A novel human cDNA with a predicted protein homologous to the carboxyl end of lysyl oxidase, an extracellular enzyme involved in the maturation of collagen and elastin, has been isolated. The homology to lysyl oxidase begins exactly at the position of the exon 1/exon 2 boundary in the mouse gene (Contente, S., Csizsar, K., Kenyon, K., and Friedman, R. M. (1993) Genomics 16, 395–400). This lysyl oxidase-like gene, which appears to be no larger than 22.5 kilobases, codes for a single polyadenylated RNA species of 2.48 kilobases and has been mapped to chromosome 15q24-q25.

Lysyl oxidase (EC 1.4.3.13) is an extracellular, copper-dependent enzyme that catalyzes the oxidative deamination of lysine residues in collagen and elastin to peptidyl α-aminoacidic-β-semialdehyde. A cDNA for mouse lysyl oxidase was first isolated as an unidentified gene (rrg) that appeared to function as a phenotypic suppressor of the ras oncogene (1). Its sequence was found to match that of rat lysyl oxidase cDNA sequence (2), and levels of lysyl oxidase activity were found to correlate with rrg expression (3). In the process of obtaining a human lysyl oxidase cDNA, we isolated clones that were not lysyl oxidase but which had considerable homology to the predicted protein of human lysyl oxidase (4, 5). A complete cDNA for this lysyl oxidase-like protein, which may be a member of a lysyl oxidase gene family, is described.

MATERIALS AND METHODS

Isolation of cDNA Clones—Phage lifts of a human umbilical artery cDNA library in AGT10 were hybridized at 42 °C against radiolabeled rrg-4b, a segment of mouse lysyl oxidase cDNA (6). Final cross-species wash conditions were at 42 °C in 0.1 × SSPE (20 × SSPE: 3 m sodium chloride, 0.23 m sodium phosphate, monobasic, 0.02 m disodium EDTA, pH 7.4) and 0.1% SDS. Phage lifts of a human fibroblast cDNA library in AGT11 were hybridized against radiolabeled 5'2a, the clone obtained from the human umbilical artery library. Final wash conditions were at 50 °C, 0.1 × SSPE, 0.1% SDS. Positively hybridizing phage were re-screened another two or three times until pure phage populations were obtained. cDNA inserts were subcloned into plasmid vector pSP72, pGEM11 (Promega), or pBCSK+ (Stratagene) for DNA sequencing.

Northern Blot Analyses—Total poly(A)+ RNAs were isolated from FS4 cells (6). Either 20 µg of total RNA or 1 µg of poly(A)+ RNA was electrophoresed on 1% agarose-formaldehyde gels along with a 0.24–9.5-kb RNA ladder (Life Technologies, Inc.) as size marker, transferred to nylon membranes as described (7), and hybridized with radiolabeled probe at 42 °C. Final wash conditions were at 50 °C in 0.1 × SSPE, 0.1% SDS. After autoradiography, the blots were rehybridized with radiolabeled λDNA to detect the size markers. Messages were sized based on the RNA ladder using the Betascope 603 blot analyzer (Betagen Corp., Waltham, MA).

Primer Extension—A synthetic oligonucleotide was radiolabeled using [γ-32P]ATP and polynucleotide kinase (7) and purified on an Elutip-D column (Schleicher & Schuell). 0.07–1.6 ng of radiolabeled oligonucleotide primer was annealed with 20 µg of FS4 total RNA at 68 °C for 10 min, followed by incubation at 30 °C overnight. Primer extension was performed as previously described (6).

Fluorescent in Situ Hybridization—As essentially described (9, 10), metaphase chromosomes derived from phytohemagglutinin-stimulated peripheral blood lymphocytes from clinically normal donors were hybridized with cDNA 5'2a labeled by nick translation with biotin-11-UTP (ENZO). Probe was included at 20 ng/µl in a 10-µl volume of hybridization solution containing 50% formamide and 10% dextran sulfate in 2 × SSC (20 × SSC: 3 m sodium chloride, 0.34 m sodium citrate, pH 7.0), pH 7.0, along with Cot-1 DNA (Life Technologies, Inc.) (300 ng/µl) and sheared salmon sperm DNA (500 ng/µl). The hybridization mixture was denatured at 70 °C for 7 min and subsequently reannealed at 37 °C for 10 min prior to hybridization for 16 h at 37 °C. Following hybridization, slides were washed at 40 °C in 50% formamide in 2 × SSC, then in 2 × SSC, each three times for 5 min. Slides were then blocked in 4 × SSC containing 3% bovine serum albumin, incubated at 37 °C for 30 min in 5 µg/ml fluorescein isothiocyanate-conjugated avidin DCS (Vector Laboratories) in 4 × SSC, 0.1% Tween 20, and 1% bovine serum albumin, 18435

The abbreviations used are: FS4, human foreskin fibroblast; kb, kilobase pair(s); bp, base pair(s).

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† To whom correspondence should be addressed. Tel.: 301-295-3482; Fax: 301-295-1640.

§ The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) L21186.
Lysyl Oxidase-like cDNA Maps to Chromosome 15q24-q25

RESULTS AND DISCUSSION

A human umbilical artery cDNA library screened at low stringency with the mouse lysyl oxidase cDNA 3rg-4b (6) in order to isolate a human lysyl oxidase cDNA yielded only two positively hybridizing phage, each containing an insert of 1.1 kb. One of these, 5/2a, was subcloned and sequenced. It contained a poly(A) stretch at an end and hybridized with a 3.3258 band at 136 bases from the 5’ end of E25. A less intense band at 137 and 138 bases was also obtained. Primer extension analysis was carried out using a synthetic oligonucleotide located 71-87 bases from the 5’ end of E25. The transcripts reported for human lysyl oxidase were sized at 2.0 kb (12). The 2.5-kb transcript reported by Svinarich et al. (12) was faint and was observed only when a probe from the 3’ end of lysyl oxidase was used; this transcript may be the gene target on the long arm of chromosome 15.

The lysyl oxidase-like mRNA therefore appears to be 2484 bases, with a 446-bp 5'-untranslated region, a 1725-bp coding region, and a 313-bp 3'-untranslated region. The lysyl oxidase-like cDNA maps to chromosome 15q24-q25. The lysyl oxidase-like cDNA therefore appears to be 2484 bases, with a 446-bp 5'-untranslated region, a 1725-bp coding region, and a 313-bp 3'-untranslated region.

The lysyl oxidase-like gene was localized using fluorescent in situ hybridization with cDNA 5/2a as a probe. A total of 41 metaphase cells were examined. Hybridization was noted on two to four chromatin at 15q24-q25 in 17 cells. No consistent background was observed at any other chromosomal position. These results suggest that the probe is identifying a single copy target on the long arm of chromosome 15. There were some sequence differences among the lysyl oxidase-like cDNAs isolated. The 3’ 1.1 kb of E25 was identical to the sequence of 5/2a with two exceptions: a G → A transition in a wobble position that did not change the amino acid sequence, and a C → T transition in the presumptive 3’-untranslated region. Two other clones (B3-45 and B4-1) obtained from the normal human fibroblast library were identical to 5/2a at these two positions, but differed from it and E25 at other locations. One difference was in the presumptive 3’-untranslated region, a 1725-bp coding region, and a 313-bp 3’-untranslated region.

Translation of the 2343-bp E25 cDNA gave an open reading frame beginning at an AUG that was flanked by a Kozak consensus (13), except for a T at -5 instead of a C. The predicted 207 carboxyl amino acids of E25 showed 75% similarity to the same region predicted from the human lysyl oxidase cDNA (4, 5) (Fig. 2). This region corresponds to exons 2-7 of the mouse lysyl oxidase (6). The homology begins exactly at the point that is the exon 1/exon 2 boundary in mouse lysyl oxidase. Only short regions of amino acid similarity were detected between the 5’ portion of E25 and the region of the human lysyl oxidase cDNA that corresponds to exon 1 in mouse (Fig. 2). This region of the lysyl oxidase-like protein is 14.2% proline (the whole protein is 10.8% proline); a search of protein databases using the BLAST network service at the National Center for Biotechnology Information revealed matches to proline-rich regions of a number of other proteins, including EBNA-2 nuclear protein (14), tensin (15) and mouse proline-rich protein (16). These matches were limited to short stretches rich in proline and are unlikely to indicate any functional relationship.

The E25 cDNA, when used as a probe on Southern blot of F84 genomic DNA, hybridized with four Eco RI bands of about 9.5, 7.2, 6.0 and 2.3 kb and with five Bam HI bands of about 9.4, 5.3, 3.6, 2.3 and 1.5 kb (data not shown). Thus the size of the lysyl oxidase-like gene appears to be 22.1 kb or less, assuming there are no undetected intron fragments or fragments containing very small exons that did not hybridize well.

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2 K. Kenyon, W. S. Modi, S. Contente, and R. M. Friedman, unpublished results.
The predicted lysyl oxidase-like protein contains 574 amino acids and would have a molecular mass of 63 kDa. By analogy to lysyl oxidase, which is synthesized as a 48-kDa protein and processed to a 32-kDa active form (17), this putative protein could be processed at the Arg-Arg-Arg at position 93-95 (Fig. 2) to a 53-kDa form, or at Arg-Arg at position 272-273 to a 34-kDa form. A signal sequence cleavage site which conforms to the (−3, −1) rule is predicted (18) between Gly-25 and Gln-26, strengthening the hypothesis that this is an extracellular enzyme.

The function of the lysyl oxidase-like gene is at present unknown. The two copper binding sites proposed for lysyl oxidase (2) are not conserved in the putative lysyl oxidase-like protein. However, the carboxyl end is Cys- and His-rich, and there are several short Cys- and/or His-containing sequences known to be copper, zinc, or iron binding in other proteins as well as a motif at position 481–503 that lacks only the final His residue of a proposed zinc binding domain of Xenopus laevis transcription factor IIIA (Cys-X7-Cys-X12-His-X2-3-His) (19). The location of the lysyl oxidase-like gene at 15q24-q25 is near one of the genes for fibrillin, a component of microfibrils, at 15q21.1 (20, 21). A second fibrillin gene has been mapped to chromosome 5q23–31 (22), the same region as lysyl oxidase (5q23-31) (23). Mutations in the 15q fibrillin gene are associated with Marfan syndrome, a systemic disorder of connective tissue (23, 24). The process of fibrillin assembly is not known, but disulfide-stabilized or cross-linked multimers of fibrillin are found in tissue (25), and Maslen et al. (21) have suggested that the 8-cysteine repeats in fibrillin could oxidize and mediate the cross-linking. It is tempting to speculate that the lysyl oxidase-like gene codes for an enzyme that is involved in the processing of extracellular matrix components such as fibrillin-containing microfibril or fibroenectin fibrils.

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