GENOME-WIDE IDENTIFICATION AND PHYLOGENETIC ANALYSES OF NOTCH 1 GENE

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Abstract. The NOTCH gene encode transmembrane receptor. It play a vital role in several process stem cell maintenance and differentiation during embryonic and adult development. When ligand bind at a specific part intracellular part of NOTCH receptor is cleaved and translocate to the nucleus from where it can bind to transcription site. NOTCH activity can promotes tissue growth and cancer in some conditions but they also suppress tumors formation in others. Their gene structure show the amount of introns and exons by a dimensions structure of NOTCH gene. Various tools or database such as Mega7, Pfam and Gene structure and display server are used to analyze their phylogeny and their chromosome positions gene structure and introns and exons. Further studies are made to target the NOTCH pathway on growth and cancer suppressor.
Keywords: *oncogenes, NOTCH 1-4 receptors, heterodimeric protein, gene*

Introduction

NOTCH gene is evolutionary conserved signaling gene that is essential for embryonic development in all metazoan organisms. It is also present in human in which it encodes single pass transmembrane receptor. Many lab studies have shown that NOTCH protein mediates a wide range of biological operations. NOTCH 1 gene make a protein called NOTCH1 (Borggrefe and Oswald, 2009). Receptor protein has such site in which certain other proteins called ligands can attach. Attachment of the ligands to NOTCH1 can send signal throughout the body for normal development of many tissues before and after birth. It can turn normal cell into cancerous and also considered as oncogenes. It involves in cell proliferation, cell differentiation, maturation and apoptosis and cell growth. NOTCH family encodes single pass transmembrane proteins. In mammals four NOTCH receptors are present NOTCH1, NOTCH2, NOTCH3, and NOTCH4 (Fleming, 1998). These are heterodimeric protein consist of N terminal extracellular portion which are non-covalently bound to transmembrane domains. These extracellular domains of protein family consist of large numbers of tandemly repeated copies of an epidermal growth factor. NOTCH receptors interact with membrane bound ligands that are that are encoded by Jagged (JAG1 and JAG2) and Delta gene family. The signal induced by ligand binding is transmitted intracellularly. The receptor/ligand interaction induces two additional proteolytic cleavages that free the intracellular domain of the Notch receptor from the cell membrane. The cleaved fragment translocates to the nucleus due to the presence of nuclear localization signals. Once in

the nucleus, the Notch intracellular domain forms a complex with the RBPSUH protein, a sequence-specific DNA binding protein (also known in mammals as CSL, CBF1 and RBP-J).

It involves in various kind of diseases such as cancer, critical congenital heart disease, carcinoma, Alagille syndrome and various others. NOTCH gene has other name AOS5, TAN1, HOVD1, HN1, Neurogenic locus Notch homolog protein. NOTCH involved in controlling of various developmental processes by controlling cell divisions. Its ability to affect the cell cycle kinetics and response to apoptotic signals that notch proteins involved in the malignant transformation of some cells. In the last few years evidence has collected on notch participation in carcinogenesis and human tumors. Notch signaling is constitutively activated in several types of cancer cells and it is regarded as an ant apoptotic and pro-oncogenic signal. Notch3 overexpression is responsible for increased in vitro tumor cell growth in human lung cancer. Moreover, increased expression of Notch3 has been observed in spontaneous human pancreatic tumors. Notch signaling also play role in the induction of terminal differentiation and growth arrest. NOTCH gene involved in broad range of disease. NOTCH pathway can cause various inherited disease. Mouse models have made for each type of disease and analyze the model lead to several potential sights. Further studies in NOTCH pathway mutant mice will lead to additional way to the pathogenesis of the disease in humans.

NOTCH receptors

NOTCH1: It is a human gene encoding single transmembrane receptors. When notch 1 activate before birth it induce radial glia differentiation. Reelin and NOTCH 1 cooperate in the development of dentate gyrus.

NOTCH2: It is a protein that is encoded by NOTCH 2 gene. It is associated with Alagile syndrome and Hajdu–Cheney syndrome. It can remove PEST domain and remove nonsense mediated mRNA.

NOTCH3: It encodes the third discovered human homologue of the Drosophila melanogaster type I membrane protein notch. In Drosophila, notch interaction with its cell-bound ligands (delta, serrate) establishes an intercellular signaling pathway that plays role in neural development.

NOTCH4: It is located on 6 chromosomes. Its biological significance has not been described. Mutation in NOTCH 4 gene associated with susceptibility to schizophrenia.

Materials and Methods

For retrieval of protein and DNA NOTCH gene from all databases of the NCBI and then FASTA sequence was downloaded by running BLAST. To find similar sequence from protein database BLAST-P was run. Protein sequence was used as a query sequence. Genomic information related to gene was taken from NCBI. Targeted sequences are further used in different databases to find out conserved domains.

Phylogenetic analysis of NOTCH gene family

Multiple sequences were done by using bioinformatics tool MEGA 7 to obtain pairwise and multiple alignment of our sequence. MEGA 7 is used for manual sequence alignment and construct evolutionary tree. Maximum likelihood were used for tree construction. A separate instrument was used to measure molecular weight and

isoelectric point. Different subfamilies were named according to their corresponding NOTCH homolog by phylogenetic analysis.

Gene structure analysis and detection of conserved motifs

Gene structure and display server are used to find the composition and positions of introns and exons and conserved elements of NOTCH gene. The sequence of NOTCH protein was given as a query sequence and downloaded all target sequence in order to analyze the conserved motifs and domains of the protein sequence among are Pan Troglodytes, Macaca Mulatta, Macaca Nemestrina, as well as Pongo Abelii by using online database Pfam (*Table 1*). This process is used to remove sequence that does not have conserved domain and motif. Position of chromosome, length of protein and genomic sequence was observed by using NCBI database (EMBL Official Portal, 2021).

Table 1. *Online databased of Pfam.*

Proposed names	Gene locus	Protein accession no	RNA accession no	Exons	Chromosome no.	Orf length	Amino acid length	Start of genomic location	Conserved domains in protein sequence
Pan Troglodytes	CK820_G0012017	XP_009455997.1	XM_009457722.3	34	9	7440	2479	10872668	Smart000034 Pfam00008 Pfam00066 Pfam07684 Pfam12796 Pfam11235 Pfam08168
Pongo Abelii	CR201_G0049823	XP_024108478.1	XM_024252710.1	34	9	7671	2556	110450912	Cd0054 Pfam00066 Pfam00088 Pfam11936 Pfam12796
Macaca Mulatta	Unknown	XP_014971839.1	XM_015116353.2	34	15	7671	2556	1699397	Cd00054 Cd00204 Pfam0066 Pfam07684 Pfam12796 Pfam06816
Macaca Nemestrina	Unknown	XP_011723094.1	XM_011724792.2	34	Unknown	7671	2556	9666387	Cd00054 Cd00204 Pfam00066 Pfam06186 Pfam12796 Pfam07684 Sd00054

Results and Discussion

NOTCH family has four members NOTCH 1, 2, 3 and 4. NOTCH is involved in hair growth. NOTCH 1 to 4 shares the high degree of structure similarity. NOTCH protein extracellular domains consist of variable number of epidermal growth factor that mediates interactions with ligands. It was originally identified in human leukemogenesis (Pancewicz and Nicot, 2011). NOTCH can act as tumor suppressor or oncogenes are not clearly identified. It is also act as growth inhibitor in keratinocytes and small lung cancer. NOTCH 1 gene can be observed by different tools to check their functions their conserved regions, introns exons, composition of elements and their evolutionary

relation with their ancestors by using different tools such as MEME, MEGA 7, Gene structure and display server.

MEME

It is used to discover motifs of DNA, RNA and protein sequences. NOTCH 1 gene protein sequences are put in MEME search box it can find their motifs the width of all the protein sequence are 50 (*Figure 1*). The E-value varies of each sequence (*Figure 2*).

Figure 1. *The protein sequences.*

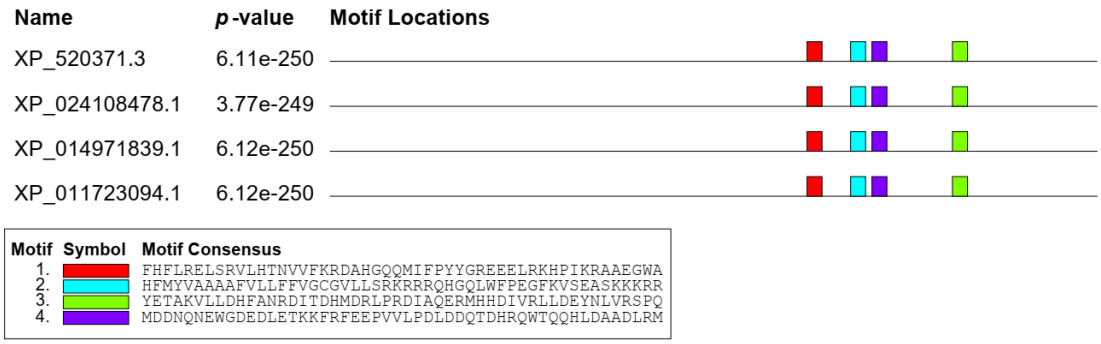

Figure 2. *The E-value sequences.*

Genome struture display server

It was performed for the visualization of NOTCH 1 gene composition, introns and exons and their conserved regions (*Figure 3*).

Figure 3. *NOTCH 1 gene composition, introns and exons.*

MEGA 7

MEGA 7 tool is used to find the evolutionary relationship between different species. *Figure 4* describes the relation of organism, species from their common ancestors. This figure depicts the molecular phylogenetic analysis by Maximum likelihood method. The analysis involved 11 amino acid sequences. All positions containing gaps and missing data were eliminated. Evolutionary analysis was conducted in MEGA 7.

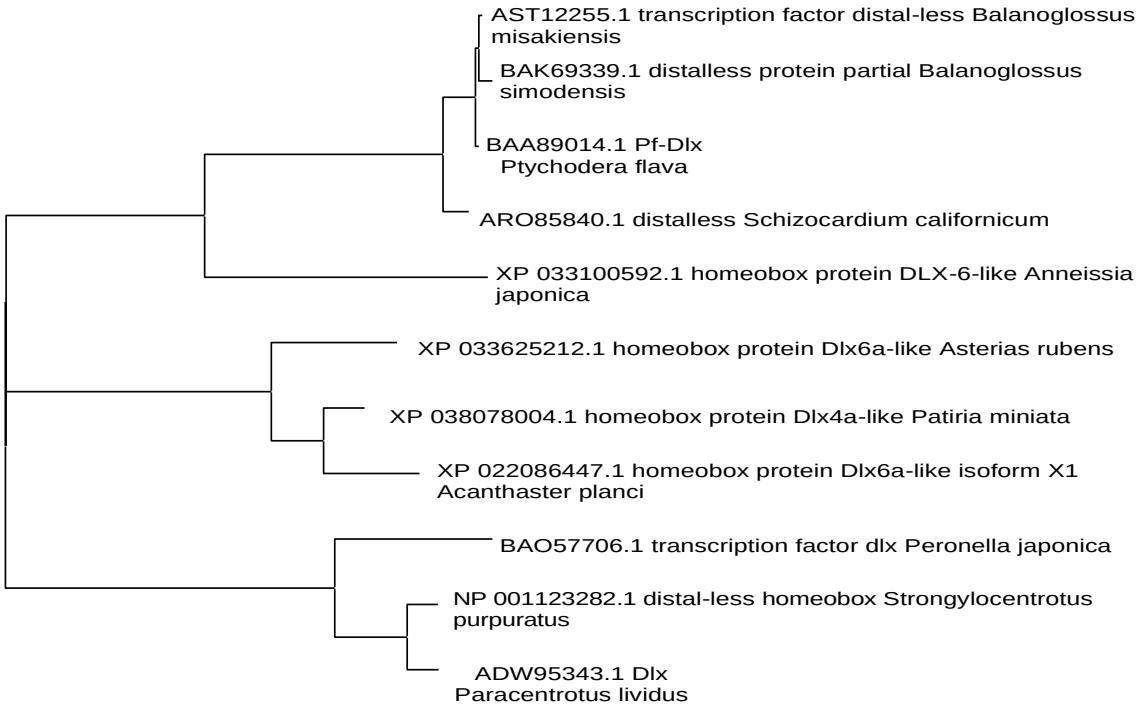

Figure 4. *Relation of organism, species from their common ancestors.*

Serial cloner

It is used for the visualization and sequence analysis. It is also used in DNA cloning. It can used genomic sequence to find out the restriction site in genes. NOTCH 1 gene mRNA and genomic sequence of different organisms are put in serial cloner to find the number of restriction sites in genes (*Figure 5*).

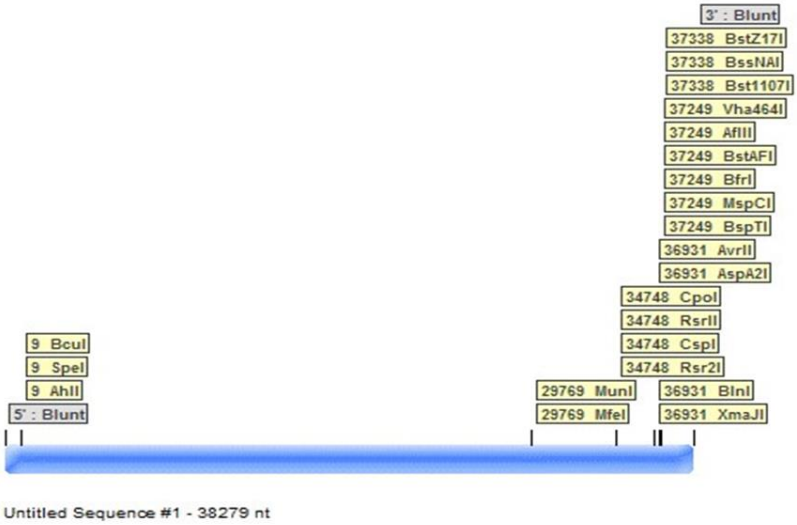

Figure 5. *NOTCH 1 gene mRNA and genomic sequences.*

Conclusion

Notch gene was first discovered in Drosophila (Egan et al., 1998). NOTCH signaling pathway is important mediator of cell fate selection which are involved in epidermal appendage formation. It is four receptors play important role in cell differentiation in early development process. NOTCH protein shares a high degree of domain topology. NOTCH signaling is mediated by intracellular domains which functions as transcription factor. Intracellular domains of NOTCH comprised different identified domains that have different functions such as regulating NOTCH activity (Gridley, 2003). All members of NOTCH are involved in cancer including breast, melanoma, and leukemia pancreatic (Demarest et al., 2008). The mutant form of NOTCH has been identified to increase NOTCH transcriptional activity. Their phylogenetic analysis depicts the relation with their ancestors.

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Conflict of interest

There are no conflict of intrest involve any parties in this research study.

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