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Short note

Cloning and characterization of a human lysyl oxidase-like 3 gene (*hLOXL3*) ☆

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Abstract

Using the PCR primers generated from human expressed sequence tag (EST), the cDNA of lysyl oxidase-like gene 3 (*LOXL3*), a new member of human lysyl oxidases gene family, was cloned from the human fetal brain mRNA. The predicted amino acid sequence of the *hLOXL3* gene was highly homologous to mLOR2. Bioinformatics analysis shows that hLOXL3 protein is also a member of the scavenger receptor cysteine-rich family, which contains a 25 amino acids signal peptide. The *hLOXL3* gene was mapped to human 2p13 locus by BLAST search and at least 14 exons were found. Expression of the *hLOXL3* gene was detected in several human tissues and especially high in spleen and testis.

Keywords: Lysyl oxidase; *hLOXL3* gene; RT-PCR

1. Introduction

Lysyl oxidase (LOX) is a copper-dependent enzyme that initiates the lysine-derived cross-links of collagens and elastin. This cross-linking converts the soluble monomers of collagen and elastin into insoluble fibers in the extracellular matrix (Kagan and Trackman, 1991). Lysyl oxidase is also known as a tumor-suppressor gene, ras recision gene (rrg), which down-regulates the ras-induced cellular transformation of NIH3T3 cells (Contente et al., 1990; Kenyon et al., 1991). The role of lysyl oxidase during urchin development and chick aortic development has also been reported (Wu et al., 1992; Butler et al., 1987). Deficiency in lysyl oxidase activity has been found in a variety of human connective tissue disorders, including cutis laxa, Menkes syndrome and Ehler's Danlos syndrome type V (Royce et al., 1980). A hypothesis was suggested that diverse functions of lysyl oxidase might be attributed to the existence of the different lysyl oxidase family members.

Four human lysyl oxidase gene family members (*hLOX*, *hLOXL*, *hLOXL2*, *hLOR*) have been reported but little is known about their functions except LOX (lysyl oxidase) protein. Human LOXL (lysyl oxidase-like) may catalyze the lysine-derived cross-linking in type III collagen (Kim et al., 1999). Human LOXL2 (lysyl oxidase-like 2) is highly expressed in reproductive tissues and has an intracellular localization, which indicating it may be responsible for those reported intracellular and intranuclear catalytic functions (Jourdan-Le Saux et al., 1999). Human LOR

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(lysyl oxidase-related) protein is a member of the SRCR (scavenger receptor cysteine-rich) family with four scavenger cysteine-rich domains. It is an extracellular protein that may be specifically involved in cell adhesion and senescence (Satio et al., 1997).

Here we reported the cloning of a new member in the human *LOX* gene family that corresponds to *mLOR2* (mouse lysyl-oxidase related 2), the *hLOXL3* (human lysyl oxidase-like 3) gene, together with its sequence characterizations and mRNA expression profile in several human tissues.

2. Materials and methods

2.1. Cloning of hLOXL3 gene

The mouse *LOR2* cDNA sequence (AF053368) was used to search the human EST database by the BLAST search program (http://www.ncbi.nlm.nih.gov/cgi-bin/BLAST). The matching ESTs corresponding to the 5′ and 3′ sequence of mouse *LOR2* was used to generate the PCR primers to clone the corresponding human gene. Human fetal brain mRNA (Clontech) was purchased and RT-PCR was performed. PCR conditions were as follows: 1 min at 94°C for denature; 30 s denature at 94°C; 30 s annealing at 60°C; and 3 min extension at 68°C for 35 cycles; a final extension at 68°C for 7 min. The main band was then excised and cloned into pGEM-T vector (Promega). DNA sequencing was performed using Big-Dye Terminator Cycle sequencing Kits (Perkin–Elmer). The complete sequence was determined and confirmed by primer walking strategy. Sequence assembly was performed with program Acembly (Sanger's center).

2.2. Bioinformatics analysis

BLAST-N searching against the genome database and the nr database of GenBank was performed to identify the chromosomal localization of the *hLOXL3* gene. STS markers matching the *hLOXL3* gene were searched against the Unigene database in NCBI. The predicted amino acid sequence was compared against the profile entries in Prosite to find the occurrence of known profiles (http://www.expasy.ch/pfscan) and signal peptide prediction was performed at http://www.cbs.dtu.dk/services/SignalP.

2.3. Assessment of hLOXL3 mRNA tissue distribution

Some samples from human multiple tissue cDNA (MTC) panels (Clontech) were chosen as PCR template. The primers for G3PDH were as follows: 5′-TGA AGG TCG GAG TCA ACG GAT TTG GT-3′; and 5′-CAT GTG GGC CAT GAG GTC CAC CAC-3′. The PCR primers and PCR conditions for *hLOXL3* were as mentioned above. Twenty-four cycles (for G3PDH) or 35 cycles (for *hLOXL3*) of amplification were performed using Elongase DNA polymerase (GIBCOBRL). The PCR products were then resolved on a 1.2% Metaphor agarose gel (FMC).

3. Results and discussion

3.1. The human LOXL3 cDNA sequence

A BLAST search using the *mLOR2* cDNA sequence against human dbEST (expressed sequence tag) was performed to identify its human homologies. Several ESTs that have considerable homology (85–90%) were found throughout the entire *mLOR2* gene. Overlapping ESTs such as AI651637 and AI917716 were found matching the 3′ sequence of *mLOR2* and a gap between 5′ and 3′ sequence also existed. Only one EST (AI752772) was found to match the 5′ sequence of *mLOR2* gene. PCR primers (A 5′-GGC ACG AGC TAG GAC TGA TCT CCA G-3′ and B 5′-CCT AGC AAC ATA TTA TAG TAA AAA ATG AGG TGG-3′) were designed from the 5′ and 3′ matched AI752772 and AI651637 sequences. The two primers generate a 2781 bp fragment by RT-PCR (reverse transcription-polymerase chain reaction) from human fetal brain mRNA (Clontech) and the full length was obtained by a walking strategy (Fig. 1a). The result showed that the gene had 88% homology to the entire sequence of *mLOR2* gene and the proteins encoded by these two genes shared 92% identity throughout the sequence. The cDNA was designated the human lysyl oxidase-like 3 gene (*hLOXL3*) in agreement with Human Genome Organization (HUGO) Nomenclature Committee.

3.2. Sequence characterization

The result of BLAST-N search against the nr database of GenBank showed the gene derived from the 2p13 genomic sequence of AC006544, spanned more than 21 kb of the genomic DNA and consisted of at least 14 exons. An STS (sequence tagged site) marker stSG4710 matched the 3′ sequence of *hLOXL3* cDNA suggesting that the gene is located between D2S292 and D2S145. The gene encoded a putative protein approximately 753 amino acids and cleavage of the predicted 25 residues in the signal peptide part would generate a mature protein of 728 amino acids. Ten cysteine residues of the lysyl oxidase domain and the catalytic domain (DIDCQWIDITDVQPGNY) and the putative copper-binding site (WEWHSCHQHYH) (Krebs and Kranetz, 1993) are

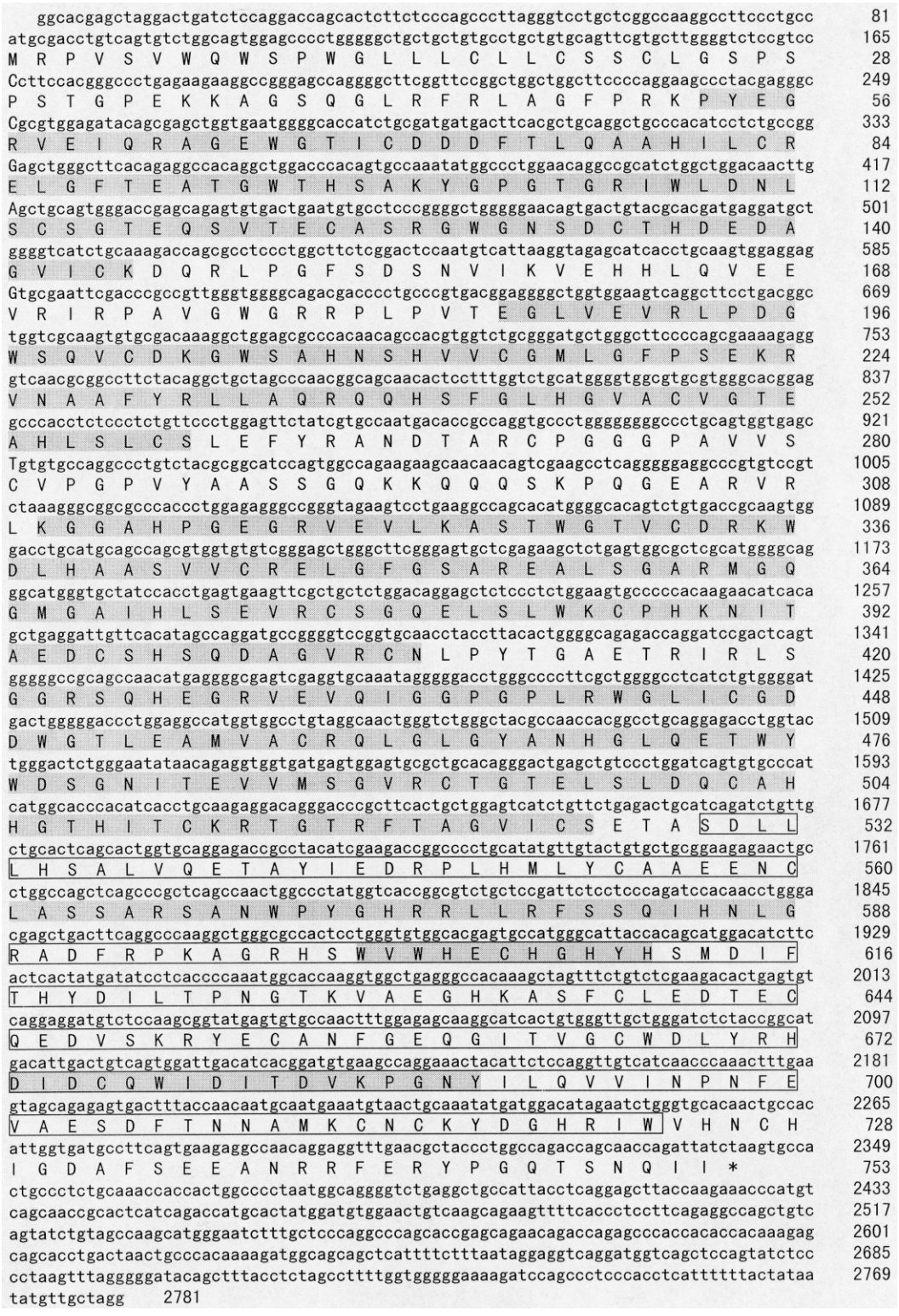

Fig. 1. Sequence and lysyl oxidase domain homology of the *hLOXL3* gene. (a) Nucleotide and deduced amino acids sequence of *hLOXL3*. Amino acids sequence is represented below the DNA sequences with the one-letter amino acid codes. Nucleotides and amino acids are numbered at the right. The asterisk represents the stop codon. The four SRCR domains are shaded and the lysyl oxidase domain is boxed. The copper binding site and the catalytic domain in the lysyl oxidase domain are also shaded. (b) Lysyl oxidase domain homology alignment of hLOXL3, mLOR2 (AAC83205), hLOR (AAB49697), hLOXL2 (AAD34343), hLOXL (NP_005567), hLOX (NP_002308). The alignment was performed by GeneDoc program (http://www.cris.com/~Ketchup/genedoc.shtml): black (100% similarity); grey (80–90% similarity); light grey (60–70% similarity). Amino acids are numbered according to their actual position in the protein. The copper binding site and the catalytic domain are underlined. The conserved 10 cysteine residues are represented by asterisk. Only hLOXL2 has a mismatch in the tenth conserved cysteine residue.

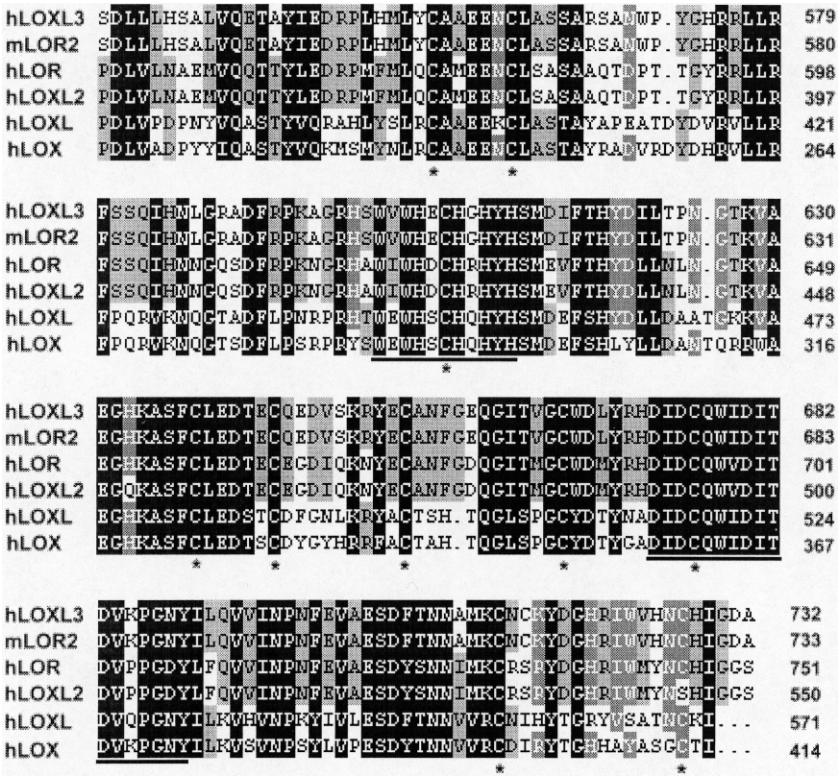

Fig. 1. (*Continued*).

conserved in hLOXL3, including the four-histidine residues involved in copper-binding co-ordination (Fig. 1b). Analysis of hLOXL3 with Profilescan identified four copies of approximately 100-amino acid SRCR domain (Fig. 1a). The SRCR domains are found in several secreted or cell surface proteins and are likely to be involved in binding to other cell surface or extracellular molecules (Resnick et al., 1994).

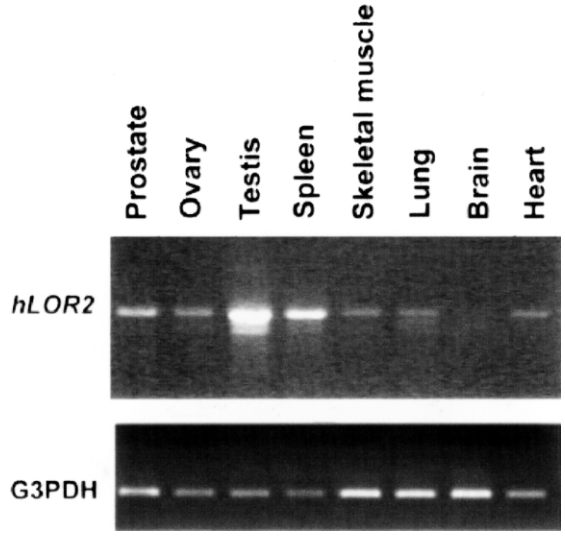

Fig. 2. Expression pattern of *hLOXL3* gene in eight human tissues. Pre-normalized cDNAs was purchased from CLONTECH and employed as a template in PCR reactions containing *hLOXL3* and G3PDH-specific primer as described in 'Materials and Methods'.

3.3. hLOXL3 gene expression

The tissue expression pattern of the *hLOXL3* gene was investigated by PCR amplification of normalized adult human cDNA. The 2781 bp main band of *hLOXL3* mRNA was detected in heart, skeletal muscle, testis, ovary, lung, spleen and prostate. Spleen and testis express the *hLOXL3* gene in high levels while no obvious band was detected in adult brain (Fig. 2). Since the *hLOXL3* gene was cloned from a human fetal brain mRNA, we speculated that *hLOXL3* gene expression may be temporally regulated. Though the specificity of two primers was examined by BLAST search against nr database (Primer B only matches to AF141307 and no cDNA was found to match primer A), more than one band was detected especially in testis and lung. This leads to the suggestion that other lysyl oxidase family members or alternatively spliced variant of the *hLOXL3* gene may exist.

Mouse *LOR2* gene is located in the mnd2 region of mouse BAC clone 245c12, which is a synteny region to 2p13 region containing *hLOXL3* gene (Jang et al., 1999). Considering their sequence homology, *mLOR2* and *hLOXL3* genes are orthologous in mouse and human.

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