ORIGINAL ARTICLE

AN *IN SILICO* PRIMARY AND SECONDARY STRUCTURE PREDICTION OF HUMAN INTERFERON ALPHA RECEPTOR 2 PROTEIN

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Background: Structural analysis of human interferon alpha receptor 2 (IFNAR-2) protein is important to determine its structure and function because that information is needed to understand the role and mechanism of IFNAR-2 protein in human immune system. Therefore, this study was conducted to find out composition of amino acids contributing in primary and secondary structure of IFNAR-2 protein. **Methods:** Protein sequences of human IFNAR-2 were retrieved from 'The Universal Protein Resource (UniProt)' and 'National Center for Biotechnology Information (NCBI)' databases. The Basic Local Alignment Search Tool (BLAST) was used to search for every IFNAR-2 protein sequence in NCBI database. Human IFNAR-2 protein sequences were further refined according to set criteria for experimental analysis. All retrieved IFNAR-2 protein sequences were aligned by using computational tool 'Clustal Omega'. Consensus protein sequence was obtained from aligned protein dataset. Furthermore, consensus protein sequence of IFNAR-2 was subjected for secondary structure prediction analysis. Protein topology was predicted by using Expert Protein Analysis System (ExPASy) server and Transmembrane Helices; Hidden Markov Model. **Results:** Alignment data set revealed that IFNAR-2 protein consisted of 515 amino acids long chain, having total 37 identical positions with 6.446% identity. Protein topology analysis predicted that human IFNAR-2 protein consists of verities of secondary structures such as alpha-helix, turn and beta sheets. Alpha-helixes mainly form three topological domains (i) inner (1–6 amino acids), (ii) outer (7–29 amino acids) and (iii) trans-membrane domain (30–515 amino acids). **Conclusion:** Human IFNAR-2 protein consists of 515 amino acids having hydrophobic, polar and aromatic characteristics. Alpha-helixes, turn, beta sheets and three topological domains constitute secondary structure and predicted topological domains contribute in the subcellular compartmentalization.

Keywords: Human IFNAR-2, *In silico* analysis, Protein secondary structure, Subcellular compartmentalization

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INTRODUCTION

The knowledge about primary and secondary structural characteristics of proteins is central to set the basis for therapeutic drug development in medical and pharmaceutical sciences.[1] Interferon alpha/beta receptor 2 (IFNAR-2) also known as IFNR-alpha binding protein, (a single-pass type I membrane protein) belongs to the type II cytokine receptor family. Interferon alpha/beta receptor 2 can associate with IFNAR-1 to form the type I interferon receptor. It is involved in IFN-mediated activation of STATs (Signal Transducer and Activator of Transcription proteins) signalling during immune response. Isoform 1 and isoform 2 of IFNAR-2 are directly involved in signal transduction.[2] The IFN-alpha/beta receptor-2 subunit is considered the primary binding chain of the receptor however, both the receptor subunits, IFNAR-1 and IFNAR-2, cooperate in the high-affinity binding of interferon (IFN) to the receptor complex.[3] Genetic variations in IFNAR-2 translated in protein structure variability, consequently influence the susceptibility to hepatitis virus infection.[2] Interferon-alpha/βeta-2 involves in the host's immune response and resists intracellular pathogenic viral infections through innate and adaptive immune systems.[4–6]. Role of IFNAR-2 protein in signalling cascade during immune response prompt us to investigate its structure. As we know that information about protein structure is crucial to translate respective mechanism of action (function) but no detailed study has been found about primary and secondary structural analysis of IFNAR-2. Therefore, we conducted this study with primary objective to find out the composition of amino acids (hydrophobic and polar amino acids) present in coding region of human IFNAR-2 protein. Second objective covered comparative protein sequence analysis by alignment, to determine variable and conserved regions, amino acids composition, and respective secondary structure formation such as chain, Beta sheet, α-Helix and turn.

MATERIALS AND METHODS

The study was conducted from Jan 2017 to Dec 2017 at Department of Biochemistry, HITEC-Institute of Medical Sciences Taxila Cantt, Pakistan after taking approval from Institutional Review Board of the Institution.

To analyse the protein structure of human IFNA-2 receptor, computational programmes (*In silico*) have been employed in this study. Protein sequences were retrieved from 'Universal Protein Resource' (Uniprot; http://www.uniprot.org/) and 'National Center for Biotechnology Information' (NCBI; http://www.ncbi.nlm.nih.gov/). The Basic Local Alignment Search Tool (BLAST) conducted against non-redundant (nr) database for every human IFNAR-2 protein sequence. The selection criteria were set as (i) include non-recombinants (ii) Isolation and clear literature records (iii) blast hits filtered with blast score >150 (iv) length >50 (v) relative identity >30%. The sequences which didn't fall in this criterion, were not selected for analysis.[7]

The Basic Local Alignment Search Tool provided a large number of data set. Only selected number of human IFNA-2 receptor sequences were further refined according to set criteria for experimental analysis. Protein blast was conducted by online tool 'Protein Basic Local Alignment Search Tool (Blastp; https://blast.ncbi.nlm.nih.gov/Blast.cgi)' by matrix 'blosum62'. Retrieved protein data set was renamed and aligned by Clustal Omega. During alignment sequences were manually analyzed and cropped to get best alignment block of sequences data set. Further selection based upon the set of consensus sequences was done for analysis. Alignment parameters such as uncharacterized protein sequences were removed from final data. Sequence which showed 100% identity was selected. Final selection contains 12 sequences. Alignment Program Clustal Omega was used**.** Clustal-Omega uses the HHalign algorithm and its default settings were set as its core alignment engine. Default parameters consisted of Gonnet transition matrix, gap opening penalty as 6 bits and gap extension as1 bit.[8,9]

Protein topology of IFNAR-2 was predicted by using protein data set. ExPASy server, TMHMM is a membrane protein topology prediction method based on a hidden Markov model (HMM) used to predict seven transmembrane helices in proteins[1,10] from the final dataset (https://www.expasy.org/) (http://www.cbs.dtu.dk/services/TMHMM-2.0/).

RESULTS

In this study we conducted primary and secondary structure prediction analysis of human IFNAR-2 protein by employing *In silico* approach. Results revealed that a set of total 12 protein sequences were identical in the aligned human IFNAR-2 sequences data set (Table-1 and Figure-1)

Alignment results of IFNAR-2 protein sequence showed that it's a 515 amino acids long chain with peptide signal. Peptide signal is a polypeptide, having 26 amino acids starting with methionine and involved in post translational modifications. Total 37 identical positions were found with 6.446% identity. Amino acids at position number 7–29 and 244–264 contribute in the formation of transmembrane region which contains more hydrophobic amino acids as compared to polar amino acids. It consists of two charged amino acids Arginine (R) and Lysine (K). It also contains aromatic amino acids which are Phenylalanine (F) and Tyrosine (Y). In whole stretch of 515 amino acids there are six cysteine (C) residues present at position numbers 39, 85, 93, 122, 207, 227, which participate in disulphide bond formation. Secondary sequence of protein consists of helix, turn and beta sheets. Amino acids involved in helix formation are Lysine (K), Asparagine (N), Cysteine (Position number 82, 83, 84), Leucine (L), Alanine (A), Isoleucine (I), Aspartic acid (D), (Position number 128, 129, 130, 131) Glutamic acid (E) (Position number 159, 160, 161) and Phenylalanine (F) (Position number 8, 10 and 196. Consensus set (among all aligned sequence) of similar amino acid region consists of hydrophobic and polar amino acids, found at position number 135–180. Asparagine was found in topological domain, contributing in glycosylation (Figure-1).

Topological domain is a section describing the subcellular compartment where each non-membrane region of a membrane-spanning protein is found. Protein Topology Prediction analysis of human IFNAR-2 revealed by TMHMM predicts transmembrane helices in proteins. Results showed that there are three Topological domains (Inner, Transmembrane and Outer) found in sequence annotation. First domain is from position number 27 to 243, second domain is 23 from position 265 to 302 and third domain is from position number 309 to 515 (Figure-2).

Table-1: Human IFNAR-2 Protein dataset

S.No.	Uniport ID	Name	Identity
1	P48551	INAR2_HUMAN	100%
2	P48551-2	INAR2_HUMAN - Isoform 2	100%
3	P48551-3	INAR2_HUMAN - Isoform 3	100%
4	C9JCUO	C9JCUO_HUMAN - Interferon alpha/beta receptor 2	100%
5	S6FRS2	S6FRS2_HUMAN - Interferon 2 (Alpha, beta and omega)	100%
6	C9JM33	C9JM33_HUMAN - Interferon alpha/beta receptor 2	100%
7	C9K067	C9K067_HUMAN - Interferon alpha/beta receptor 2	100%
8	HOY3Z8	HOY3Z8_HUMAN	100%
9	H7C3V1	H7C3V1_HUMAN	100%
10	Q9BUAO	Q9BUAO_HUMAN - Interferon 2	99.6%
11	F8WDE1	F8WDE1_HUMAN - Interferon alpha/beta receptor 2	98.4%
12	H7C176	H7C176_HUMAN - Interferon alpha/beta receptor 2	92.7%

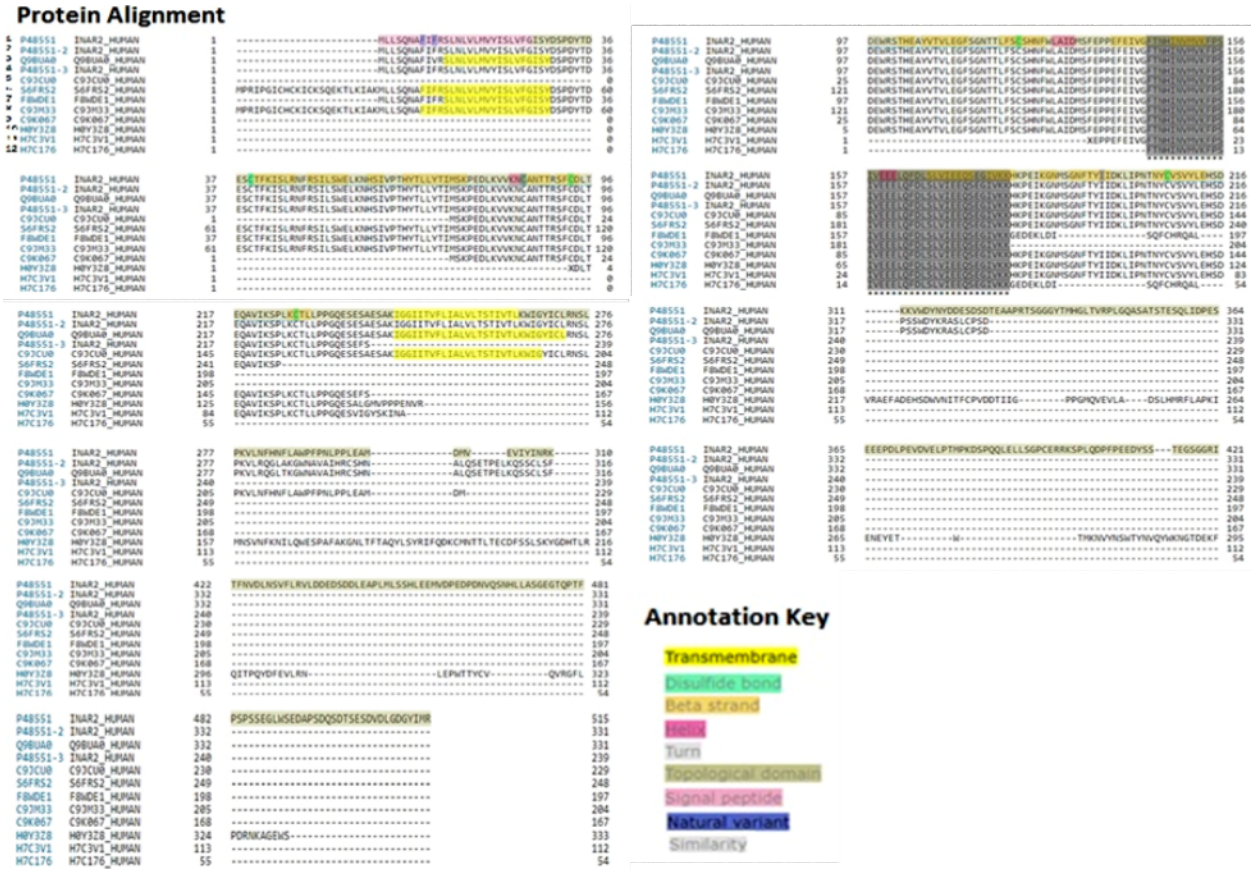

Figure-1: Alignment; Human IFNAR-2 protein

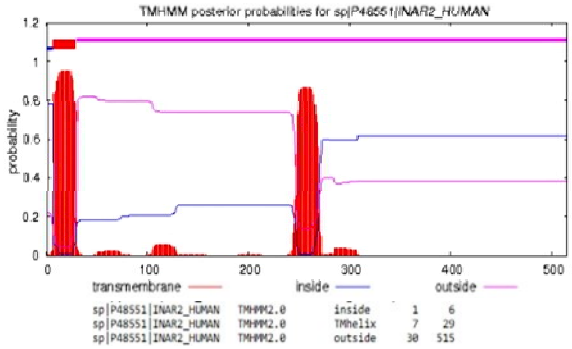

Figure 2: Protein topology prediction for human IFNAR-2 protein

DISCUSSION

Type 1 interferon (IFNs) belongs to the homologous cytokines, that elicit immune response against viral infection and develops anti-proliferative state in infected cells. This family include IFN-α, -β and -ω, which binds to receptors cell surface. These cell surface receptors have trans-membrane proteins known as type 1 IFN-α receptor and type 2 IFN-α receptor proteins, which associate with each other upon binding with receptor and form interferon-alpha receptor (IFNAR) complex.[11] This IFNAR complex is novel among cytokine receptors in mediating signalling, by >5 different ligands.[12]

Our study predicted primary and secondary structural features of human IFNA-2 receptor for the first time, as literature search indicated lack of information about primary protein features and amino acid compositional analysis of this biological important signalling protein of immune system. This analysis revealed that human IFNAR-2 polypeptide chain consists of amino acids having hydrophobic, polar and aromatic characteristics. Information about nature and composition of amino acids in polypeptide chain is very crucial because primary structure translate/interpret/ direct secondary structure formation. Intrinsic propensities of amino acids, their position in polypeptide chain and periodicity of non-polar and polar amino acids affect the physiochemical characteristics and respective function of protein.[13] Therefore, based on our predicted analysis IFNAR-2 protein possess more hydrophobic and less hydrophilic character. IFNAR-2 protein form helical structure. Previously IFNAR-2 protein has also been categorized in II helical cytokine receptor (hCR) family due to its characteristic feature[12] as predicted in our study as well. Predicted inner, outer and trans-membrane loops provide information about its sub cellular localization that mostly it presents in plasma membrane. This feature conveys respective functions,

i.e., interaction with membrane bound receptors and trigger downstream signalling cascade.[2]

Our findings from *in silico* analysis provides the insight about the structural features and nature of human IFNAR-2 protein, which has implications for further research about complex structural and functional analysis of human IFNA-2 receptor particularly in context with therapeutics and *de novo* protein design, in future.

CONCLUSION

In silico analysis revealed that human IFNA-2 receptor protein long chain consists of 515 amino acids having hydrophobic, polar and aromatic characteristics. Secondary structures of human IFNA-2 receptor protein includes helix, turn, beta sheet and topological protein domains, which play important role in receptor function.

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