres of the PCR products from the two cells were run on a 1.5% agarose gel. The fragments highly or specifically displayed in 4C cells were excised and purified. This DNA was reamplified with the same combination of primers and then subcloned into Pinpoint Xa1-T vector (Promega, USA).

cDNA library screening

The colonies of full-length cDNA were screened by PCR. Human Testis Large-Insert cDNA Library (Clontech, Calif) was first converted into plasmid cDNA Library, and then an arrayed cDNA library in 96-well plates was made according to the method of Munroe [13, 14]. In this arrayed cDNA library 1.54×10^6 colonies were screened by PCR.

Northern blot analysis

Multiple tissue northern (MTN) blots (Clontech) were hybridized with the ^{32}P-labeled probes. The probe corresponding to 1378–1634 bp of H-Lse was used for hybridization. After stringent wash, the blot was placed on the storage phosphor screen (Packard, USA) and exposed for 3 hours in the dark. The signal was detected at the Cyclone storage phosphor system (Packard).

Chromosome mapping of H-Lse

The Stanford TNG Radiation Hybrid Panel (Research Genetics, Huntsville, Ala) was used to map the chromosomal localization of HSE with primers HSEmapF (5′-*ATGAACACCGTCTCCACC*-3′) and HSEmapR (5′-*AAATCTGAAGGACCCATC*-3′), according to the manufacturer's instructions. After 35 cycles of amplification, the reaction products were separated on a 1.5% agarose gel. The positive amplification was labeled as 1 and the negative one was labeled as 0. The results were analyzed through the Stanford genome center web server to determine the probable chromosomal location.

In situ hybridization of human testis

RNA DIG-labeled probes were made by in vitro transcription. T7 and SP6 promoter sequences were incorporated into the two sides of the templates (195–553 bp of H-Lse) by PCR, sense and antisense probes were made using DIG-RNA labeling mix (Roche, USA) according to the manufacturer's instructions. After fixation, paraffin embedding, mounting, and sectioning, sections of human embryonic and adult testes were prehybridized in hybridization buffer (DIG Easy Hyb, Roche, Germany) at 42°C for 2 hours. Hybridization was carried in hybridization buffer containing appropriate probes at 65°C for 16 hours in humidity chamber.

Subcellular localization of H-Lse

Subcellular localization of HSEI and HSEII was performed by the method of green fluorescent protein. pEGFP-C2-HSEI AND pEGFP-C2-HSEII were constructed using two sets of primers (HSEI: 5′-*GGGGAATTCAATGATATGGTGCTGCAG*-3′ and 5′-*GGGGTCGACATTTAGCAACATTGCTCTG*-3′; HSEII: 5′-*GGGGAATTCATGGTCGCGCCGGGGCTTG*-3′ and 5′-*GGGGTCGACATTTAGCAACATTGCTCTG*-3′) and EcoRI/SalI restriction sites of pEGFP-C2. Recombinant vectors were transfected into BxPC-3 cells (BxPC-3 cell is a cell lineage of adenocarcinoma from pancreas) by Lipofectin reagent (Gibco BRL). Cells were imaged 40 hours after transfection on the fluorescence microscope.

RESULTS

Identification of H-Lse by differential display RT-PCR

After being stained with PI and measured by the FCM, three groups of cells in seminiferous tubules of human adult testis were detected (Figure 1), 2C and 4C cells were subsequently sorted. A clone was identified by differential display RT-PCR, which was highly expressed in the 4C cells (Figure 2) and with high homology (86%) to a mouse lysosome sialic acid 9-O-acetylesterase. The clone was named H-Lse.

Structural characteristic of H-Lse

In the two rounds of screening in the arrayed cDNA library, the plasmid containing full-length H-Lse

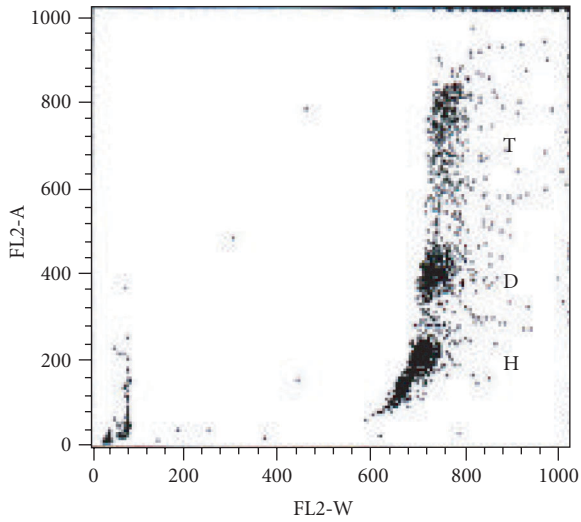


FIGURE 1. Flow sorting of 2C cells and 4C cells of seminiferous tubules from human testis. Three populations of cells are identified according to fluorescence intensity (FL2-A) and width of the emitted fluorescence (FL2-W). 4C and 2C cells are sorted: T, 4C; D, 2C; H, 1C.

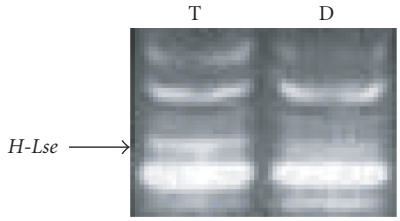


FIGURE 2. DD-RT-PCR results of 4C and 2C cells. *H-Lse* is highly expressed in 4C cells.

(GenBank accession number: AF303378) was found. *H-Lse* is 2750 bp in length, encoding a putative protein of 523 amino acids with a molecular weight of 58 kd. Its isoelectric point is 7.19. The N terminus (1–18 aa) of the protein is a region containing hydrophobic amino acid residues, which may be a signal peptide. By comparison of the protein sequences (Figure 3), we hypothesized that H-Lse is the human counterpart of mouse lysosome sialic acid 9-O-acetylesterase.

Chromosome localization of H-Lse

After PCR amplification, the results can be shown as a pattern (0000000010001010000001100000100000001100000100000100000100100000001000000000010010010000001). Retrieving results from the Stanford genome center web server shows that HSE is localized in the human 11q24 (Figure 4).

Northern blot analysis of human tissue

The distribution of *H-Lse* in various human tissues was analyzed by Northern blot (Figure 5) and the results showed the presence of three distinct mRNA species at approximately 2.7 kb, 6.0 kb, and 7.5 kb. The expected transcript of *H-Lse* was approximately 2.7 kb and it was consistently expressed in all the tissues examined with high expression found in the testis, prostate, and colon. The transcript of approximately 7.5 kb was exclusively expressed in the colon. The transcript of approximately 6.0 kb was distributed in the testis, colon, small intestine, prostate, and thymus, with the highest level of expression found in the testis.

Developmental and spermatogenic stage-specific localization of H-Lse

To examine a possible role of H-Lse in testicular development and/or spermatogenesis, in situ hybridization experiments were conducted to compare H-Lse expression in human embryonic and adult testes since spermatogenesis is not initiated in the embryo and there is no meiosis in embryonic seminiferous tubules. The results showed that no signal was detected in the embryonic testis, while positive signals were detected in spermatocytes but not spermatogonia in the seminiferous tubules of adult testis. Signals were associated with germ cells but not other somatic cells in the testis, that is, Sertoli and Leydig cells. Negative control of sense probes confirmed the specificity of the results (Figure 6).

Lysosomal localization of H-Lse

The subcellular localization of H-Lse fusion proteins was visualized by transiently transfecting H-Lse gene fused with GFP into BXPC-3 cells. As shown in Figure 7, the control cells transfected with GFP protein alone exhibited fluorescence evenly distributed throughout the cytoplasm, while GFP-H-Lse fusion protein was compartmentalized in numerous large dense-core vesicles in the cytoplasm.

DISCUSSION

Spermatogenesis is a developmental program that occurs in mitotic, meiotic, and postmeiotic phases. In the mitotic phase, spermatogonia proliferate to expand the quantity of germ cells; in the meiotic phase, spermatocytes accomplish chromosomal synapsis and genetic recombination before two meiotic divisions; and in the postmeiotic phase, haploid spermatids are remodeled into spermatozoa by the processes of acrosome formation, nuclear condensation, flagellar development, and loss of the majority of cytoplasm. Under the control of intrinsic and extrinsic factors, spermatogenesis is characterized by the expression of a spectrum of genes that are cell-type-specific or stage-specific. They are thought to play an essential role in spermatogenesis at particular stages. For example, *MutS homologue 5* is required for chromosome pairing, *CPEB* and *SCP3* are required for synaptonemal complex assembly and chromosome synapsis in primary spermatocytes [15, 16, 17].

In the present study, we have identified a gene, *H-Lse*, from human adult testis with high homology to

```
m-Cse   1   ------------------------------------------------------------ 1
m-Lse   1   MVSPGPVFGIVLLIIARVSRSAGIGFRFASYIDNYMVLQKEPSGAVIWGFGTPGATVTVT 60
h-Lse   1   MVAPGLVLGLVLPLILWADRSAGIGFRFASYINNDMVLQKEPAGAVIWGFGTPGATVTVT 60

m-Cse   1   ------------------------------------MAQQTLGTMNFTLRVHDVLFGDV 23
m-Lse   61  LCQGQETIMKKVTSVKEPSNTWMVVLDPMKPGGPFEVMAQQTLGTMNFTLRVHDVLFGDV 120
h-Lse   61  LRQGQETIMKKVTSVKAHSDTWMVVLDPMKPGGPFEVMAQQTLEKINFTLRVHDVLFGDV 120

m-Cse   24  WLCSGQSNMQMTVSQIFNASKELSDTAAYQSVRIFSVSLIQSEEELDDLTEVDLSWSKPT 83
m-Lse   121 WLCSGQSNMQMTVSQIFNASKELSDTAAYQSVRIFSVSLTQSEEELDDLTEVDLSWSKPT 180
h-Lse   121 WLCSGQSNMQMTVLQIFNATRELSNTAAYQSVRILSVSPIQAEQELEDLVAVDLQWSKPT 180

m-Cse   84  AGNLGHGNFTYMSAVCWLFGRYLYDTLQYPIGLVSSSWGGTYIEVWSSRRTLKACGVPNT 143
m-Lse   181 AGNLGHGNFTYMSAVCWLFGRYLYDTLQYPIGLVSSSWGGTYIEVWSSRRTLKACGVPNT 240
h-Lse   181 SENLGHGYFKYMSAVCWLFGRHLYDTLQYPIGLIASSWGGTPIEAWSSGRSLKACGVPKQ 240

m-Cse   144 RDERVGQPEIKPMRNECNSEESSCPFRVVPSVRVTGPTRHSVLWNAMIHPLQNMTLKGVV 203
m-Lse   241 RDERVGQPEIKPMRNECNSEESSCPFRVVPSVRVTGPTRHSVLWNAMIHPLQNMTLKGVV 300
h-Lse   241 GS--------------------------IPYDSVTGPSKHSVLWNAMIHPLQNMTLKGVV 274

m-Cse   204 WYQGESNADYNRDLYTCMFPELIEDWRQTFHYGSQGQTDRFFPFGFVQLSSYMLKNSSDY 263
m-Lse   301 WYQGESNADYNRDLYTCMFPELIEDWRQTFHYGSQGQTDRFFPFGFVQLSSYMLKNSSDY 360
h-Lse   275 WYQGESNINYNTDLYNCTFPALIEDWRETFHRGSQGQTERFFPFGLVQLSSDLSKKSSDD 334

m-Cse   264 GFPEIRWHQTADFGHVPNPKMPNTFMAVAIDLCDRDSPFGSIHPRDKQTVAYRLHLGARA 323
m-Lse   361 GFPEIRWHQTADFGHVPNPKMPNTFMAVAIDLCDRDSPFGSIHPRDKQTVAYRLHLGARA 420
h-Lse   335 GFPQIRWHQTADFGYVPNPKMPNTFMAVAMDLCDRDSPFGSIHPRDKQTVAYRLHLGARA 394

m-Cse   324 VAYGEKNLTFQGPLPKKIELLASNGLLNLTYDQEIQVQMQDNKTFEISCCSDRHCKWLPA 383
m-Lse   421 VAYGEKNLTFQGPLPKKIELLASNGLLNLTYDQEIQVQMQDNKTFEISCCSDRHCKWLPA 480
h-Lse   395 LAYGEKNLTFEGPLPEKIELLAHKGLLNLTYYQQIQVQKKDNKIFEISCCSDHRCKWLPA 454

m-Cse   384 PVNTFSTQTLILDLNACLGTVVAVRYAWTTWPCEYKQCAVYHTSSMLPAPPFIAQISHRG 443
m-Lse   481 PVNTFSTQTLILDLNACLGTVVAVRYAWTTWPCEYKQCAVYHTSSMLPAPPFIAQISHRG 540
h-Lse   455 SMNTVSTQSLTLAIDSCHGTVVALRYAWTTWPCEYKQCPLYHPSSALPAPPFIAFITDQG 514

m-Cse   444 I-------- 444
m-Lse   541 I-------- 541
h-Lse   515 PGHQSNVAK 523
```

Figure 3. Alignment of amino acid sequences of H-Lse, mouse cytosolic sialic-acid-specific 9-O-acetylesterase (m-Cse), and mouse lysosome sialic-acid-specific 9-O-acetylesterase (m-Lse). Residues in the black boxes represent the identical region of the three proteins and residues in the gray boxes represent the conserved region.

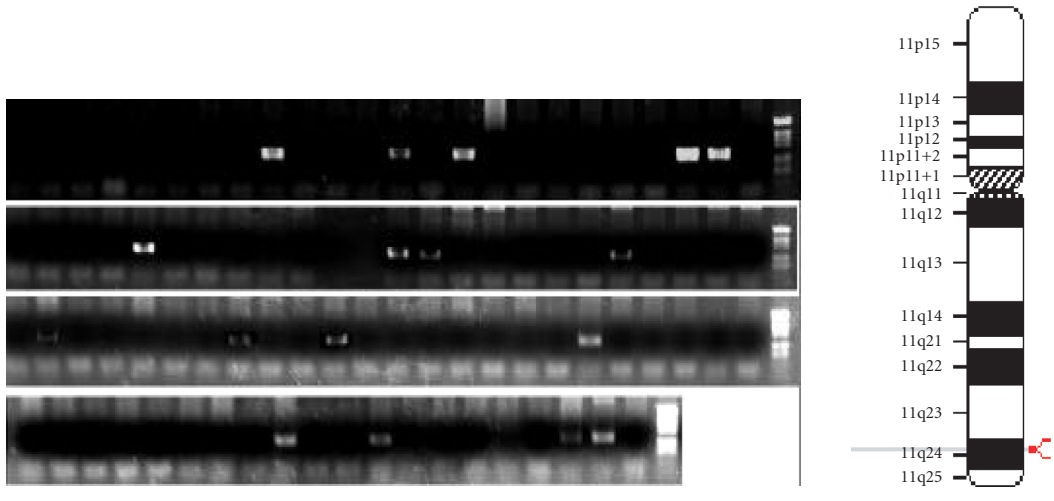


Figure 4. The results of radiation hybrid of *H-Lse* gene. (a) The PCR amplification of 90 clones of the Stanford TNG Radiation Hybrid Panel. (b) The scheme of human chromosome localization of *H-Lse* gene.

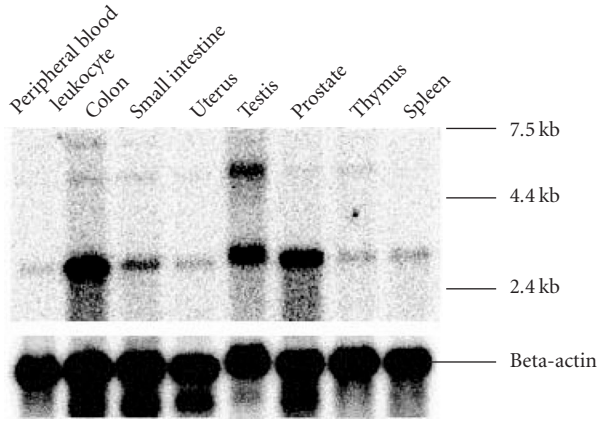


FIGURE 5. Northern blot of *H-Lse* gene. Three distinct mRNA species at approximately 2.7 kb, 6.0 kb, and 7.5 kb are detected in MTN membranes; beta-actin is the control.

mouse *Lse*. The subcellular localization of GFP-H-Lse fusion protein showed that H-Lse is concentrated in vesicle-like structures in the cytoplasm, presumably the lysosomes, indicating that H-Lse is likely to be the lysosome form of sialic-acid-specific 9-O-acetylesterase. However, our northern blot analysis shows transcripts of different sizes with different tissue distributions. They may be the tissue-specific transcripts of H-Lse since others have reported that there are multiple transcripts of mouse sialic-acid-specific 9-O-acetylesterase [2, 10, 18]. The wide distribution of the expected transcript of H-Lse, approximately 2.7 kbp, in all tissues examined suggests an essential role of H-Lse in cellular functions.

As the most common modification of sialic acids found in mammalian cell surface sialoglycoconjugates, 9-O-acetylation and de-O-acetylation play an important role in the transition of antigenicity and transformation of intercellular cross-talk. 9-O-acetylation of sialic acids can mask some epitopes. For example, natural ligands of the B-cell adhesion molecule CD22 beta can be masked by 9-O-acetylation of sialic acids [19]. Similarly, it can inhibit binding of sialoadhesin, a macrophage-restricted and sialic-acid-dependent adhesion molecule [20]. On the other hand, 9-O-acetylation of sialic acids can form novel epitopes. Influenza virus C haemagglutinin specifically requires 9-O-acetylated sialic acids for binding to host cells [21]. Incubation of red blood cells with sialate 9-O-acetylesterase rendered the erythrocytes resistant against agglutination by influenza C virus [22]. O-acetylation of disialoganglioside GD3 by human melanoma cells has been reported to create a unique antigenic determinant [23]. Modifications of sialic acids may be an important mechanism underlying the interaction/cross-talk between different types of cells. The essential role of sialic acids modification in cellular communications may explain the presently observed wide distribution of H-Lse in all examined tissues.

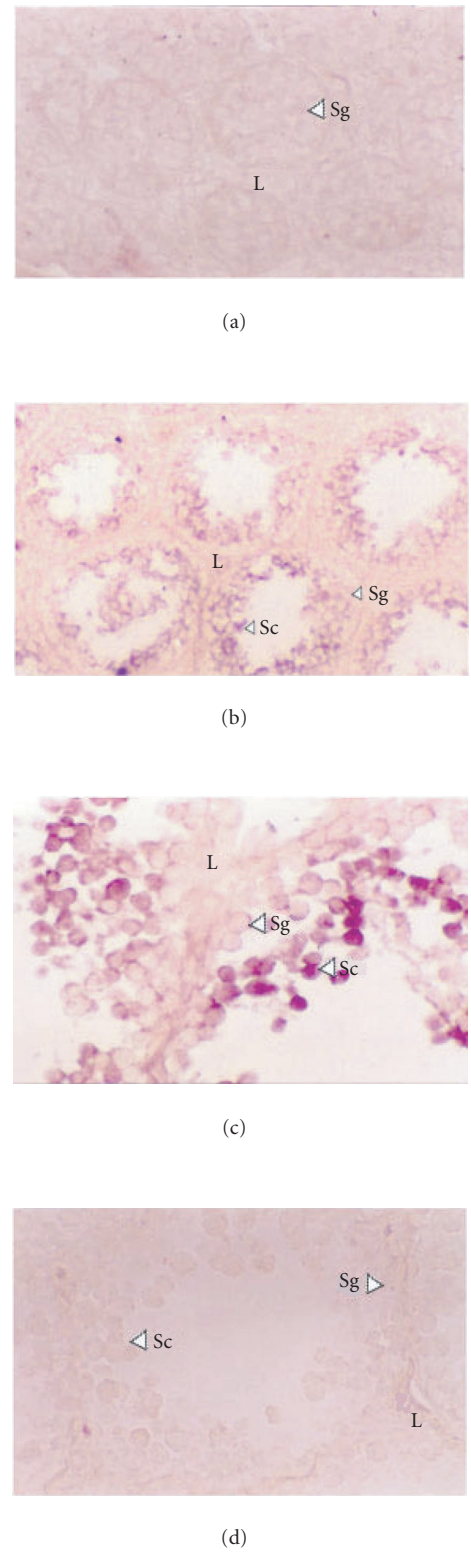
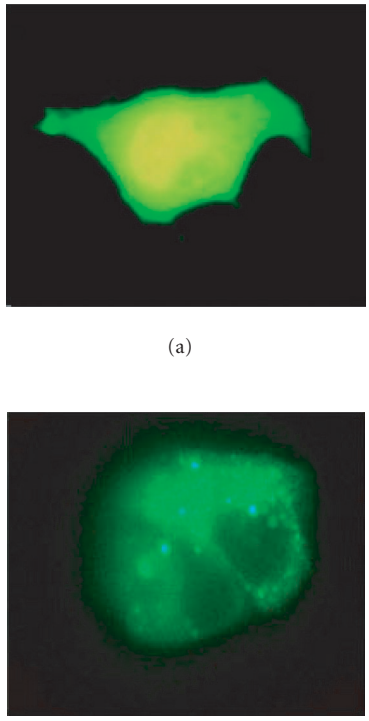


FIGURE 6. Detection of H-Lse mRNA in human embryonic and adult testes by in situ hybridization with antisense probes (a), (b), and (c) and sense probe (d). (a) Human embryonic testis (6 months) (400×); (b) human adult testis (100×); (c), (d) human adult testis (400×): Leydig cells (L), spermatogonia (Sg), and spermatocytes (Sc).



(a)

(b)

FIGURE 7. Distribution of GFP-H-Lse fusion protein in BXPC-3 cells. (a) GFP protein is evenly distributed throughout the cytoplasm. (b) GFP-H-Lse is compartmentalized in numerous large dense-core vesicles in the cytoplasm.

The present study suggests that the expression of H-Lse is developmentally regulated and spermatogenic stage-specific. The evidence for this includes: (1) lack of expression in embryonic testis; (2) association of high level of mRNA detected by DD-RT-PCR with the 4C but not 2C cells in adult testes; and (3) detection of in situ hybridization signal in spermatocytes but not spermatogonia or other somatic cells. In the absence of spermatogenesis, embryonic testis contains only two distinct cell types, spermatogonia and Sertoli cells, while the seminiferous epithelium of adult testis consists of germ cells at different stages of spermatogensis. The 4C cells found in adult testis include the primary spermatocytes and spermatogonia of G_2/M stage, while 2C cells include spermatogonia of G0/G1 stage, secondary spermatocytes, and Sertoli cells. The absence of H-Lse mRNA in embryonic testis and the high level of its mRNA in the 4C cells of adult testis suggest that its expression is restricted to spermatocytes, particularly the primary spermatocytes. Together with the in situ hybridization results showing mRNA of H-Lse restricted to spermatocytes, but not spermatogonia, Sertoli cells or interstitial cells, these data suggest that H-Lse is likely to be involved in the process of spermatogenesis, although its role in testicular development cannot be entirely ruled out. Unfortunately, due to the deformation of the available human testes, we were not able to make further distinction between primary and secondary spermatocytes. What has been clearly shown by the present data is that H-Lse is only present at a stage beyond spermatogonia, suggesting its possible role in the differentiation of germ cells.

Interestingly, the processes of 9-O-acetylation and de-O-acetylation of sialic acid have been implicated in organogenesis and cellular differentiation, since alteration of these processes could lead to interruption of cellular development such as embryogenesis. Transgenic mice constitutively overexpressed the 9-O-acetyl-sialic-acid-specific esterase of influenza C that has been found to arrest embryo development at the two-cells stage. It has also been reported that in vitro development of embryonic stem cells shows that the expression level of Lse is low at the initiation of the development, and followed by an increase at later stages [24]. In transgenic mice with selective expression of 9-O-acetyl-sialic-acid-specific esterase in retina and the adrenal gland, these organs showed various abnormalities in organization, while all other tissues appeared normal [25]. Lse has also been considered to play a key role in the differentiation of B lymphocyte [2], since it is expressed in late but not early B lymphocyte. The presently observed development-dependent pattern of H-Lse expression is consistent with that found in other cell types: absence or low expression at early stage of differentiation but high at later stages. Taken together, 9-O-acetyl esters in sialic acids appear to be important for development or cellular differentiation.

Spermatogenesis is a multiple-staged continuous progress of cellular differentiation. It has been reported that some cell surface glycoconjugates are modified during the early steps of spermatogenesis, and influence the differentiation of spermatogenic cells [26]. As nine-carbon sugars commonly found in many glycoproteins of spermatogenic cells, sialic acids represent a target for cell surface modification, that is, removal of 9-O-acetyl esters by enzymes such as Lse. Modification of sialic acids may result in alteration in cell-cell communication, that is, Sertoli cells and germ cells interaction, thereby influencing the differentiation of spermatogenic cells. Thus, future studies on the presently identified H-Lse may provide insight into molecular mechanisms underlying testicular development and/or germ cell differentiation during spermatogenesis in humans.

ACKNOWLEDGMENT

The research is supported by the National 973 fund of China, no G1999055901.

REFERENCES

[1] Rosenberg A, Schengrund C. *Biological Roles of Sialic Acids*. New York, NY: Plenum Press; 1976.

[2] Schauer R. *Sialic Acids: Chemistry, Metabolism and Function*. *Cell Biology Monographs*. New York, NY: Springer-Verlag; 1982.
[3] Kansas GS. Selectins and their ligands: current concepts and controversies. *Blood*. 1996;88(9):3259–3287.
[4] Powell LD, Varki A. I-type lectins. *J Biol Chem*. 1995;270(24):14243–14246.
[5] Varki A. Sialic acids as ligands in recognition phenomena. *FASEB J*. 1997;11(4):248–255.
[6] Drzeniek R. Substrate specificity of neuraminidases. *Histochem J*. 1973;5(3):271–290.
[7] Varki A, Diaz S. A neuraminidase from *Streptococcus sanguis* that can release O-acetylated sialic acids. *J Biol Chem*. 1983;258(20):12465–12471.
[8] Schauer R. Biosynthesis and function of N- and O-substituted sialic acids. *Glycobiology*. 1991;1(5):449–452.
[9] Varki A. Diversity in the sialic acids. *Glycobiology*. 1992;2(1):25–40.
[10] Takematsu H, Diaz S, Stoddart A, Zhang Y, Varki A. Lysosomal and cytosolic sialic acid 9-O-acetylesterase activities can be encoded by one gene via differential usage of a signal peptide-encoding exon at the N terminus. *J Biol Chem*. 1999;274(36):25623–25631.
[11] Mays-Hoopes LL, Bolen J, Riggs AD, Singer-Sam J. Preparation of spermatogonia, spermatocytes, and round spermatids for analysis of gene expression using fluorescence-activated cell sorting. *Biol Reprod*. 1995;53(5):1003–1011.
[12] Liang P, Pardee AB. Differential display of eukaryotic messenger RNA by means of the polymerase chain reaction. *Science*. 1992;257(5072):967–971.
[13] Green ED, Olson MV. Systematic screening of yeast artificial-chromosome libraries by use of the polymerase chain reaction. *Proc Natl Acad Sci USA*. 1990;87(3):1213–1217.
[14] Munroe DJ, Loebbert R, Bric E, et al. Systematic screening of an arrayed cDNA library by PCR. *Proc Natl Acad Sci USA*. 1995;92(6):2209–2213.
[15] Edelmann W, Cohen PE, Kneitz B, et al. Mammalian MutS homologue 5 is required for chromosome pairing in meiosis. *Nat Genet*. 1999;21(1):123–127.
[16] Tay J, Richter JD. Germ cell differentiation and synaptonemal complex formation are disrupted in CPEB knockout mice. *Dev Cell*. 2001;1(2):201–213.
[17] Yuan L, Liu JG, Zhao J, Brundell E, Daneholt B, Hoog C. The murine SCP3 gene is required for synaptonemal complex assembly, chromosome synapsis, and male fertility. *Mol Cell*. 2000;5(1):73–83.
[18] Stoddart A, Zhang Y, Paige CJ. Molecular cloning of the cDNA encoding a murine sialic acid-specific 9-O-acetylesterase and RNA expression in cells of hematopoietic and non-hematopoietic origin. *Nucleic Acid Res*. 1996;24(20):4003–4008.
[19] Sjoberg ER, Powell LD, Klein A, Varki A. Natural ligands of the B cell adhesion molecule CD22 beta can be masked by 9-O-acetylation of sialic acids. *J Cell Biol*. 1994;126(2):549–562.
[20] Kelm S, Schauer R, Manuguerra JC, Gross HJ, Crocker PR. Modifications of cell surface sialic acids modulate cell adhesion mediated by sialoadhesin and CD22. *Glycoconj J*. 1994;11(6):576–585.
[21] Herrler G, Klenk HD. The surface receptor is a major determinant of the cell tropism of influenza C virus. *Virology*. 1987;159(1):102–108.
[22] Schultze B, Gross HJ, Brossmer R, Klenk HD, Herrler G. Hemagglutinating encephalomyelitis virus attaches to N-acetyl-9-O-acetylneuraminic acid-containing receptors on erythrocytes: comparison with bovine coronavirus and influenza C virus. *Virus Res*. 1990;16(2):185–194.
[23] Cheresh DA, Reisfeld RA, Varki AP. O-Acetylatoin of disialoganglioside GD3 by human melanoma cells creates a unique antigenic determinant. *Science*. 1984;225(4664):844–846.
[24] Guimaraes MJ, Lee F, Zlotnik A, McClanahan T. Differential display by PCR: novel findings and applications. *Nucleic Acids Res*. 1995;23(10):1832–1833.
[25] Varki A, Hooshmand F, Diaz S, Varki NM, Hedrick SM. Developmental abnormalities in transgenic mice expressing a sialic acid-specific 9-O-acetylesterase. *Cell*. 1991;65(1):65–74.
[26] Saez FJ, Madrid JF, Aparicio R, Alonso E, Hernandez F. Glycan residues of N- and O-linked oligosaccharides in the premeiotic spermatogenetic cells of the urodele amphibian *Pleurodeles waltl* characterize by means of lectin histochemistry. *Tissue Cell*. 2000;32(4):302–311.

* Corresponding author.
E-mail: shajh@njmu.edu.cn
Fax: +86 25 666 2908; Tel: +86 25 666 2908