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Characterization of human CRB gene product by the use of bioinformatic tools

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Abstract

Carbonyl reductase is a monomeric, cytosolic enzyme that catalyzes the two-electron reduction of a wide range carbonyc compounds. We intend to make a silico analysis of CRB gene in different vertebrate species. The homology is analysed with NCBI BLASTp, a multiple alignment is carried out by Clustal Omega and phylogenetic tree is constructed by Mega 6. CRB protein is highly conserved in the considered species. No transmembrane regions or signal peptides were detected. Subcellular localization analysis revealed that human CRB1 was a cytoplasmatic protein (62.5%). Results showed an entire open reading frame of 887 bp encoding 295 aminoacids. This gene is expressed in different tissues, but is highly expressed in small intestine, liver and colon

Keywords: carbonyl reductase, expression, bioinformatic, in silicoclonning

Introduction

Human Carbonyl reductase gene (CRB1) is located on chromosome 21 (21q22.13) and consist on 3 exons. It encodes a monomericcytosolicenzyme carbonyl reductase, that belongs to the short-chain dehydrogenases/reductases (SDR) family, and function as NADPH-dependent oxidoreductasesof a great variety of carbonyc compounds. (http://www.ncbi.nlm.nih.gov). The enzyme is widely distributed in human tissues and also occurs in many other species. It is displayed great variability in CBR1 expression in human liver [4] and heart [5] tissues. CBR1 also plays an important role in the metabolism of the anticancer anthracyclines. Taket al.[9]have shown that CBR1 is a good molecular target for the development of anticancer drugs for human hepatocellular carcinoma (HCC) patients. CBRs might be involved in a variety of cellular and molecular reactions associated with drug metabolism, detoxication, drug resistance, mutagenesis, and carcinogenesis.

Nowdays the data on GenBank are quite abundant. Therefore, this data can be used to compare biomolecules anddraw the relationship between different species. The aim of this study is in silico analysis of CRB gene in different species and phylogenetic relationship among vertebrates, by the use of bioinformatic tools.

Materials and methods

Homology search

BLASTp software [1, 2] at NCBI (http://www.ncbi.nlm.nih.gov) was used to search homologues protein sequence to human CRB1, applying human CRB amino acid sequence as a query against the SwissProt protein databases. CRB sequences of human and other species were downloaded and then aligned using ClustalW software [6, 11] at the EBI site (http://www.ebi.ac.uk).

Primary analysis of the protein is carried out using ScanSitepI/Mw. SignalPwas used for detection of possible signal peptide, while for the detection of transmembrane region was used TMPRED program (http://www.ch.embnet.org/software/TMPRED_form.html). Subcellular localization of human CRB1 protein was indicated by PSORT.

Evolutionary Analysis

Neighbor-joining (NJ) phylogenetic trees were constructed with Jones-Taylor- Thomton (JTT) distances, using MEGA6 molecular evolutionary genetics analysis software [10]. In order to assess the reliability of the tree, 500 bootstrap replicates were applied.

Spatio temporal expression.

The expression profiles of human CRB gene in multiple tissues was determined by BioGPS software [13].

Results and discussion

Homology Search

BLASTp analysis revealed that CRB is conserved in different species. Tab 1 shows that human CRB protein is very close to *Pan troglodytes* (99%), and *Maccaca mulatta*(96%). The lowest homology displayed *Danio rerio* (67%). The length of CRB cDNA ranged from 997 bp (*Ratus norvegicus*) to 3831bp (*Danio rerio*) and the length of CRB protein sequences ranged from 276 aa (*Danio rerio*) to 289 aa (*Sus scrofa*).

Table 1BLASTp results from different vertebrate species

Species	**Protein accession number**	**pI**	**cDNA length**	**Number of aminoacids**	**% of identity with human**	**Chromosome position**
Homo sapiens (Human)	NP_001748	8.55	1321 bp	277	100	21q22.13
Pan troglodytes (Chimpanzee)	XP_531449	8.55	1382 bp	277	99	21
Macacamulatta (Rhesus macaque)	EHH16984.1.	8.55		277	96	
Canis lupus familiaris (dog)	XP_852675	7.65	1189 bp	277	89	31
Bostaurus (cattle)	NP_001029685	8.50	1034 bp	277	89	1
Musmusculus (house mouse)	NP_031646	8.53	1081 bp	277	88	16 C4
Rattusnorvegicus (Rat)	NP_062043	8.21	997 bp	277	86	11q11
Susscrofa (Pig)	NP_999238	7.58	1230 bp	289	84	?
Oryctolaguscuniculus (Rabbit)	NP_001076218	6.72	1280 bp	277	84	?
Daniorerio (zebrafish)	NP_919387	7.57	3831 bp	276	67	1

Protein sequence analysis

Multiple alignment results (figure 1) shows that CRB protein is conserved in the investigated species. CRB protein in *Sus scrofa* was longer than in other species, which have the same length of 276-277 aminoacids. The pI value of the protein in the investigated organisms ranged from 6.72 to 8.55. No signal peptide was found in all organisms. No transmembrane domain was found in human CRB1 protein. Analysis of cDNA sequence by ORF finder at NCBI (http://www.ncbi.nlm.nih.gov/gorf/gorf.html) revealed an entire open reading frame of 887 bp encoding a protein of 295 aminoacids.

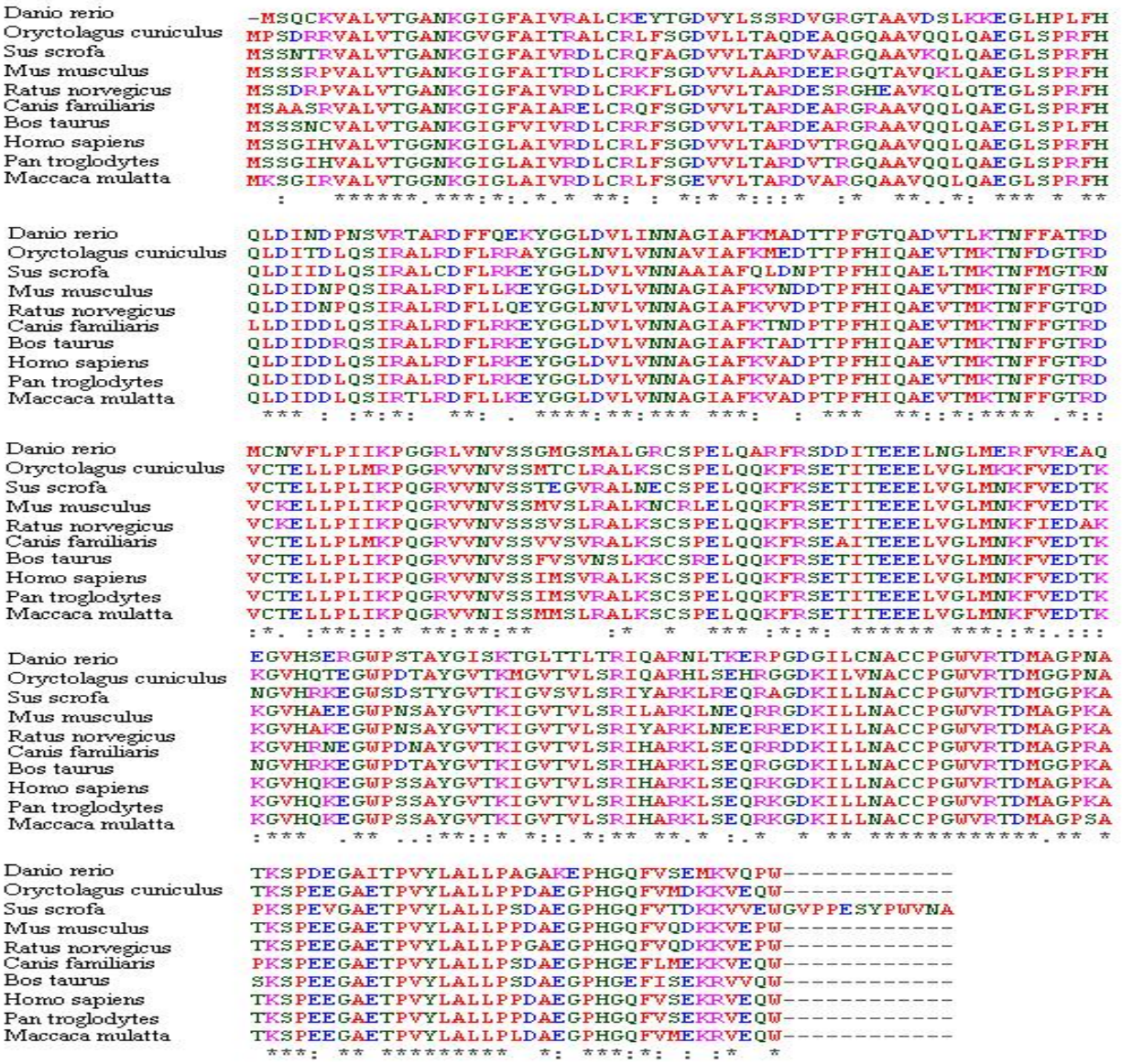

Figure 1 Multiple alignment of vertebrate CRB protein

The cellular prediction indicated that the human CRB1 protein was a most probable cytoplasmaticproteim (65.2%), having a low probability to locate in nucleus (13.0%) and mitochondria (21.7%).

Phylogenetic analysis

Phylogenetic tree was constructed with MEGA6. As shown in Figure 2, CRB protein from *Homo sapiens*, chimpanzee (*Pan troglodytes*) and then monkey (*Maccaca mulatta*) have the highest similiarity. Also rat (*Rattus norvegicus*) and mouse (*Mus musculus*) CRB proteins, are closely related while zebrafish (*Danio rerio*) shows the lowest similiarity.

Expression patern

BioGPS software was used to determinethe expression profiles of human CBR1 in multiple tissues. The results show that human CBR1 is expressed in different tissues, but displays ahigh expression level in small intestine, liver and colon (Figure 3).

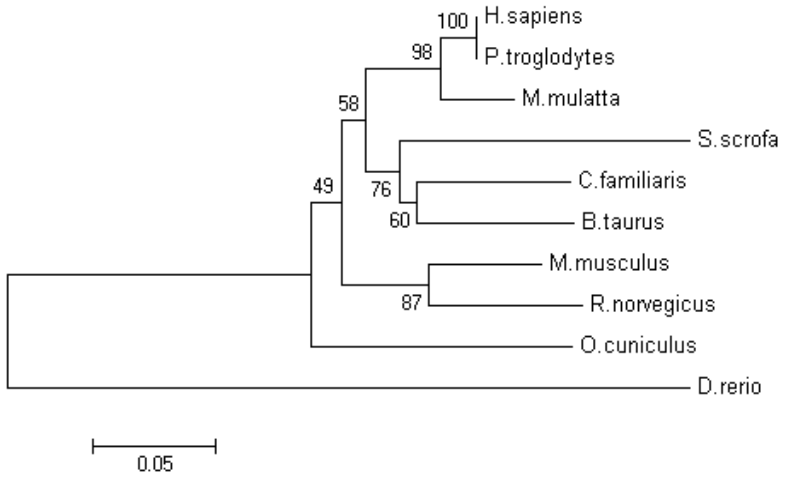

Figure 2 Phylogenetic tree of CBR protein.

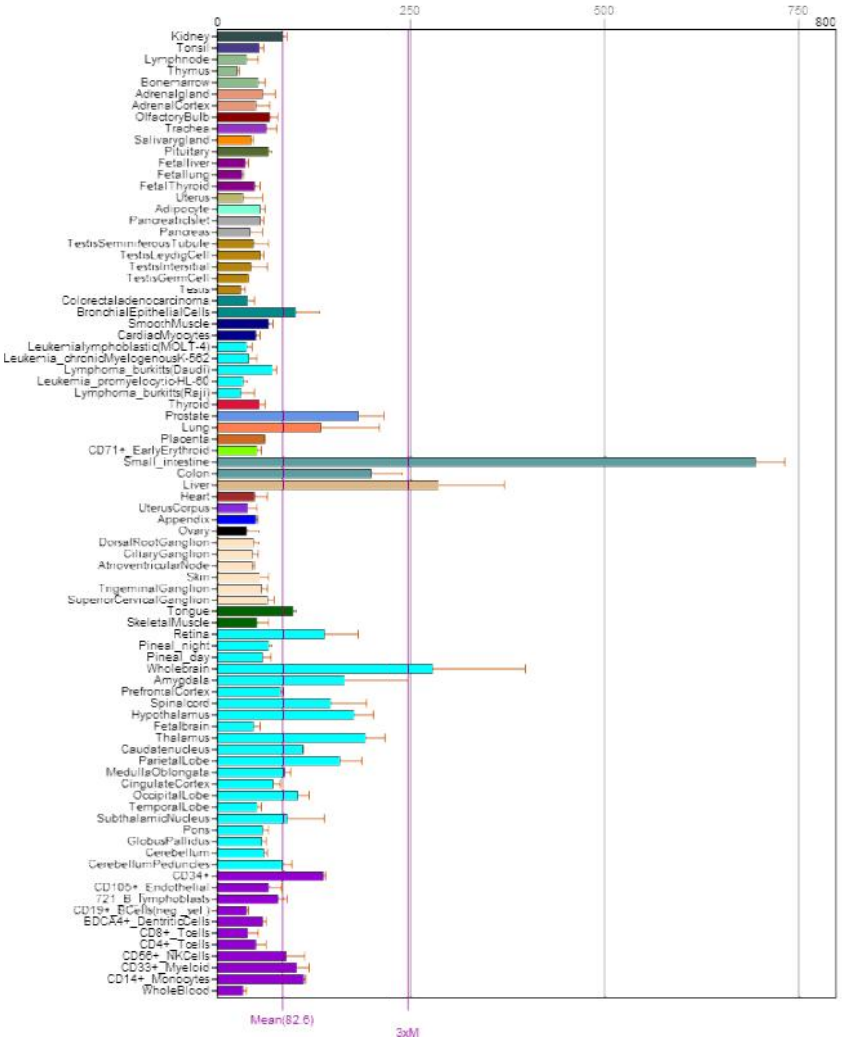

Figure 3 Expression profiles of human CRB transcripts

In silico cloning is a recent method having a lot of advantages like low cost, high efficacy, easy operation [3, 14]. It is a convenient technique forcloning novel gene [6, 7].

The BLASTp results provided here, indicate that CRB protein occurred in different vertebrate species showing a high level of conservation ranging from 67 to 99% (Tab 1). The results indicate that CRB

gene has been well conserved in different species. Phylogenetic tree shows that human CRB protein displayed the highest level of homology to *Pan troglotydes* and *Maccaca mulatta*, but the lowest level to *Danio rerio, Oryctolagus cuniculus*

Wirth et al. [12] report the immunohistochemical localization of the enzyme in normal human tissues and high concentrations were found in many organs. Nishimuta et al. [8] have concluded that CBRs might have higher metabolic activities in human intestine than in human liver. Our analysis carried out by BioGPS software, reveal that CRB gene is expressed in different tissues, showing the highest level of expression in small intestine.

To our knowledge, it was the first time of human CRB protein characterization with in silico cloning and the analysis of relationship between different vertebrate species.

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