# Cloning and characterization of a fourth human lysyl oxidase isoenzyme

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We report here the complete cDNA sequence and exon-intron organization of the human lysyl oxidase-like (LOXL)3 gene, a new member of the lysyl oxidase (LO) gene family. The predicted polypeptide is 753 amino acids in length, including a signal peptide of 25 residues. The C-terminal region, residues 529–729, contains a LO domain similar to those in the LOX (the first characterized LO isoenzyme), LOXL and LOXL2 polypeptides. It possesses the putative copper binding sequence, and the lysine and tyrosine residues that form the lysyltyrosyl quinone cofactor. The N-terminal region, which is similar to that in LOXL2 but not those in LOX and LOXL, contains four subregions similar to scavenger receptor cysteine-rich domains and a putative nuclear localization signal. Recombinant LOXL3, expressed in HT-1080 cells, was secreted into the culture medium but was not detected by immunofluorescence staining in nuclei. The LOXL3 mRNA is 3.1 kb in size and is expressed in many tissues, the highest levels among the tissues studied being seen in the placenta, heart, ovary, testis, small intestine and spleen.

Key words: collagen, elastin, extracellular matrix, lysyl oxidase, lysyl oxidase-like protein.

## INTRODUCTION

Lysyl oxidase (LO; EC 1.4.3.13) is an extracellular copper-dependent amine oxidase that has long been known to catalyse the first step in the formation of the lysine- and hydroxylysine-derived crosslinks in collagens and lysine-derived crosslinks in elastin [1,2]. Recently, multiple novel biological functions have been attributed to this enzyme. These include tumour suppressor activity and growth control, and are believed to involve additional substrates which may be intracellular or even intranuclear [3–16].

Three isoforms of human LO have been characterized so far. The gene for the first characterized LO isoenzyme, traditionally known as *LOX*, is located on human chromosome 5q23.3-q31.2 [17]. The LOX mRNA encodes a 50 kDa precursor, which is secreted from the cell and processed in the extracellular space to an enzymically active 30 kDa form [18]. This form has been purified to homogeneity from several animal species and from human placenta [1,19–21]. In addition to the crosslinking of collagen and elastin molecules, it has been shown in many studies that LOX probably also acts as a tumour suppressor [4,15]. A variety of cultured, malignantly transformed human cells exhibit very low levels of LO mRNA and activity relative to their non-transformed counterparts [3,9]. The mouse *LOX* gene is also known as the *rrg* or *ras* recision gene, and its product is able to function as a phenotypic suppressor of the *ras* oncogene [4]. This feature has now been verified by several investigators [9,10,15,22–27].

The second LO isoform, LOXL, is also an extracellular enzyme. Its gene has been mapped to human chromosome 15q23, and it encodes a 63 kDa polypeptide [28], although proteins with molecular masses of approximately 68, 52, 42 and 30 kDa have been identified in the culture media of human, murine and bovine cells and in tissues [29]. The LOXL protein has been localized to sites of *de novo* fibrosis in the liver and shown to be co-regulated with the expression of the gene encoding type III collagen, whereas the *LOX* gene was co-regulated with that encoding the pro$\alpha$1(I) chain of type I collagen, suggesting that the LOX and LOXL polypeptides may have different functions [30].

The gene for the third LO isoform, called *LOXL2* or *WS9-14*, is located on human chromosome 8p21.2-p21.3 [31] and encodes a 87 kDa polypeptide [32]. *LOXL2* is expressed especially in senescent fibroblasts and several adherent tumour cell lines, but is down-regulated in several non-adherent tumour cells, suggesting that it may be involved in cell adhesion and that loss of this protein may be associated with the loss of tumour cell adhesion and may therefore play a role in metastasis [32]. The presence of a signal peptide and four scavenger receptor cysteine-rich domains (SRCR) suggests that LOXL2 is an extracellular protein which may bind to other cell-surface or extracellular matrix molecules [32].

All these LO isoforms show extensive similarity in their C-terminal parts, which contain the putative copper binding site [33] and the lysine and tyrosine residues that form the lysyltyrosyl quinone cofactor [34].The N-terminal regions of these proteins, however, show no significant similarity. We report in the present study on a new member of the emerging *LO* gene family that encodes a protein referred to as LOXL3.

## MATERIALS AND METHODS

### Search for expressed sequence taqs and high throughput genomic sequences

The BLAST program (version 2.0) was used to search all available databases for sequences that are similar to those of the coding regions of the human LOX [17], LOXL [28] and LOXL2 [31,32] cDNAs. Two high throughput genomic sequences (GenBank®

Abbreviations used: LO, lysyl oxidase; LOX, first characterized LO isoenzyme; LOXL, LO-like protein; SRCR, scavenger receptor cysteine-rich domain; RACE, rapid amplification of cDNA ends.

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The nucleotide sequence reported in this paper will appear in DDBJ, EMBL, GenBank® and GSDB Nucleotide Sequence Databases under accession number AF282619.

accession numbers AC005033 and AC005041) similar to sequences of these cDNAs were found and, based on these, oligonucleotides were synthesized.

### Isolation of cDNA clones

The PCR primer pairs HLO406F (5′-AGCAACACTCCTTTG-GTCTGCATGGG-3′)/HLO406R (5′-CCTCCCCCTGAGGC-TTCGACTGTTGTTG-3′) and HLO402F (5′-TGTGCTGCG-GAAGAGAACTGCCTGGC-3′)/HLO404R (5′-ATGTGGC-AGTTGTGCACCCAGATTCTATGTC-3′) were used to obtain 204-bp and 527-bp products from a human placenta λgt11 cDNA library (Clontech). These were then used to screen the same placenta library, and two positive clones were obtained and characterized. To obtain the 5′end of the cDNA, the primers LO4cDNA1F (5′-ATGCGACCTGTCAGTGTCTGGCAGT-GGAGCCC-3′) and HLO406R (above) were used for PCR with human placenta and fetus cDNA pools (Marathon Ready cDNA; Clontech). The PCR was performed using the Advantage 2 PCR Enzyme System kit (Clontech), and reaction conditions entailing 30 cycles at 94 °C for 30 s, 94 °C for 10 s and 68 °C for 2 min. The PCR products were sequenced directly. 5′ Rapid amplification of the cDNA ends (RACE) was performed using the human placenta and fetus cDNA pools, decribed above, as templates, and the Advantage 2 PCR Enzyme System kit with the oligonucleotide pairs, AP1 (5′-CCATCCTAATACGACTC-ACTATAGGGC-3′) and HLO406R, for 40 cycles at 94 °C for 2 min, 94 °C for 15 s, 65 °C for 1 min and 72 °C for 1 min; and AP1 and LO4RNA1R (5′-CCTGTTCCAGGGCCATATTTG-GCACTGTG-3′) for 40 cycles at 94 °C for 2 min, 94 °C for 15 s, 65 °C for 1 min and 72 °C for 1 min. All the products were subcloned into the plasmid pUC18 (SureClone; Amersham Pharmacia Biotech).

### Characterization of the exon–intron organization of the *LOXL3* gene

The high throughput genomic sequences were compared with cDNA sequences obtained using the exon prediction softwares GRAIL 1.3, FGENES and Genie. To verify the position of the first intron, PCR was performed using the placenta and fetal cDNA pools, as described above, and PCR enzyme with the oligonucleotide pairs HLO4prom4F (5′-AGGACCAGCACT-CTTCTCCCAGCCCTTAGG-3′) and HLO406R for 50 cycles at 94 °C for 4 min, 94 °C for 15 s, 65 °C for 1 min and 72 °C for 1 min, and HLO4prom4F and LO4RNA1R (5′-CCTGTTCC-AGGGCCATATTTGGCACTGTG-3′) for 50 cycles at 94 °C for 4 min, 94 °C for 15 s, 65 °C for 1 min and 72 °C for 1 min. These reactions yielded products of size 971 bp and 371 bp respectively. The PCR products were sequenced directly.

### DNA sequencing

DNA sequencing was performed using an automated sequencer (ABI Prism 377; Applied Biosystems). DNASIS and PROSIS version 6.00 softwares (Pharmacia) were used to analyse the sequence data.

### Northern-blot analysis

Human multi-tissue Northern blots I and II (Clonetech) containing 2 μg of poly(A)$^+$ RNA per sample were hybridized using UltraHyb solution (Ambion) under the stringent conditions specified by the manufacturer. The 971 bp PCR product from the 5′ end of the cDNA obtained in PCR using primers HLO4prom4F and HLO406R was used as a probe.

### Recombinant expression of the LOXL3 polypeptide

Recombinant expression of the LOXL3 cDNA was carried out using the mammalian expression vector pcDNA3.1/V5-HisA (Invitrogen). A cDNA construct, generated by PCR with the human fetus cDNA pool as the template, covered nucleotides 75–2333, which correspond to the open reading frame of the LOXL3 cDNA. The construct contained, at its 3′ end, sequences encoding the V5 epitope and a His tag, which was used for antibody staining of the recombinant protein. All the nucleotides in the construct were verified by sequencing. The plasmid was then transfected into human HT-1080 fibrosarcoma cells (American Type Culture Collection), using the Fugene6™ transfection reagent (Boehringer Mannheim) according to the manufacturer's instructions.

### Cell culture

The LOXL3/pcDNA3.1-V5/HisA-transfected HT-1080 cells were cultured in Dulbecco's modified Eagle's medium (Life Technologies Inc.) containing 10 % (v/v) fetal-bovine serum, 400 μg/ml G-418 sulphate and 100 units/ml penicillin and streptomycin, at 37 °C in air/$CO_2$ (19:1). Lysates were prepared from confluent plates by removing the medium, the cell layers were rinsed with PBS, and the cells were suspended by incubation for 5 min in Hanks balanced salt solution containing 0.05 % trypsin and 0.53 mM EDTA. After the addition of an equal volume of 10 % fetal-bovine serum/Dulbecco's modified Eagle's medium, the cells were sedimented at 500 ***g*** and the cell pellet was resuspended, washed twice in PBS and re-sedimented. The resulting pellet was suspended in PBS and the cell residue was lysed by adding a solution of 62.5 mM Tris/HCl (pH 6.8)/10 % (v/v) glycerol/2 % (w/v) SDS/5 % (v/v) 2-mercaptoethanol, and heating at 100 °C for 5 min. For medium samples, confluent plates were cultured for 16 h as above, except that the medium contained no FBS. The samples for SDS/PAGE were concentrated 20–50-fold using MicroSep™ centrifugal 10 kDa concentrators (Filtron). All samples were analysed by SDS/PAGE (8 % polyacrylamide) and immunoblotting.

### Western blotting

Western-blot analyses were performed as described previously [35,36]. A monoclonal anti-V5 antibody (Invitrogen), specific for the V5 epitope in the LOXL3 expression construct, was used to monitor the expression patterns in samples of the cell culture medium and cell lysates.

### Immunofluorescence staining of the recombinant LOXL3 protein

LOXL3-transfected HT-1080 cells, seeded on to glass coverslips and grown to the desired density, were fixed for 5 min in pre-cooled methanol at −20 °C and incubated in 2 % (w/v) BSA/PBS (pH 7.2) for 30 min, to reduce non-specific staining. A monoclonal anti-V5 antibody was applied at an appropriate dilution, the samples were incubated for 1 h at room temperature, and then extensively washed with PBS. FITC-conjugated goat anti-mouse secondary antibody was diluted according to the manufacturer's instructions (DAKO) and was allowed to bind to the specimens for 1 h at room temperature. After extensive washing with PBS, the coverslips were mounted on to microscope slides with Immu-Mount (Shandon) and viewed and photographed using a light microscope (Leitz Aristiplan). The specificity of the staining was confirmed by staining native HT-1080 cells as described above. All of the control stainings resulted only in a faint, uniform background staining.

## RESULTS AND DISCUSSION

### Cloning of the fourth human LO isoform LOXL3

A search of all the available human databases for sequences similar to those of the coding regions of the human LOX, LOXL and LOXL2 polypeptides [17,28,31,32], identified two high throughput genomic sequences AC005033 and AC005041. Two oligonucleotide pairs were then used to obtain PCR products of 240 and 527 bp from a human placenta cDNA library, and these were used as probes to screen the same library. Two clones that covered nucleotides 825–2574 of the LOXL3 cDNA were obtained and sequenced. To obtain the 5′ end of the cDNA, the sequences of AC005033 and AC005041 and the two cDNA clones were used to find proper primers for PCR. The boundaries of the exons of the *LOXL3* gene were predicted using the GRAIL 1.3, FGENES and Genie programs, and several primers were synthesized and tested. Two of these were used to obtain a 913 bp PCR product, which included an overlapping 5′ region of the cDNA. To obtain a 5′RACE product, the primers AP1 and HLO406R were used in the first round of PCR, and AP1 and LO4RNA1R in the second round, yielding a product of 388 bp. This same 5′RACE product was obtained from both human placental and human fetal cDNA pools.

The full-length cDNA was at least 2574 bp in size and contained an open reading frame of 2226 bp. The 5′ non-coding region was 74 bp in length and the 3′ non-coding region at least 238 bp. The cloned cDNA had no polyadenylation signal, which indicated that the actual size of the 3′ non-coding region was greater than 238 bp.

### Amino acid sequence of LOXL3 and its comparison with those of LOX, LOXL and LOXL2

The open reading frame encoded a 753 amino acid polypeptide (Figure 1). A putative signal peptide was present in its N-terminus, the most likely cleavage site, based on the computational parameters of Nielsen et al. [37], being between residues 25 and 26. Thus the first amino acid of the processed polypeptide is probably serine, and the size of the processed polypeptide is 728 residues (Figure 1). The calculated molecular mass of the processed polypeptide is 80.3 kDa.

The four non-processed LO-like polypeptides varied in length from 417 to 774 residues, LOX being the shortest and LOXL2 the longest one. All four polypeptides had a C-terminal LO domain located between residues 529 and 729 in the LOXL3 sequence (Figure 1). The degree of amino acid sequence identity between the LOXL3 and LOXL2 polypeptides within this domain was 69 %, whereas the corresponding identities between LOXL3 and LOXL and between the LOXL3 and LOX were 53 % and 51 % respectively. This domain contains the copper binding sequence [33] WVWHECHGHYH (residues 601–611 in the LOXL3 sequence), with four histidine residues that supply the N-ligands for the co-ordination complex of this cation in all LO isoforms (Figure 1). The tyrosine residue (Tyr-689 in the LOXL3 sequence), which together with Lys-634 forms the lysyltyrosyl quinone cofactor [34], is located in the sequence DIDCQWIDITDVKOGNY, which is entirely conserved between LOXL3 and LOX (Figure 1). The ten cysteine residues, characteristic of all LO isoforms, are likewise entirely conserved.

Although the N-terminal region of the LOXL3 polypeptide shows no similarity to those of LOX and LOXL, the LOXL2 polypeptide is similar to LOXL3, even in this region; the degree of amino acid sequence identity between the full-length LOXL3 and LOXL2 polypeptides being 55 %. This suggests that the two polypeptides form a subfamily within the family of LO-related

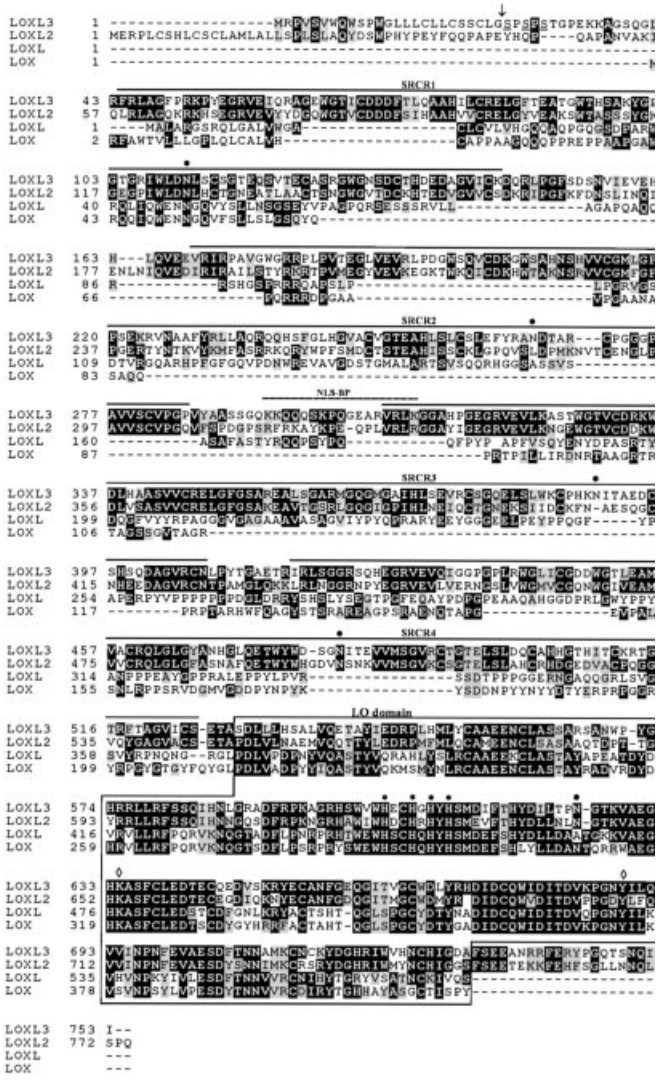


**Figure 1 Comparison of the amino acid sequence of human LOXL3 with those of the human isoforms LOX [17], LOXL [28] and LOXL2 [32]**

The predicted signal peptide cleavage site of LOXL3 is indicated by an arrow, the SRCRs are overlined, the three putative O-glycosylation sites are underlined, the five putative N-glycosylation sites are indicated by black dots and the LO domain, corresponding to the LOXL3 sequence, is boxed. The four histidine residues supplying the nitrogen ligands for the copper co-ordination complex are indicated by asterisks and the lysine and tyrosine residues that form the lysyltyrosyl quinone cofactor by open diamonds.

proteins. The N-terminal regions of the LOXL3 and LOXL2 polypeptides each contain four subregions similar to SRCRs [32,38]. The N-terminal region of the LOXL3 polypeptide also contains a bipartite nuclear localization signal [39], KKQQQS-KPQGEARVRLKG (residues 293–311), which is not found in any other LO-like polypeptide (Figure 1). The processed LOXL3 polypeptide contains three putative O-glycosylation sites (Ser-26, Ser-28 and Ser-30) in its extreme N-terminus that are not found in any other LO-like polypeptide, and it also has five potential N-glycosylation sites, whereas the LOX, LOXL and LOXL2 polypeptides have three, one and three such sites respectively (Figure 1). Only the extreme C-terminal one of the five sites is located in a position identical to that of its counterpart in the LOXL2 polypeptide (Figure 1).

**Table 1 Exon–intron organization of the *LOXL3* gene**

Capital letters denote exons and lower case letters introns. Bold letters indicate the nucleotides at the splice junctions.

| Exon no. | Exon-intron boundaries | | | Exon size (bp) | Intron size (bp) |
|---|---|---|---|---|---|
| 1 | | TCCTGCTCGGCCAAG | **gt**agggg | 61 ≤ | 1229 |
| 2 | cacccag GCCTTCCCTGCCATG<br>M | ATGGCCCTGGAACAG<br>G P G T (G) | **gt**gagca | 325 | 1973 |
| 3 | tccac**ag** GCCGCATCTGGCTGGA<br>R I W L D | TCCAATGTCATTGAG<br>S N V I E | **gt**ctgca | 164 | 601 |
| 4 | ccccc**ag** GTAGAGCATCACCTG<br>V E H H L | CGCGGCCTTCTACAG<br>A A F Y (R) | **gt**gaggg | 215 | 12440 |
| 5 | cccct**ag** GCTGCTAGCCCAACG<br>L L A Q R | AAGCCTCAGGGGGAG<br>K P Q G E | **gt**gtgtg | 220 | 237 |
| 6 | tctat**ag** GCCCGTGTCCGTCTA<br>A R V R L | CTCGCATGGGGCAGG<br>R M G Q (G) | **gt**gaggt | 181 | 140 |
| 7 | gccct**ag** GCATGGGTGCTATCC<br>M G A I H | GGGGCAGAGACCAGGC<br>G A E T R | **gt**cagtc | 155 | 240 |
| 8 | actac**ag** ATCCGACTCAGTGGG<br>I R L S G | AACCACGGCCTGCAG<br>N H G L Q | **gt**gagtg | 168 | 133 |
| 9 | tcccc**ag** GAGACCTGGTACTGG<br>E T W Y W | TCATCTGTTCTGAGA<br>I C S E (T) | **gt**gagtg | 163 | 517 |
| 10 | ggtgc**ag** CTGCATCAGATCTGT<br>A S D L L | GCACGAGTGCCATGG<br>H E C H (G) | **gt**gagag | 247 | 96 |
| 11 | cccac**ag** GCATTACCACAGCAT<br>H Y H S M | CTGAGTGTCAGGAGG<br>E C Q E (D) | **gt**gagtt | 116 | 79 |
| 12 | cctcc**ag** ATGTCTCCAAGCGGT<br>V S K R Y | AACTACATTCTCCAG<br>N Y I L Q | **gt**acctg | 137 | 106 |
| 13 | ctcac**ag** GTTGTCATCAACCA<br>V V I N P | ACAACTGCCACATTG<br>N C H I (G) | **gt**atttg | 112 | 202 |
| 14 | tctgc**ag** GTGATGCCTTCAGTG...AACCAGATTATCTAA<br>D A F S E...N Q I I | | – | 312 ≤ | – |

## Exon–intron organization of the *LOXL3* gene

The *LOXL3* gene has 14 exons and spans about 22 kb of genomic DNA. 5′ RACE suggested that this gene may have its first intron within the 5′ untranslated region. To verify the existence of this intron, PCR was performed with pools of human placental and fetal cDNAs using two pairs of primers. These reactions gave products with the expected sizes of 971 and 371 bp, thus indicating the presence of this intron.

Exons 2–13 varied in size from 112 to 247 bp, and the introns varied from 79 to 12440 bp, intron 4 being the longest (Table 1). All the exon–intron boundaries showed the consensus sequence (C/T)AG–exon–GT(A/G), except those of exons 3 and 7, in which the sequence at the 5′-splice-donor site was GTC. The locations of introns 1–10 were identical or very similar to those of introns 4–13 in the human *LOXL2* gene [31], and those of introns 9, 10 and 12 were identical to those of introns 1, 3 and 5 in the *LOX* and *LOXL* genes [40,41].

The 3′ untranslated region of the *LOXL3* gene had a TTATATAAAAA sequence 213 bp downstream of the 3′ end of the cloned cDNA, a TAAATATAT sequence 837 bp downstream of the 3′ end and a GCAATAAAGT sequence 904 bp downstream of the 3′ end, all of which may act as polyadenylation signals. Information gained from the high throughput sequences AC005033 and AC005041 suggests that the *LOXL3* gene is located on chromosome 2p13.

## Expression of LOXL3 mRNA in various human tissues

Northern blots of various human tissues containing 2 $\mu$g of poly(A)$^+$ RNA in each lane were hybridized with a 971 bp PCR product from the 5′ end of the LOXL3 cDNA as a probe (Figure 2). Only a single mRNA of about 3.1 kb was identified. At 2.8 kb, this corresponded well to the size expected for an mRNA lacking the poly(A)$^+$ tail, if the first of the three potential polyadenylation signals (see above) is used. The use of either of the other two polyadenylation signals would result in a larger mRNA size than was found here. Distinct hybridization signals were obtained with poly(A)$^+$ RNAs from all the tissues examined, the highest expression levels among these tissues being seen in the placenta, heart, ovary, testis, small intestine and spleen.

## Recombinant expression of the LOXL3 polypeptide in human HT-1080 cells

Human HT-1080 fibrosarcoma cells were transfected with an expression construct encoding the open reading frame of the LOXL3 cDNA. The construct also contained sequences coding

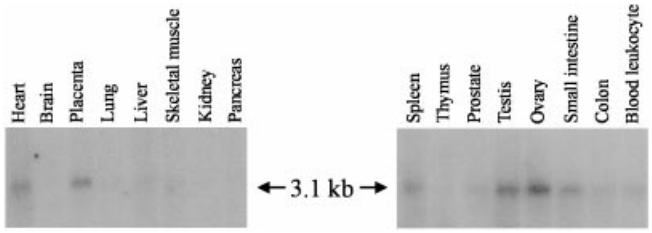


**Figure 2 Northern blot of LOXL3 mRNA in various human tissues**

Each lane contains 2 $\mu$g of poly(A)$^+$ RNA from the adult tissue indicated. The probe was a 971 bp PCR product from the 5′ end of the cDNA. The position and size of the LOXL3 mRNA are indicated with arrows.

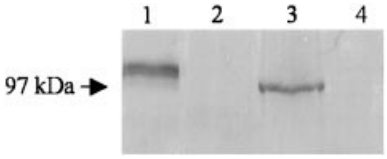


**Figure 3 SDS/PAGE analysis of LOXL3/V5His recombinant protein expression in the HT-1080 cell line using anti-V5 antibody**

Lane 1, concentrated medium from LOXL3-transfected cells; lane 2, concentrated medium from non-transfected cells; lane 3, cell lysate from LOXL3 transfected cells; lane 4, cell lysate from non-transfected cells. The expressed LOXL3 protein is indicated by an arrow.

for the V5 epitope and a histidine tag containing six histidine residues in its 3′ end, and this made it possible to study the expression by antibody staining for the V5 epitope.

Western blots of lysates from the transfected HT-1080 cells showed the expression of a 97 kDa polypeptide (Figure 3, lane 3), whereas no staining was seen in lysates from non-transfected cells (Figure 3, lane 4). This size is slightly larger than the calculated overall molecular mass of approx. 83.6 kDa for the processed LOXL3 peptide, including its V5-epitope and the histidine tag (these add approx. 2.3 kDa to the molecular mass). Analysis of concentrated medium samples showed the presence of two major polypeptides slightly larger in size than the polypeptide present in the cell lysates, and a very minor 97 kDa polypeptide (Figure 3, lane 1). The larger sizes of the polypeptides present in the cells and medium relative to the calculated molecular mass are probably due to the use of some of the potential glycosylation sites present in the LOXL3 polypeptide.

The extracellular location of the LOXL3 polypeptide and the presence in this polypeptide of four SRCRs suggest that LOXL3 may be involved in binding and crosslinking to other cell-surface and extracellular-matrix proteins. These domains are found in diverse secreted and cell-membrane-associated proteins, such as the lymphocyte glycoproteins CD5 [42] and CD6 [43], and the type I macrophage scavenger receptor [44]. The cysteine-rich domains of CD5 and CD6 have been shown to be responsible for binding to their ligands [45,46]. The LOX and LOXL polypeptides have been shown to be processed to LO forms in the extracellular matrix [18,29], whereas no data are available on any proteolytic processing of LOXL2. The present data indicate that the recombinant LOXL3 polypeptide is not processed to a shorter form in the medium of HT-1080 cells or in the presence of human primary skin fibroblast medium (results not shown). The presence of the SRCRs in the LOXL3 and LOXL2 polypeptides raises the possibility that these polypeptides may exert their effects without any proteolytic processing, but additional studies in other cell systems are needed to elucidate this aspect.

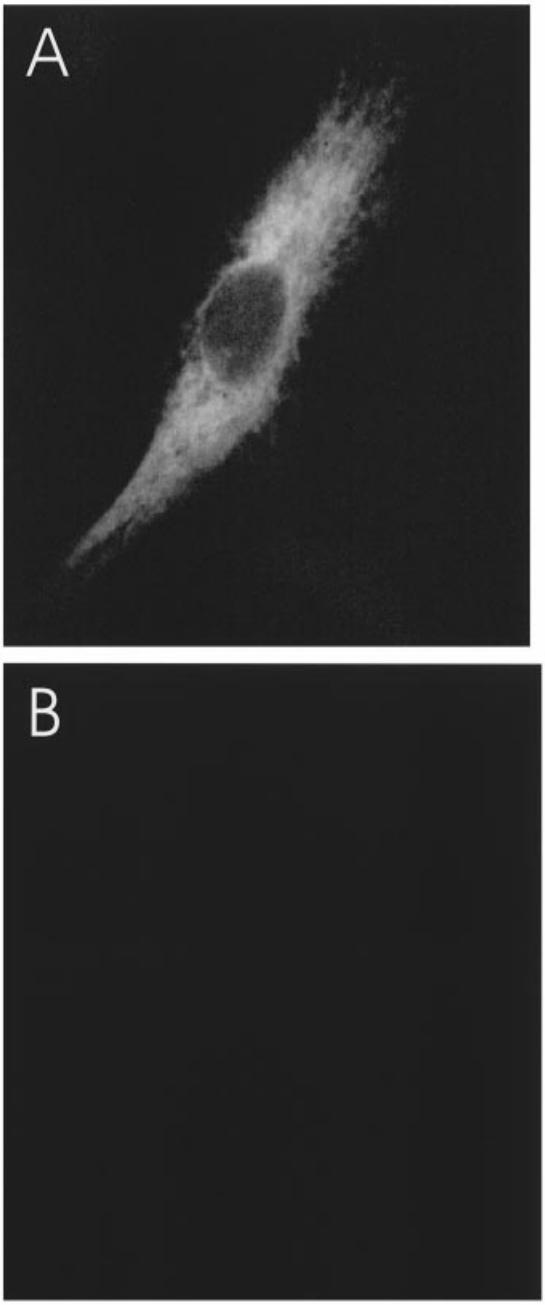


**Figure 4 Immunofluorescence staining of the LOXL3/V5His recombinant protein in a transfected HT-1080 cell line (A) and non-transfected HT-1080 cells (B)**

Magnification $\times$ 50.

### Immunofluorescence staining of HT-1080 cells expressing recombinant LOXL3

Immunofluorescence staining of the transfected HT-1080 cells by a monoclonal antibody specific for the V5 epitope, generated to

the C-terminal end of the recombinant polypeptide, gave a strong cellular signal but no signal could be detected in the nuclei (Figure 4). This suggests that LOXL3 may not contribute to the LO activity identified in the nucleus, which was shown to be due, at least in part, to the LOX polypeptide [14]. This result is somewhat surprising, as LOXL3 has a putative nuclear localization signal which is not present in any other member of the LO family. These bipartite signals are usually not highly conserved, however, and consist of two adjacent basic amino acids (Arg or Lys), a spacer region of any ten residues and at least three basic residues (Arg or Lys) in the five positions after the spacer region [39,47]. The rat LOX has a sequence, RRR-DSATAPRADGAAAQPR, in its N-terminal propeptide region, which is similar to the nuclear localization signal identified in the N-myc protein [14,46] and might thus explain the nuclear location of the LOX polypeptide. The results of the present study do not exclude the possibility that the non-recombinant LOXL3 polypeptide may be found in the nuclei of cells of some other type, and thus further studies are needed to either exclude or identify a nuclear location.

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