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Abstract: We identified and characterized the rat DVL2 gene using bioinformatics. In addition to the structure and chromosomal localization of the rat DVL2 gene, the transcribed and translated protein product of the gene was analyzed in silico. Results showed that the rat DVL2 gene consists of 15 exons and is located on the rat genomic contig WGA1854.3 on chromosome 10. Database searches using the rat DVL2 amino acid sequence as a query showed a number of homologous protein sequences in different species, including *M. musculus, P. troglodytes, C. familiaris, H. sapiens, B. taurus, D. rerio, X. laevis,* and *T. nigroviridis*. DAX, PDZ signaling, and DEP-conserved domain structures were identified within the rat DVL2 protein.

Key Words: Bioinformatics, DVL2, rat genome, Wnt signaling

Biyoinformatik Metodlar Kullanarak Sıçan DVL2 Geninin Tanımlanması ve Karakterizasyonu

Özet: Bu çalışmada biyoinformatik yaklaşımlar kullanarak sıçan DVL2 genini tanımladık ve karakterize ettik. Sıçan DVL2 geninin yapı ve kromozomal lokalizasyonuna ek olarak genin kodladığı protein de in silico olarak analiz edildi. Sonuçlarımıza göre sıçan DVL2 geni 15 eksondan oluşmuş ve 10. kromozomdaki genomik kontig WGA1854.3 üzerinde bulunmaktadır. Sıçan DVL2 proteininin *M. musculus, P. troglodytes, C. familiaris, H. sapiens, B. taurus, D. rerio, X. laevis* ve *T. nigroviridis* türlerindeki homologları ve bunlar arasındaki homoloji oranları amino asit duzeyinde belirlendi. Rat DVL2 proteininde DAX, PDZ-Sinyalizasyon ve DEP korunmuş domeyn yapıları olduğu belirlendi.

Anahtar Sözcükler: Biyoinformatik, DVL2, rat genomu, Wnt sinyalizasyonu

Introduction

Wnt signaling is conserved in various species and plays important roles in development and differentiation (1-5); however, abnormal Wnt signaling may cause abnormalities, such as cancer (6). Wnt signals are transduced through frizzled receptors. They transduce signals to the intracellular space by activating disheveled (DVL) proteins (7). The DVL family proteins are multi-domain, intracellular transducers with important signaling functions (8). All DVL protein family members contain 3 highly conserved domains (9): N terminal DAX (also called DIX) (10), central PDZ (11), and C terminal DEP (12) domains. There are 3 DVL genes (DVL1, DVL2, and DVL3) in the human (13) and mouse genomes (14-16), and 2 DVL genes (DVL1 and DVL3) in the rat genome. Recently, the rat DVL2 gene has been grossly predicted in silico and added to the NCBI Genebank database (GeneID: 303251); however, in this annotation, no information about the gene, such as structure, sequence length, the predicted protein sequence, or functional domains, is present. Therefore, we aimed to identify and further characterize it.

Herein, we analyzed the rat DVL2 gene, its structure, the chromosomal localization, predicted peptide sequence, and domain structures. We also analyzed its homologues in other species and their evolutionary relationship.

Materials and Methods

Identification DVL2 gene in rat genome

New gene fragments were identified using in silico tools and database searches, as previously described (17-20). To identify the related genomic clones, a TBLASTN search through the NCBI server (http://www.ncbi.nlm.nih.gov/blast) against non-redundant databases was

performed using the amino acid sequences of human DVL2 as the query sequence. Within the determined genomic region, putative CDS was determined by using the Wise2 program (http://www.ebi.ac.uk/wise2). To identify the complete mRNA, BLASTn searches against ESTdb were performed using the nucleotide sequence of putative CDS of rat DVL2 as a query sequence. This process was repeated several times for the precise determination of the mRNA sequence.

In order to establish the genomic organization of the DVL2 gene, the mRNA sequence was BLASTed against the genome of *R. norvegicus*. Using Genomatix software (http://www.genomatix.de) and inspecting the gt-ag rule of intronic sequence, usage of the exons was defined.

Deducing and analyzing the amino-acid sequence of DVL2 gene

Prediction of the coding region was performed using the ORF Finder program (http://www.ncbi.nlm.nih.gov/projects/gorf/). The BLASTP program (http://www.ncbi.nlm.nih.gov/blast/) at NCBI was used to identify the homologous proteins in other species.

Amino-acid sequence alignments were performed using the ClustalW program (http://www.ebi.ac.uk/clustalw). RPS-BLAST (http://www.ncbi.nlm.nih.gov/blast) was used for protein domain application analysis.

Phylogenetic analysis

The phylogenetic trees were constructed using the neighbor-joining method (NJ) with Jones-Taylor-Thornton (JTT) distances. NJ searches were conducted using the MEGA3 program (21). The reliability of internal branches was assessed using 500 bootstrap replicates.

Expression Analysis

The ESTs corresponding to the rat DVL2 gene were searched for with the BLASTn program (http//www.ncbi.nlm.nih.gov/blast). Then, the tissue sources of ESTs corresponding to the new gene were listed.

Results

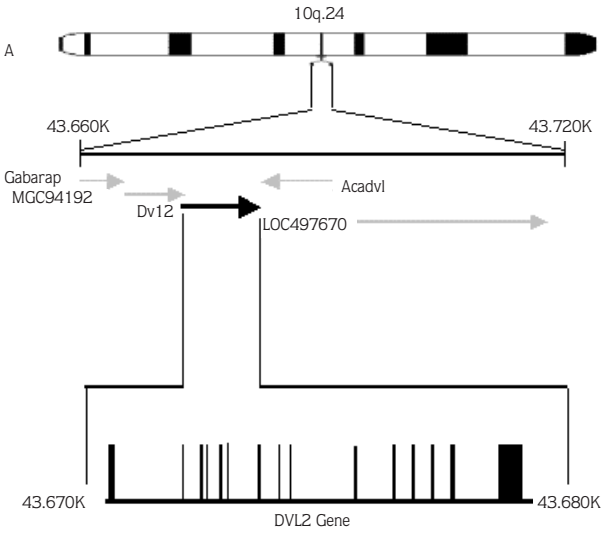
Identification of rat DVL2 gene

TBLASTN searches using human DVL2 mRNA revealed that the rat DVL2 gene fragments were located within the rat genome contig WGA1854.3 on chromosome 10 at the nucleotide position 43.670 kb-43.680 kb, and that they were about 10 kb in size (Figure 1a). The precise exon-intron boundaries were determined by examining the consensus sequence of exon-intron boundaries and the codon usage within the coding region (Figure 1b).

Analyses of deduced amino-acid sequence

Rat DVL2 mRNA was found to consist of at least 34 bp of 5' UTR, 2208 bp of coding region, and at least 575 bp of a 3' UTR region (Figure 2). The rat DVL2 mRNA was predicted to encode a peptide of 736 amino acids.

The BLASTP program revealed that rat DVL2 has 98%, 96%, 96%, 92%, 94%, 78%, 77%, and 74% total amino acid identity with the corresponding proteins of *M. musculus* (CAI35165), *H. sapiens* (AAH14844), *C.*



B

Exon No	Nucleotide sequence around exon-intron boundaries			
1	**5'-UTR**	GGGCGG...	TTTTGG	**GT**AAGC...
2	... CTGC**AG**	GGTGGT...	TCCTGG	**GT**AAGG...
3	... CCCT**AG**	CTGGTG...	CTTCCA	**GT**GAGT...
4	... GTGT**AG**	CCCTAA...	ATGGCG	**GT**GAGG...
5	... ACAC**AG**	CTGGTG...	GAGTAG	**GT**ATGG...
6	... TATC**AG**	GTTCAG...	GAGAGG	**GT**GAGG...
7	... CTGC**AG**	ACCTCA...	ACATGG	**GT**ATGG...
8	... CCAC**AG**	AGAAAT...	TTGCAG	**GT**GTGT...
9	... TTGC**AG**	GTAAAC...	GCCAGG	**GT**AAGT...
10	... CCAC**AG**	CCCCAT...	CCCGAA	**GT**GAGT...
11	... TACC**AG**	ATGAGC...	CTGATG	**GT**GAGC...
12	... TGTC**AG**	GCTGTG...	TTCTAG	**GT**ATTC...
13	... TCCC**AG**	GCTCGG...	AGAGTT	**GT**AAGT...
14	... TTCC**AG**	ACCTAG...	GTGAGG	**GT**AAGT...
15	... CAAC**AG**	GGAGCC...	TTATAA	**3'-UTR**

Figure 1. (A) Structure and chromosomal localization of the rat DVL2 gene. (B) Exon–intron boundaries of the rat DVL2 gene.

familiaris (XP_546582), *P. troglodytes* (XP_511978), *B. taurus* (XP_870854), *X. laevis* (AAH90218, XDSH), *D. rerio* (NP_997813), and *T. nigroviridis* (CAG00725), respectively. ClustalW alignment of these proteins displayed very high similarity in the N terminal half and more diversity at the C terminus (Figure 3).

RPS-BLAST results revealed that rat DVL2 proteins include DAX, PDZ signaling, and DEP-conserved domains spanning from amino acid 13 to 93, 265 to 352, and 433 to 505, respectively (Figure 2).

Phylogenetics analysis

We constructed the phylogenetic tree of the DVL2 proteins from the species given above with MEGA3 (Figure 4). The results indicated that DVL2 is conserved among all organisms investigated, with very high conservation among the mammals.

Expression of rat DVL2

We investigated differential tissue and organ expression of rat DVL2 by analyzing the sources of rat DVL2 ESTs, as defined by the BLASTn program (Table).

Discussion

The DVL genes of vertebrates are homologous with the Drosophila DSH gene and they are highly conserved throughout evolution. The products of DVL genes are required for the transduction of Wnt signals from the cell surface to the cytoplasm. Although the mechanism of Wnt signaling from frizzled receptors to cytoplasm is not understood very clearly, DVL proteins are considered the intersection of Wnt signaling (24). Therefore, identification of Wnt-signaling components in experimental animals, such as the rat, is very important for understanding of the

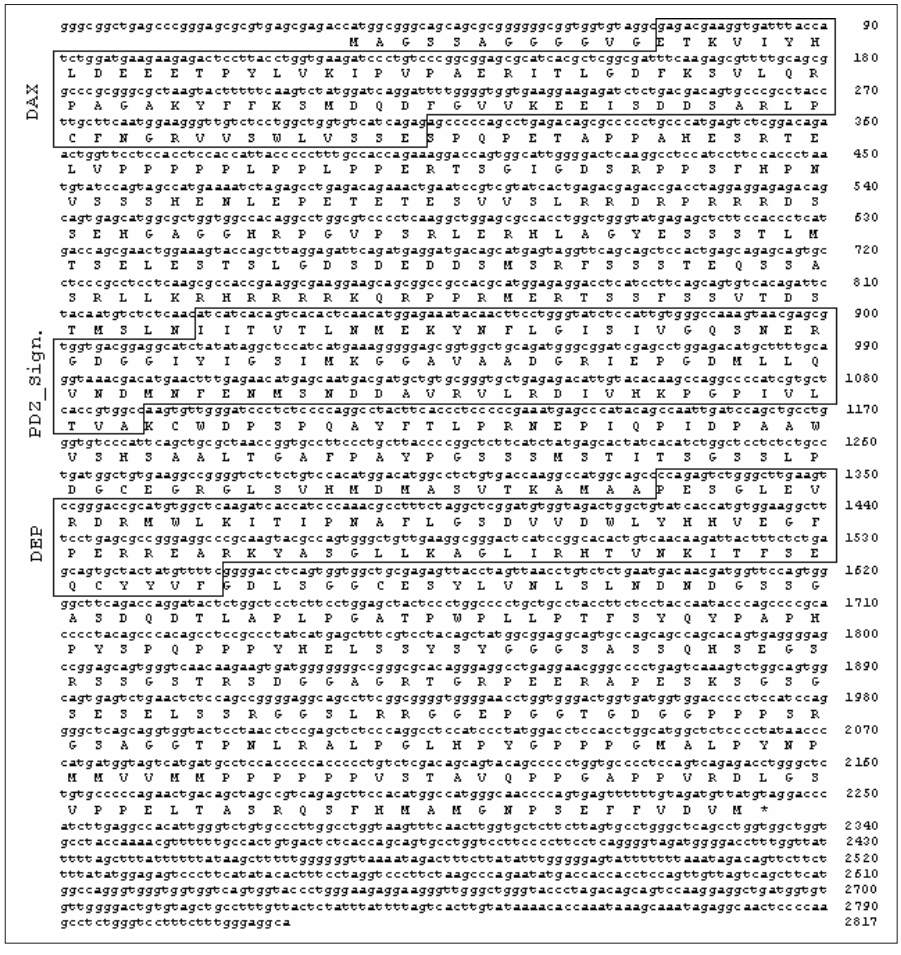


Figure 2. Rat DVL2 mRNA and proteins. Rat DVL2 proteins include DAX, PDZ signaling, and DEP-conserved domains. These domains are boxed.

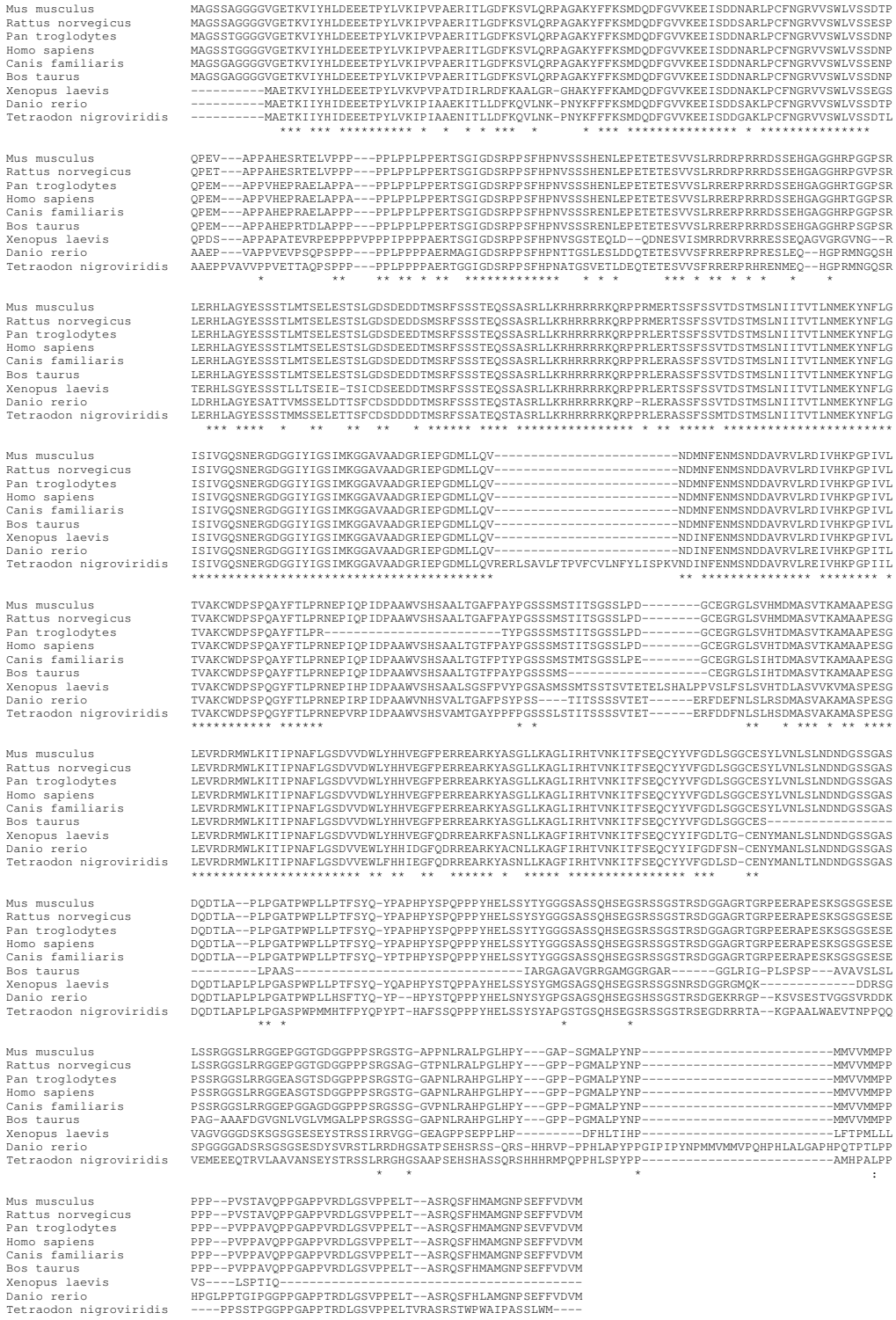


Figure 3. Alignment of homologous proteins to rat DVL2 proteins. Amino acid residues conserved among these species are shown by an asterisk below the alignment.

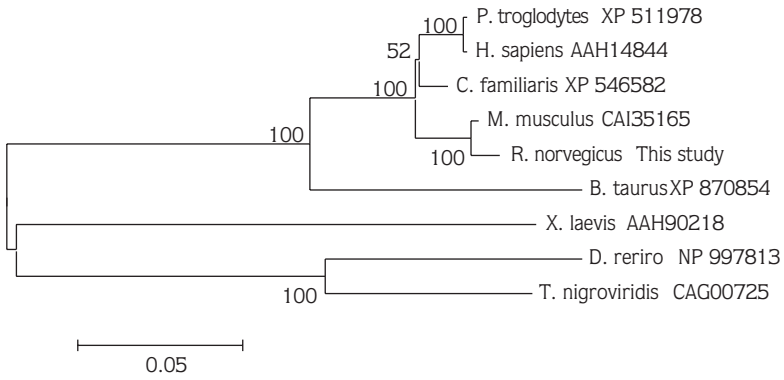


Figure 4. Phylogenetic tree reconstructed by the MEGA3 program. The abbreviation of species names and the corresponding GeneBank accession numbers of sequences are indicated. Branch lengths indicate evolutionary relationship.

Table. Rat DVL2 ESTs and expressed organs.

	Accession no.	Source of EST		Accession no.	Source of EST
1	CO557967	Testis	10	BF543944	Heart
2	CO557148		11	AI712638	
3	BP473037	Pancreatic islet	12	BE100058	
4	BP472111		13	CK844954	
5	BP480453		14	CB544369	Brain
6	BF556436	Whole embryo	15	CB770815	
7	CD372507		16	AA998681	Unknown
8	DN947987	Prostate	17	CR465333	
9	CB773237	Pituitary	18	CB717514	

molecular basis of the developmental process and carcinogenesis that are related to Wnt signaling.

Bioinformatics is an emerging and rapidly growing research area that has attracted great attention from medical, biological, and information scientists (22). Bioinformatic applications have an important role in the confirmation of laboratory-based work and in de novo analysis (23).

We identified and characterized the rat DVL2 gene using bioinformatics in this study. We discovered that the rat DVL2 gene contains 15 exons. The complete coding sequence of the DVL2 gene was determined by assembling 15 exons within genomic contig WGA1854.3 on rat chromosome 10 (Figure 1a). Rat DVL2 mRNA was found to consist of least 34 bp of 5' UTR, 2208 bp of coding region, and at least 575 bp of a 3' UTR region. The rat DVL2 polypeptide, 736 aa, was predicted from the nucleotide sequence of the putative rat DVL2 mRNA (Figure 2).

All DVL protein family members contain 3 highly-conserved domains. The N-terminal DIX domain contains ~ 80 amino acids and is involved in the regulation of Wnt signaling through binding to axin (10,25). The central PDZ domain contains ~90 amino acids and is involved in cell-cell interactions (11,26). The C-terminal DEP domain contains ~90 amino acids and is implicated in the planar cell polarity pathway in the fly to control the polarity of epithelial cells in the plane orthogonal to their apicobasal axis (27,28). A recent study of the DEP domain also demonstrated that this domain is involved in frizzled-7-induced translocation of DVL1 to the plasma membrane (24). In the RPS-BLAST search we found that the predicted rat DVL2 proteins contain these conserved functional domains. Their physiological role has yet to be determined in experimental settings.

By using the MEGA3 program we confirmed that

DVL2 proteins are highly conserved among all organisms investigated. This result clearly indicates that DVL2 proteins have an imperial function in cell signaling.

Data mining techniques are powerful tools for discovering and analyzing novel genes (22); however, results of bioinformatic studies need to be confirmed by laboratory experimentation (19,20).

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