PHYLOGENETIC ANALYSIS OF THE p53 AND p63/p73 GENE FAMILIES

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Key words: p53, p63/p73, phylogenetic analysis, multiple alignment, positive selection, purifying selection

SUMMARY

Motivation: Proteins of the relative families p53 and p63/p73 are transcriptional factors that are involved in the signaling pathway in cells. The wide spectrum of their functions includes cell cycle arrest and apoptosis in response to DNA damage. The p53 protein also participates in development of particular tissues during embryogenesis. Thus, it is of high importance to establish the relation between structure, function and evolution of these proteins.

Results: In the current computational study, the evolutionary mode of the p63/p73 protein family was investigated. The results obtained were compared with our previous of the phylogenetic analysis of the p53 protein. Evidence was obtained, indicating that the evolutionary history of the p63/p73 proteins has been under positive selection. An attempt was made to associate the current evidence with the previous for positive selection in the p53 family.

INTRODUCTION

The p53 protein is an important tumor suppressor because of its ability to produce apoptosis in the tumor cells. This protein can also arrest the cell cycle and allows cell repair before the start of replication. The p53 protein also plays an important role in the embryonic development of vertebrates by regulating the proliferation and apoptosis of the cells (Levine *et al*., 2004). The p53 protein is a relative of the p63/p73 protein family, which is somewhat structurally similar to p53, but differs from it by the presence of the C-terminal domain. The proteins of this family can induce cell cycle arrest and apoptosis, but they are of greater importance in embryogenesis than p53. Establishment of the relation between structure, function and evolution of these two families is of importance because it would provide a better understanding of the mechanism of their antitumor activity and involvement in embryogenesis (Saccone *et al*., 2002).

The aim of this study was to define the relation between the functional significance of particular amino acid residues of the p63/p73 protein family, their position in the protein structure, and the evolutionary mode of the codons, that correspond to the residues. Another aim was to compare the current results with those previously obtained for the p53 family (Benson *et al*., 2006).

METHODS AND ALGORITHMS

Amino acids and the nucleotide sequences for the p63/p73 proteins of 9 vertebrate species were taken from the GenBank database (Benson *et al*., 2006) like in our previous study for the p53 protein (Pintus *et al*., 2006). The species and accession numbers of the corresponding database entries are listed in Table 1. We obtained a multiple alignment of the amino acid sequences using the ClustalW program, version 1.7 (Li *et al*., 2003) applied to build the phylogenetic tree using the PHYML (Guindon *et al*., 2003) package, version 2.4.4.

Also, a multiple nucleotide alignment was obtained on the basis of the residue alignment using the *ad hoc* Perl program. With this program, instead of amino acid residues, their corresponding codons from the nucleotide sequences of the corresponding genes were inserted in the alignment text. Accordingly, each gap of the alignment was replaced by 3 gaps of the nucleotide alignment. Search for the adaptive branches of the phylogenetic tree and the adaptive codons in the nucleotide sequences was performed using the codeml program from the PAML package, version 3.14 (Yang *et al*., 2000).

Table 1. Accession numbers of the GenBank entries for p63 and p73 proteins in different species

p63		p73	
NM_011641	*Mus musculus*	Y19234	*Mus musculus*
NM_019221	*Rattus norvegicus*	XM_342992	*Rattus norvegicus*
NM_204351	*Gallus gallus*	XM_417545	*Gallus gallus*
NM_003722	*Homo sapiens*	NM_005427	*Homo sapiens*
XM_845322	*Canis familiaris*	XM_546740	*Canis familiaris*
BC076530	*Danio rerio*	NM_183340	*Danio rerio*
AF314148	*Xenopus laevis*	AF043641	*Barbus barbus*
XM_867115	*Bos Taurus*	XM_593064	*Bos taurus*
XM_516946	*Pan troglodytes*	Y11419	*Cercopithecus aethiops*

IMPLEMENTATION AND RESULTS

It was found that p73 evolution was associated with positive selection at the time when the Carnivora and Artiodactyla ancestors diverged from each other. The codon that predominantly accumulated nonsynonymous substitutions was detected in the coding sequence of the p53 gene. In the human p53 protein this codon occupies position 556T.

Evidence was obtained indicating that during the evolutionary history of the p63 protein positive selection acted at the time of divergence between Synapsida and Reptilia (dN/dS = 1.9352). The dendrograms for both p73 and p63 proteins are depicted in Fig. 1 and 2, respectively.

DISCUSSION

Our previous study demonstrated that positive selection during the divergence of Synapsida an Amniota, in general, acted also throughout the evolutionary history of the p53 protein, a relative of the p63/p73 protein family. It is known that homoiothermy and viviparity first appeared among synapsids. These evolutionary acquirements have made necessary changes in the genetic regulation of ontogeny, and this, in turn, might have caused adaptive changes in the p53 and p63/p73 families. However, the appearance of the terrestrial animals and the emergence of homoiothermy demanded a higher metabolic rate that most likely acted as a carcinogen.

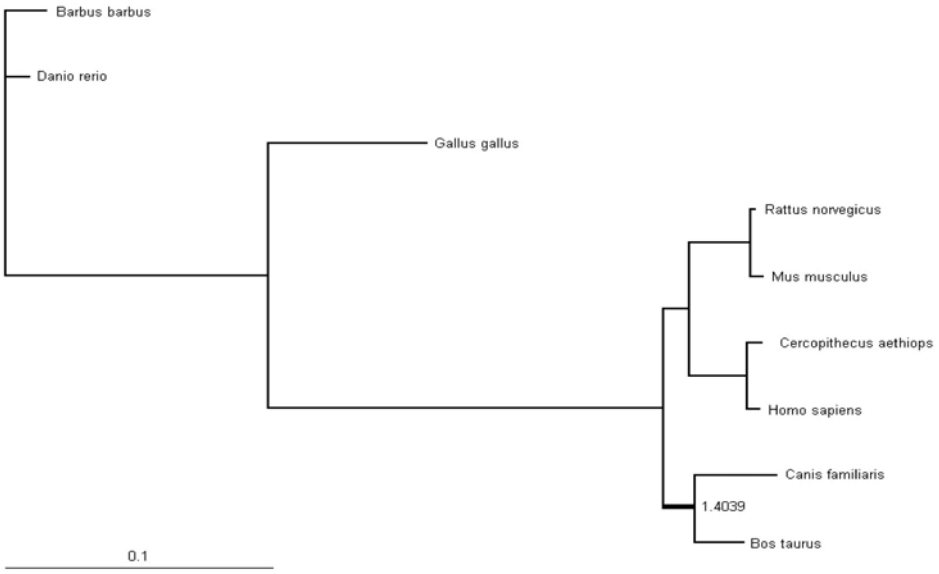

Figure 1. Dendrogram of p73 protein. The branch that corresponds to positive selection is shown in bold and the dN/dS ratio is indicated.

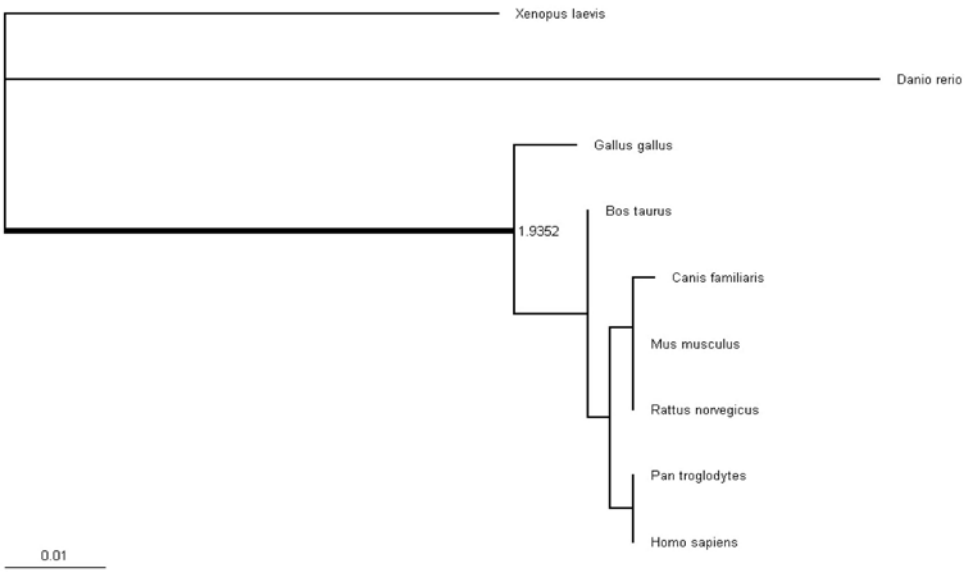

Figure 2. Dendrogram of p63 protein. The branch that corresponds to positive selection is shown in bold and the dN/dS ratio is indicated.

These conclusions are consistent with the idea that the p53 protein has descended from an ancestor common to p63/p73, and it has lost during its evolution the C-terminal SAM domain at about the time of the divergence of teleosts and amphibia (Saccone *et al*., 2002). In the meantime, p53 function underwent profound specialization possibly because of the increase in the rate of tissue regeneration in the advanced vertebrates leading to higher risk of tumor formation (Yang *et al*., 2002).

ACKNOWLEDGEMENTS

The authors are grateful to I.V. Lokhova for bibliographical support and A.N. Faddeva for translation of the abstract into English. The work was supported in part by Russian Foundation for Basic Research (Nos 04-01-00458, 05-04-49283, 06-04-49556), the State contract No. 02.434.11.3004 of 01.04.2005 and No. 02.467.11.1005 of 30.09.2005 with the Federal Agency for science and innovation "Identification of promising targets for the action of new drugs on the basis of gene network reconstruction" federal goal-oriented technical program "Study and development of priority directions in science and technique, 2002–2006, Interdisciplinary integrative project for basic research of the SB RAS No. 115 "Development of intellectual and informational technologes for the generation and analysis of knowledge to support basic research in the area of natural science", CRDF Rup2-2629-NO-04.

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