Brief Report

# Phylogenetic analysis of otospiralin protein

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## Abstract

**Background:** Fibrocyte-specific protein, otospiralin, is a small protein, widely expressed in the central nervous system as neuronal cell bodies and glia. The increased expression of otospiralin in reactive astrocytes implicates its role in signaling pathways and reparative mechanisms subsequent to injury. Indeed, otospiralin is considered to be essential for the survival of fibrocytes of the mesenchymal nonsensory regions of the cochlea. It seems that other functions of this protein are not yet completely understood.

**Materials and Methods:** Amino acid sequences of otospiralin from 12 vertebrates were derived from National Center for Biotechnology Information database. Phylogenetic analysis and phylogeny estimation were performed using MEGA 5.0.5 program, and neighbor-joining tree was constructed by this software.

**Results:** In this computational study, the phylogenetic tree of otospiralin has been investigated. Therefore, dendrograms of otospiralin were depicted. Alignment performed in MUSCLE method by UPGMB algorithm. Also, entropy plot determined for a better illustration of amino acid variations in this protein.

**Conclusion:** In the present study, we used otospiralin sequence of 12 different species and by constructing phylogenetic tree, we suggested out group for some related species.

**Key Words:** Fibrocyte-specific protein, otospiralin, phylogenetic tree

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## INTRODUCTION

Fibrocytes are mesenchymal cells derived from monocyte precursors, which co-express markers of hematopoietic cell antigens and monocytic lineage as well as fibroblast products.[1,2] These cells accelerate tissue repair through stimulation of cell proliferation, migration, re-epithelialization and angiogenesis.[2,3] These cells exert potent antigen-presenting properties during the inflammatory phase of wound healing.[2] These cells are proposed as potent candidate cells in the treatment of chronic nonhealing wounds.[3] Indeed, accumulated recruitment of fibrocytes has been demonstrated in various disease states such as atherosclerosis,[1] circulating fibrocytes differentiate into mature tissue resident fibrocytes.[4] The secreted protein by tissue resident fibrocytes, known as Fibrocyte-derived protein or otospiralin, is a recently discovered protein, which its clear function remained elusive.[5] This protein is a small protein, widely expressed in the central nervous system as neuronal cell bodies and glia.[5] The increased expression of otospiralin in reactive astrocytes implicates its role in signaling pathways and reparative mechanisms subsequent to injury.[5] Indeed, otospiralin is producing by fibrocytes of the mesenchymal nonsensory regions of the cochlea and is essential for their survival.[6-9] Otospiralin, a 6.4 KDa protein, structurally has been predicted to

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compose a single N-terminal trans-membrane helix domain and a C-terminus cytosolic tail.[5,7] *OTOSP*, the human gene encoding otospiralin, spans 1630 nucleotides and contains four exons. It encodes a 567-nucleotide cDNA and is located on chromosome two at position q37.3.[5] Twelve residues of this protein are homologs to a motif within the N-terminal end of the p30 core shell nucleocaspid protein of type C retroviruses (gag p30).[10,11] These highly conserved residues in mammals and fishes are part of the 16 amino acid stretch suggesting the principal functional importance of this region.[10] Establishing a relation between structure, function and evolution of this protein is of paramount importance because it would provide better understanding of further possible mechanisms that the protein involves in. By now, there is no information about the ancestral origin of otospiralin and its evolutionary events. General information about this protein is really limited. Thus, the aim of this study is to construct the phylogenetic tree of otospiralin and approaching to a theoretical out-group for this protein for shedding some light on its evolution.

## MATERIALS AND METHODS

### Sequences, alignment, and construction of phylogenetic tree

Amino acids and the nucleotide sequences for the otospiralin protein of 12 vertebrate species were taken from National Center for Biotechnology Information database (http://www.ncbi.nlm.nih.gov). The accession numbers of the corresponding database entries and species names are listed in Table 1. Alignments were applied in order to build a phylogenetic tree using the Mega 5 program, version 5.05.[11] For this purpose MUSCLE algorithm was used.[12] To reach a more rational phylogenic tree, we omitted partial and repetitive sequences of the same species.

## RESULTS

### Phylogeny estimation

Negative score for the opening gap was – 2.9 and gap extension score was 0. Hydrophobicity multiplier was 1.2 and clustering method one and two was UPGMB [Figure 1a and b]. Neighbor-joining tree constructed using Mega 5. Boot strap method used for test of phylogeny and MP search method was close-neighbor-interchange on random trees. The multiple sequence alignment of the otospiralin

**Table 1: Accession numbers of NCBI entries for Otospiralin in different species**

| Accession number | Name of species |
|---|---|
| EAW71179.1, AAI05086.1, AAL47489.1, NP683764.1,AAY14739.1 | *Homo sapiens* |
| XP 001088580.1 | *Macaca mulatta* |
| XP 516192.2 | *Pan troglodytes* |
| NP 001107997.1, DAA30857.1 | *Bos taurus* |
| XP 534625.2 | *Canis familiaris* |
| NP 631927.1, AAL47487.1 | *Rattus norvegicus* |
| NP 00116646.1, AAL47488.1, | *Cavia porcellus* |
| NP 694754, AAL47490.1, AAI32499.1 AAI32495.1, AAI00324.1, EDL39996.1 | *Mus musculus* |
| XP 001365725.1 | *Monodelphis domestica* |
| XP 001506615.1 | *Ornithorhynchus anatinus* |
| NP 001087267.1, NP 001165110.1 | *Xenopus laevis* |
| NP 001165109.1 | *Xenopus (Silurana) tropicalis* |

NCBI: National Center for Biotechnology Information

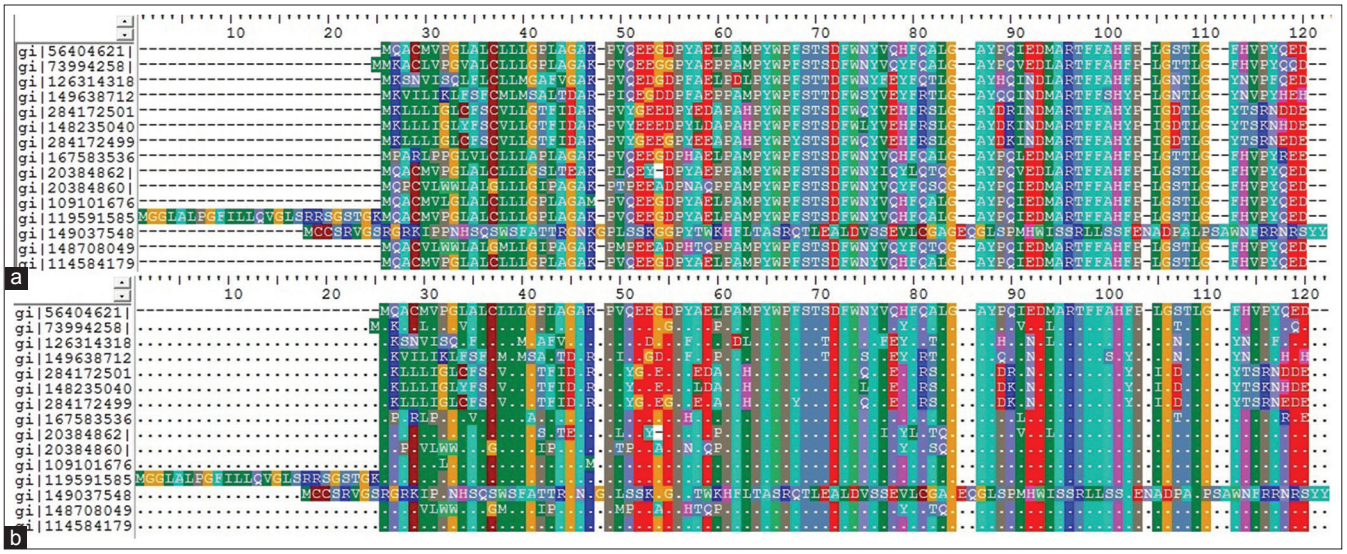


**Figure 1:** (a) Multiple sequence alignment of otospiralin protein. Alignment shows that there are more amino acids in human otospiralin. (b) Conserved and nonconserved regions in otospiralin protein. Conserved amino acids are masked

proteins are depicted in Figure 1a and b, respectively. Constructed phylogenetic tree is depicted in Figure 2.

### Determination of entropy

Entropy plot indicates variation in different positions of multiple sequence alignment. Moreover, entropy increases by increasing variation. BioEdit 7.0.8 (Ibis Biosciences) was used for determining entropy plot of otospiralin protein [Figure 3].

### Entropy plot

Entropy plot touches scale of 1 several times. The most variable part is from amino acid position 27–36. Also in other positions 42–45, 50, 53–54, 59, 93–94, 99, 117–118, 122–123 and 126–127 scale 1 is touched with entropy plot. This indicates that otospiralin protein has several variable parts. Based on the entropy plot we can say that this protein has a high rate of variance in special amino acid positions. It seems that point mutations, which found in different sequences are related to the function of otospiralin in different species. Using point mutation, each organism modified the sequence of this protein for better function. Knowing conserved amino acids helps to find key role of them in protein function.

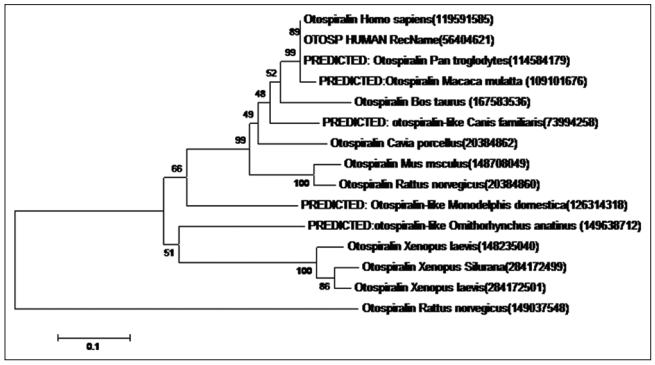


**Figure 2:** Neighbor joining tree of otospiralin. Tree shows that there is a high similarity between human and *Macaca mulatta* otospiralin

## DISCUSSION

Based on accession numbers provided in Table 1, there are repetitive and partial sequences available for some species. Alignment results show that there are four conserved amino acids (P, P, W, and F) in all of otospiralin sequences. Meanwhile, two other Glu is conserved in all sequences except *Rattus norvegicus* (149037548). Furthermore, human otospiralin (119591585) and *R. norvegicus* (149037548) have more amino acids. It shows that these sequences have additional exon on messenger RNA (mRNA) sequence of this gene, or maybe mRNA splicing of these sequences performs in different manners. The most similar sequence to human otospiralin is *Macaca mulatta* (109101676). Pairwise alignment of these two sequences shows that there are additional 21 amino acids in the beginning of human sequence and two different amino acids in the rest of the sequence. So, this study suggests that because of the high similarity between human and *M. mulatta* otospiralin, *M. mulatta* can be used as a proper animal model for some kind of human diseases, which are related to otosperalin. Also, it is probable that the evolutional changes of otospiralin are caused by point mutations and same exons expressed in this protein sequence because length of the three integrated sequences are similar and just one amino acid deletion exist in the sequence of *Cavia porcellus*. Finally, following the changes, which are caused by point mutations, *Xenopus* genus otospiralin was appeared.

## CONCLUSION

P, P, W and F are three conserved and probably key amino acids in otospiralin sequences and except one sequence (149037548), two other G are conserved. It is probable that the position of these amino acids in

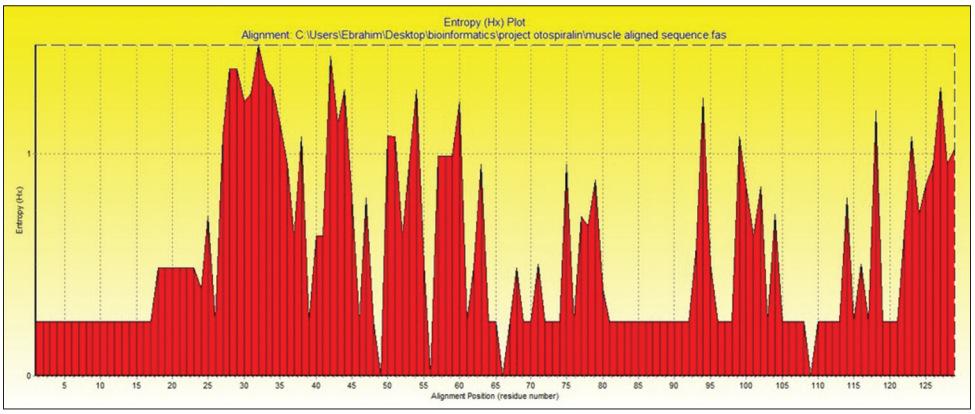


**Figure 3:** Entropy plot of otospiralin. Plot indicates that there are consensus and nonconsensus regions in this protein

three-dimension structure of otospirallin is highly conserved and have important role in its function.

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