

# Categorizing Molecular Features of NOTCH4 Involved in Breast Cancer

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## ABSTRACT

Breast cancer is one of the leading causes of cancer death in women. It is observed that hormonal, lifestyle and environmental factors that may increase the risk of breast cancer and often begins with cells in the milk-producing ducts (invasive ductal carcinoma), glandular tissue called lobules (invasive lobular carcinoma) or in other cells or tissue within the breast. The major signaling pathways involved in the breast cancer are Estrogen pathway, MAPK signaling pathway, PI3K/AKT signaling pathway, Notch signaling pathway, Wnt signaling pathway and P53 signaling pathway. Over the past decade, abnormal activation of Notch signaling in breast cancer has been stated by many different groups. In invasive breast cancer, the elevated expression of Notch signaling pathway components has been reported, including Jagged1-2, Dll1, Dll3, and Dll4, Notch receptor (Notch1 to Notch4). It is observed that increased JAG1-NOTCH4 signaling in human breast tumors is an important stimulator of cancer stem cells. The present investigation deals with the thorough understanding of molecular features of NOTCH4 protein emphasizing its key role in triggering the cancer pathway, by using different bioinformatics tools. The detailed insights into molecular features and the functional elements of NOTCH4 by analyzing its physicochemical parameters, secondary and tertiary structure prediction, domain analysis and intermolecular interactions, it can be considered as one of the potent drug target in breast cancer and can contribute to a novel alternate and promising treatment strategy for breast cancer through computer aided drug designing.

**Keywords :** Cancer, Signal pathways, NOTCH, Drug target, *Insilico*.

## I. INTRODUCTION

Breast cancer is a leading cause of cancer death in women worldwide (A. J. Redig et al., 2013). Age, reproductive factors, family history of breast disease, early menarche, late menopause, obesity in postmenopausal women, high concentration of endogenous estrogen (Emmanuel N. Kontomanolis et al., 2018), genetic pre-disposition and environmental factors have been associated with an increased risk for the development of breast cancer (Rupen Shah et al., 2014). Over the past few years, significant

advances have been made in the sighting of new drugs for treating breast cancer. Improved understanding of the biological signaling pathways that triggers the breast cancer has allowed the development of more effective and personalized approach to treatment (Christy W. S. Tong et al., 2018).

Notch signaling is an evolutionarily conserved pathway that regulates cell-fate determination during development and maintains adult tissue homeostasis. Abnormal Notch signaling is related with several

congenital developmental diseases and various types of cancer (Han et al., 2011). Over the past decade, abnormal activation of Notch signaling in breast cancer has been stated by many different groups. In mammals, there are four Notch receptors (Notch1, Notch2, Notch3, Notch4) and five ligands (Jagged1, Jagged2, Delta-like1 (Dll1), Dll3, and Dll4) (Ahmet Acar et al., 2015).

Notch4 plays a particular key role in controlling breast cancer stem cell (Ahmet Acar et al., 2015). Jagged1, expressed in cancer cells, has been shown to trigger Notch signaling in adjacent endothelial cells to stimulate angiogenesis (Ahmet Acar et al., 2015). Increased JAG1-NOTCH4 signaling in human breast tumors is a significant stimulator of cancer stem cells. In this role, Notch4 appears to be mainly important, as knockdown of Notch4 has significant effect on breast cancer stem cell numbers than Notch1 knockdown. Pharmacologic and genetic inhibition of the Notch signaling can reduce breast Cancer stem cell activity in vitro and tumor formation in vivo. Hence, it is very clear that activation of Notch plays a key role in breast cancer. Therefore it represents a very striking therapeutic target (Ahmet Acar et al., 2015). In this review the present investigation deals with detailed insights into molecular features of Notch4 that it could be one of the potent drug target in breast cancer.

## II. METHODS

### 1. Sequence retrieval:

The UniProt knowledgebase (<https://www.uniprot.org>) is the core of the consortium activities. Merging of Swiss-Prot, TrEMBL and PIR-PSD to form the UniProt knowledgebase in order to provide a fundamental database of protein sequences with annotations and functional information. Sequence of NOTCH4

protein was retrieved by UniprotKB database and stored in FASTA format, with its accession number.

### 2. Primary analysis:

ExPASy (<http://www.expasy.org>) has worldwide standing as one of the main bioinformatics resources for proteomics. The different physicochemical parameters of NOTCH4 protein were computed using ExPASy's ProtParam tool. ProtParam is a tool which computes the various physical and chemical parameters for a given protein. The computed parameters include the molecular weight, theoretical pI, amino acid composition, atomic composition, extinction coefficient, estimated half-life, instability index, aliphatic index and grand average of hydropathicity (GRAVY).

### 3. Secondary structure prediction:

The Self-Optimized Prediction method With Alignment (SOPMA) (<https://npsa-prabi.ibcp.fr>) is a tool to predict the secondary structure of a protein. Based on the query (primary sequence of a protein), SOPMA will predict its secondary structure. The secondary structure of NOTCH4 was predicted by using SOPMA.

### 4. Tertiary structure prediction:

SWISS-MODEL (<http://swissmodel.expasy.org>) is a server for automated homology modeling of three-dimensional (3D) protein structures. The 3D structure of NOTCH4 was generated by this server. Template 5uk5.1A was selected having sequence identity 58.76% to generate the 3D structure of NOTCH4.

### 5. Structure validation:

PROCHECK (<https://www.ebi.ac.uk/thornton-srv/software/PROCHECK/>) checks the stereochemical quality of a protein structure, producing a number of PostScript plots analyzing its complete and residue-by-residue geometry. It includes PROCHECK-NMR for checking the quality

of structures solved by NMR. The build 3D model quality was checked using PROCHECK. It is a way to visualize energetically allowed regions for backbone dihedral angles  $\psi$  against  $\phi$  of amino acid residues in protein structure.

**6. Structure visualization:**

RasMol is a program for molecular graphics visualisation originally developed by Roger Sayle. The predicted 3D structure of NOTCH4 was visualized by using RasMol, showing different display features and labels.

**7. Intermolecular Interaction of proteins:**

STRING (<http://string-db.org/>) is a biological database and web resource for prediction of protein-protein interactions. The STRING database contains information from numerous sources, including experimental data, computational prediction methods and public text collections. The analysis of protein protein interaction for NOTCH4 was done by using string database.

**8. Domain analysis of NOTCH4:**

Pfam (<https://pfam.xfam.org/protein/>) is a complete collection of protein domains and families, characterized as multiple sequence alignments and as profile hidden Markov models. The current release of Pfam (22.0) contains 9318 protein families. Domain analysis of NOTCH4 was done by using Pfam database.

**III. RESULTS**

**1. Sequence retrieval:**

Sequence of the NOTCH4 was retrieved by UniprotKB database and stored in FASTA format with accession number.

Accession number - Q99466.

**2. Primary analysis:**

Physicochemical parameters of NOTCH4 was analyzed by using ExPASy's ProtParam tool which are as shown in TABLE I.

TABLE I  
Physicochemical analysis of NOTCH4

Properties	Values
Number of Amino acid	2003
Molecular weight	209621.86
Theoretical pI	5.43
Total no. of negatively charged residues Asp+Glu	194
Total no. of positively charged residues Arg+Lys	131
Instability Index(II)	52.48
Aliphatic index	58.33
GRAVY	-0.293

**3. Secondary structure prediction:**

Secondary structure of NOTCH4 was predicted by using SOPMA. Secondary structural elements like alpha helix, extended strand, beta turn and random coils were enlisted in TABLE II.

TABLE II  
Secondary structure prediction of NOTCH4

Secondary structure	No. of residues	Percentage
Alpha helix (Hh)	282	14.08%
$3_{10}$ helix (Gg)	0	0.00%
Pi helix (Ii)	0	0.00%
Beta bridge (Bb)	0	0.00%
Extended	173	8.64%

strand (Ee)		
Beta turn (Tt)	119	5.94%
Random coil (Cc)	1429	71.34%

**4. Tertiary structure prediction:**

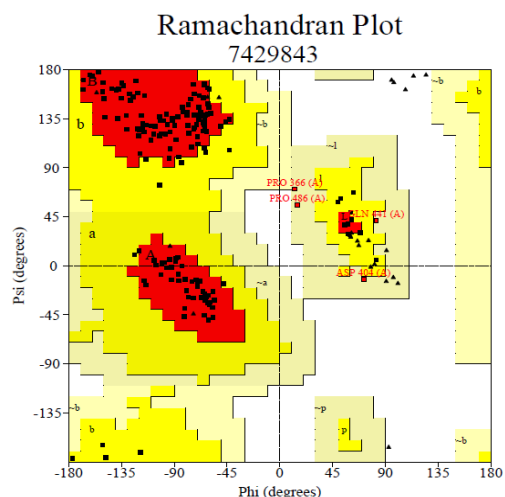
3D structure of NOTCH4 was predicted by SWISS-MODEL. 5uk5.1A template was selected which shows sequence identity 58.76% to generate 3D structure of NOTCH4. The generated structure was visualized by RasMol.

**5. Structure validation:**

Structure validation was done by using PROCHECK which checks the stereochemical quality of a protein structure, by PROCHECK statistics as shown in TABLE III. It also generates the Ramchandran plot to visualize energetically allowed regions for backbone dihedral angles  $\psi$  against  $\phi$  of amino acid residues in protein structure as shown in figure 1.

TABLE III  
PROCHECK statistics of NOTCH4

Ramchandran plot	No. of residues	percentage
Most favoured regions [A, B, L]	131	86.8%
Additionally allowed regions [a, b, l, p]	18	11.9%
Generously allowed regions [ $\sim$ a, $\sim$ b, $\sim$ l, $\sim$ P]	2	1.3%
Disallowed regions	0	0.0%



**Figure 1 :** Ramchandran plot of NOTCH4

**6. Structure visualization:**

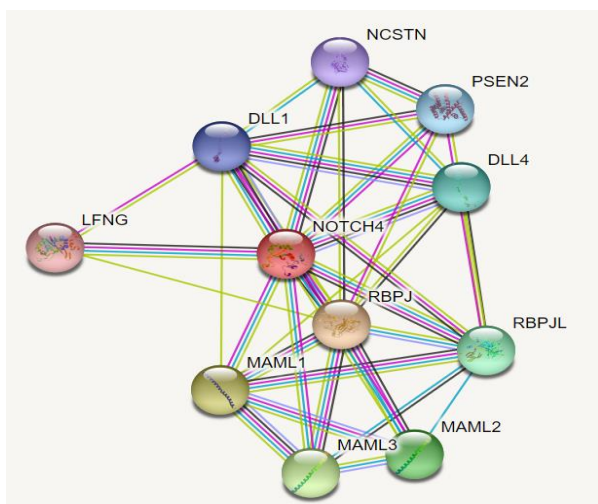
The predicted 3D structure of NOTCH4 by SWISS-MODEL was visualized by the RasMol. RasMol is a program for molecular graphics visualization which can show the structure in different styles and colours by using different commands. 3D structure of NOTCH4 was shown in figure 2.



**Figure 2.** Molecular visualization of NOTCH4 by RasMol

### 7. Intermolecular Interaction of proteins:

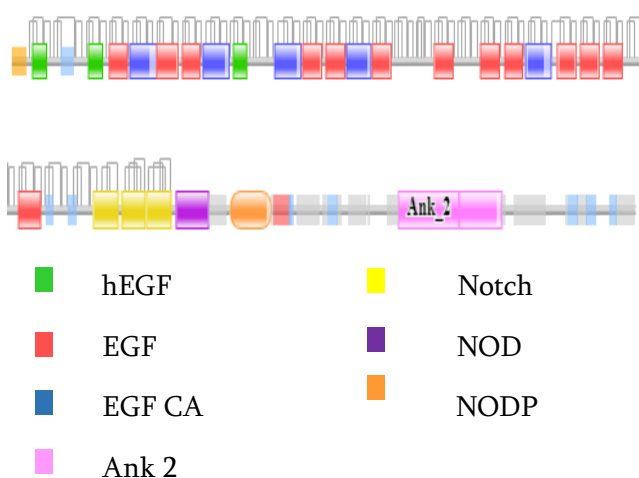
Protein protein interactions of NOTCH4 was visualized by using STRING biological database. Which shows the intermolecular interaction of NOTCH4 with other proteins as shown in figure 3.



**Figure 3.** Protein interactions of NOTCH4

### 8. Domain analysis of NOTCH4:

Domain analysis of NOTCH4 was done by using Pfam database. After analysing NOTCH4 it shows seven major domains as Human growth factor- like EGF, EGF- like domain, calcium binding EGF domain, NOTCH protein, NOD, NODP, Ankyrin repeats which are represented by different colours as shown in figure 4.



**Figure 4.** Domains of NOTCH4 shown by different colours

### IV. CONCLUSION

Breast cancer is death leading cause in women. Notch signalling pathway plays important role in proliferation and invasiveness of breast cancer. Elevated expression of JAG1-NOTCH4 is the important stimulator of breast cancer stem cells. Protein interaction network reveals that NOTCH4 is the one of the main protein that trigger the breast cancer and the present investigation states that NOTCH4 protein is unstable, non-polar and acidic. Secondary structure analysis reveals that NOTCH4 contains more random coils than the alpha helix which shows protein does not have stable confirmation and PROCHECK statistics shows that NOTCH4 contains 86.8% of most favoured regions, shows the predicted model was accurate. Understanding the importance of NOTCH4 in the Notch signalling pathway, it can be considered as one of the potent therapeutic target in breast cancer. Attempts are also underway for the identification of anticancer peptides from the scorpion venom as natural inhibitors of NOTCH4 and hence contribute for new and improving methods of breast cancer diagnosis and treatment.

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