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SHORT COMMUNICATION

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Isolation and characterization of a human cDNA encoding a protein homologous to the 7.2-kDa protein (subunit X) of bovine ubiquinol-cytochrome C reductase

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Abstract Through large-scale sequencing of clones randomly selected from a library of human cDNAs, we have isolated a novel human gene termed *hUQCR10*. Its open reading frame encodes 63 amino acids that share 88.5% identity with the sequence of bovine ubiquinol-cytochrome C reductase 7.2-kDa protein (subunit X). A single 0.6-kb transcript was expressed in all human tissues examined, but was particularly abundant in heart and skeletal muscle, tissues that consume a large amount of oxygen. The gene product therefore may play a significant role in the cellular respiratory system. In support of this hypothesis, our immunohistochemical analysis revealed that the hUQCR10 protein is located in mitochondria. A homology search using computer programs determined the chromosomal localization of the gene at 22q12.

Key words Ubiquinol-cytochrome C reductase · Subunit X · cDNA library screening · Mitochondria · Respiratory chain

Introduction

The respiratory chain contains three large enzyme complexes. One of these, ubiquinol-cytochrome C reductase (otherwise known as complex III of the respiratory chain, or cytochrome bc1 complex) is an oligomeric electron-transfer entity that is present in the inner mitochondrial membrane of eukaryotes and in the plasma membrane of bacteria (Schagger et al. 1986; Xia et al. 1997; Iwata et al. 1998). This complex accepts electrons from ubiquinone and passes them to cytochrome C. In bovine heart mitochondria, the ubiquinol-cytochrome C reductase complex consists of 11 different polypeptide subunits. One of these, the 7.2-kDa protein (subunit X) consists of single-transmembrane helices with their NH2-termini on the matrix side (Schagger et al. 1983). Subunit X maintains contact with cytochrome c1 and the iron-sulfur protein (ISP, subunit V), and may also play an essential role in the proper assembly of the bc1 complex.

Here we report the isolation, tissue expression, and subcellular localization of a novel human cDNA which encodes 63 amino acids that share 88.5% sequence identity with subunit X of the bovine bc1 complex. Immunocytochemical analysis demonstrated its localization in human mitochondria.

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Materials and methods

Cell lines

The monkey kidney-cell line COS-7 was purchased from the American Type Culture Collection (ATCC, Rockville, MD, USA). The cells were cultured in DMEM (GIBCO-BRL Grand Island, NY, USA), supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic/antimycotic solution (Sigma Chemical St. Louis, MO, USA). The cells were incubated at 37°C in an atmosphere containing 5% CO_2 at 90% relative humidity.

Isolation and DNA sequencing of a cDNA clone

We have been determining the nucleotide sequences of cDNA clones randomly selected from a human cDNA library derived from the HT-29 colon-cancer cell line. By comparing the 5′ partial DNA sequences of these cDNA clones with known DNA sequences in the database, we

identified a clone, clone 177, that encoded a protein highly homologous to the bovine ubiquinol-cytochrome C reductase 7.2-kDa protein (UQCR10), the tenth subunit of the cytochrome bc1 complex. Nucleotide sequences were determined with a Perkin-Elmer (Norwalk, CT, USA) automated DNA sequencer according to the manufacturer's instructions.

Northern-blot analysis

Human multiple-tissue blots (Clontech, Palo Alto, CA, USA) were hybridized with the reverse transcriptase (RT)-polymerase chain reaction (PCR) product of clone 177, and labeled with the Megaprime labeling system (Amersham Pharmacia Biotech, Uppsala, Sweden), as a probe. Prehybridization, hybridization, and washing were performed according to the supplier's recommendations. The blots were autoradiographed with intensifying screens at −70°C for 24 h.

Construction of expression vectors of Hemagglutinin (HA)-tagged hUQCR10 and transfection

Full-length hUQCR10 cDNA was cloned into a mammalian expression vector, pcDNA3.1 (Invitrogen, Carlsbad, CA, USA), along with an HA-epitope tag (YPYDVPDYA) (Wilson et al. 1984). Cultured COS-7 cells were plated in 6-cm culture dishes (2×10^5 cells per dish) 24 h before transfection. Expression vectors (4 μg per 6-cm culture dish) were transfected, using FuGENE6 according to the manufacturer's instructions (Boehringer Mannheim, Tutzing, Germany). The cells were harvested 16–24 h after transfection and re-plated on multi-well chamber slides.

Immunocytochemical analysis

Transiently transfected COS-7 cells re-plated on chamber slides were fixed with phosphate-buffered saline (PBS) containing 4% paraformaldehyde, then rendered permeable with PBS containing 0.1% Triton X-100 for 3 min at 4°C. After being washed with PBS, the cells were incubated at room temperature for 30 min in 2% bovine serum albumin, and subsequently incubated with anti-epitope (HA) rat monoclonal antibodies (Boehringer Mannheim) at 37°C for 1 h. Incubation with fluorescein isothiocyanate (FITC)-conjugated secondary antibodies (Jackson Immuno Research, West Grove, PA, USA) at 37°C for 40 min was followed by three washes with PBS. For counterstaining, anti-mitochondria antibody (Calbiochem, La Jolla, CA, USA) and rhodamine-conjugated secondary antibodies (Leinco Tech, St. Louis, MO, USA) were used in the same procedure. Nuclei were stained with 4′,6-diamidine-2′-phenylindole dihydrochloride (DAPI; Boehringer Mannheim) and observed under a fluorescence microscope (Nikon Eclipse E800; Nikon, Tokyo, Japan).

Results

Isolation and sequencing of cDNA, chromosomal localization, and genomic structure

The nucleotide and deduced amino acid sequences of the novel human gene, termed *hUQCR10*, are shown in Fig. 1. The cDNA sequence consists of 446 nucleotides with an open reading frame of 189 nucleotides encoding a 63-amino-acid peptide of approximately 7.2 kDa (DDBJ/EMBL/GenBank accession no. AB028598). An in-frame termination codon (TAG) is located nine nucleotides upstream of the first methionine (ATG), and the polyadenylation signal, AATAAA, begins 21 bases upstream of the polyadenylation site.

A homology search, using the FASTA and BLASTN programs, revealed that the nucleotide sequences of this cDNA were identical to parts of the genomic DNA sequence present in a BAC clone, bk256d12 (AC005529), that had been assigned to chromosome 22q12. A comparison of cDNA and genomic DNA sequences defined the genomic structure, which appears to span a genomic region of about

Fig. 1. Nucleotide sequence of the *hUQCR10* gene (*top lines*) and deduced amino acid sequence (*bottom lines*). The termination codon (TAG) is indicated by an *asterisk* and a polyadenylation signal (aataaa) is *underlined*

Table 1. Exon-intron boundary sequence of the *hUQCR10* gene

Exon no.	Exon length (bp)	cDNA position	Splice acceptor	Splice donor	Intron no.	Intron length (bp)
1	180	1–180		TCAACGAGGGG gtgtgagggc	1	2128
2	266	181–446	taaaatgcag AAGCTGTGGA			

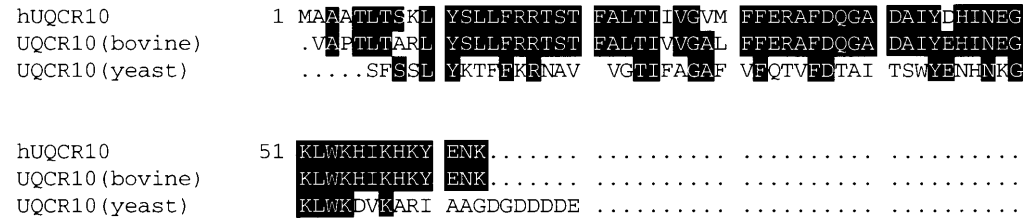

Fig. 2. Alignment of the predicted amino acid sequences of *hUQCR10* (open reading frame [ORF] of clone 177) with the amino acid sequences of *UQCR10* (bovine), and *UQCR10* (yeast) (SwissProt accession numbers P00130 and P22289, respectively). *Shading* indicates conserved residues

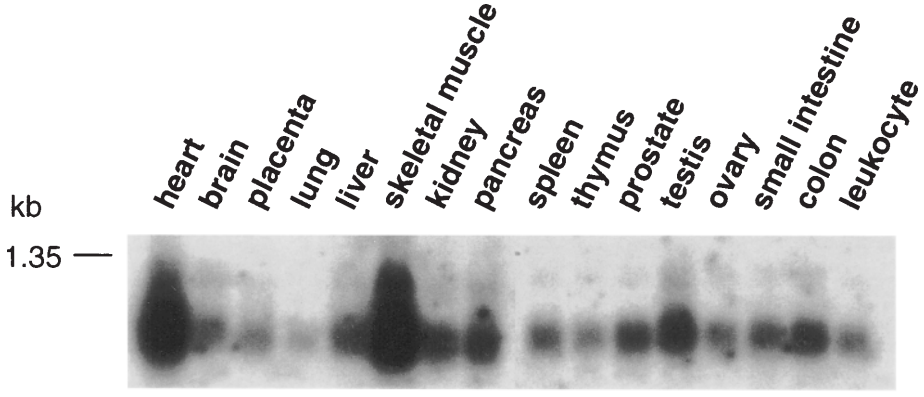

Fig. 3. Northern-blot analysis of *hUQCR10* in various human tissues. A molecular-size marker is indicated at the *left*

2.5 kb and consists of two exons. The exon-intron boundaries are consistent with the GT/AG rule (Table 1). A FASTA search for homologies between the predicted amino acid sequence and archived proteins revealed 88.5% identity with bovine ubiquinol-cytochrome C reductase 7.2-kDa protein, subunit X of the cytochrome bc1 complex (UQCR10; Fig. 2). Northern-blot analysis of *hUQCR10*, using the RT-PCR product of clone 177 as a probe, detected a single transcript of about 0.6 kb in all human tissues examined, but expression was significantly more abundant in heart and skeletal muscle, tissues that consume oxygen at a high level (Fig. 3).

Localization of hUQCR10 in mammalian cells

To determine the subcellular localization of hUQCR10 protein, COS-7 cells transiently transfected with plasmids designed to express HA-tagged hUQCR10 were cultured on slide chambers and stained by immunocytochemistry with anti-HA antibody. This experiment revealed that hUQCR10 proteins were located in the cytoplasm, in a granular pattern (Fig. 4A), suggesting localization in mitochondria. We then counterstained cells with a monoclonal antibody to human mitochondria (Fig. 4B) and confirmed that hUQCR10 protein co-localized with mitochondrial protein (Fig. 4C).

Discussion

We have described here the isolation and characterization of a novel human gene, *hUQCR10*, which is homologous to bovine ubiquinol cytochrome C reductase 7.2-kDa protein (subunit X). This enzymatic complex in bovine heart mitochondria consists of 11 different polypeptide subunits; subunit X is considered to play a role in the proper assembly of the cytochrome bc1 complex. In the human, ten subunits of this complex had already been identified and their primary structures determined (Islam et al. 1994, 1997; Duncan et al. 1993, 1994a, 1994b; Anderson et al. 1981; Suzuki et al. 1988; Hosokawa et al. 1990; Ohta et al. 1987). The human counterpart of bovine subunit X was the only component remaining to be identified.

Northern-blot analysis showed abundant expression of *hUQCR10* in heart and skeletal muscle. Since ubiquinol-cytochrome C reductase is involved in the respiratory chain,

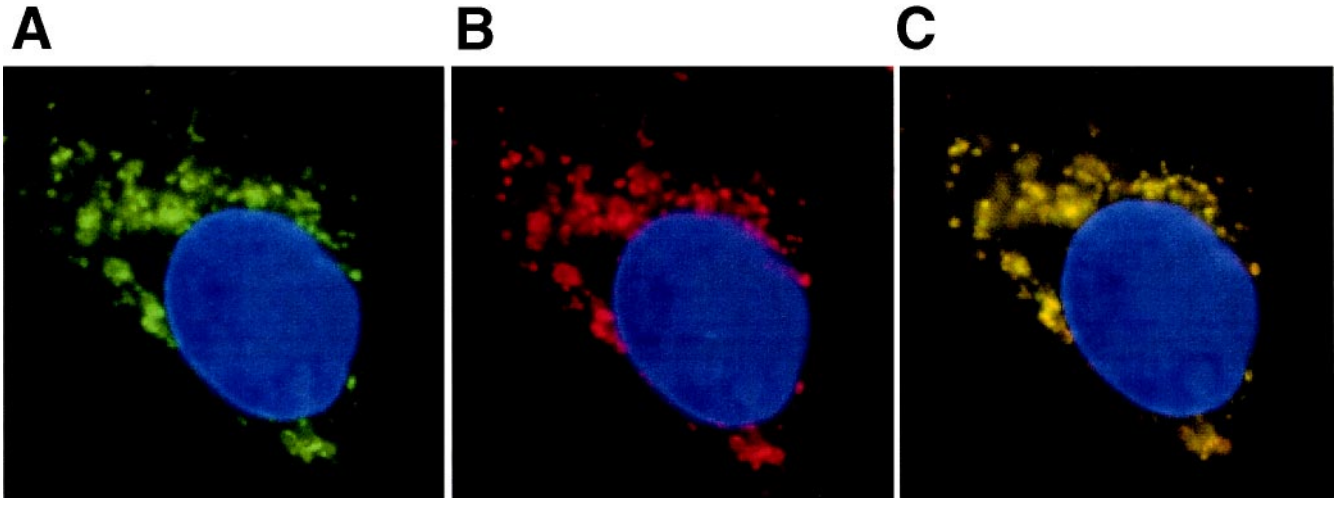

Fig. 4A–C. Subcellular localization of hUQCR10 protein. **A** A COS-7 cell transfected with HA-tagged hUQCR10 expression vector shows strong signals in the cytoplasmic region, in a granular pattern. **B** Anti-mitochondria counter-stain defines the mitochondria in the cell; the signal overlaps with that of HA-tagged hUQCR10 protein. **C** Superimposition of the two labeling methods

this observation was consistent with the high consumption of ATPs for the contraction and relaxation of muscles in those organs. The subcellular localization of *hUQCR10* in mitochondria, which we detected by immunocytochemical analysis, strongly supported our conclusion that the product of the gene reported here is a component of ubiquinol cytochrome C reductase.

Mitochondria play crucial roles in the regulation of apoptosis (Green and Reed 1998), and cytochrome C is a key molecule in this process. As the *hUQCR10* gene appears to be involved in the regulation of the redox status of cytochrome C (Kluck et al. 1997), further investigations into its function should bring new insights not only into the functions of the mitochondrial respiratory chain but also into the mechanism of apoptosis.

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