PROTEIN-PROTEIN INTERACTION ANALYSIS OF HUMAN INTERFERON ALPHA RECEPTOR 2 (IFNAR-2) PROTEIN USING STRING SERVER

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ABSTRACT

Objective: To study the functional and molecular interactions of IFNAR-2 within biological networks

Study Design: Computational analysis: STRING software

Place and Duration of Study: Department of Biochemistry, HITEC-Institute of Medical Sciences, Taxila Cantt, Pakistan, from Dec 2017 to Jun 2018.

Methodology: Protein sequence of IFNAR-2 protein was obtained from 'National Centre for Biotechnology Information (NCBI)' database and STRING analysis conducted by applying specific parameters including (1) Text mining (2) Experiments (3) Databases (4) Co-expression (5) Neighborhood (6) Gene fusion and (7) Co-occurrence for identifying protein-protein interactions and molecular associations. Maximum number of interactors was set at 20 and highest confidence level was set at 0.900.

Results: Protein-protein interaction analysis translate that human IFNAR-2 protein has high level of interactions with a set of proteins of similar size, drawn from the genome. This set represents a partial biologically connected group of proteins. This information has a potential to set the basis for further experimental investigations in more integrated and biologically linked pathway-oriented perspective that results in more targeted outcomes.

Conclusion: Functional and molecular enrichment through STRING analysis revealed that IFNAR-2 protein has strong associations and serves as a key player in antiviral response of immune system.

Keywords: IFNAR-2 protein, Search Tool for the Retrieval of Interacting Genes/Proteins (STRING), Protein-protein interactions, Molecular associations.

INTRODUCTION

Interferon (IFN) receptors are assemblies of trans-membrane glycoproteins, belonging to the helical cytokine receptor (hCR) family, which, in response to ligand, activates the signal transduction pathways. The IFNAR2 genes encode multiple iso forms that contribute to the potential complexity of the receptor[1-3]. The structure of the IFNAR-2 protein binding ectodomain (IFNAR2-EC), is the first identified helical cytokine receptor structure that provides the molecular basis for IFN binding[4]. Later on, three dimensional structure of IFNAR-2 protein was identified by NMR which predicted that core of ligand binding domain consists of hydrophobic aliphatic amino acids[5]. A previous study based upon insilico software analysis reported that IFNAR-2 protein is a 515 amino acids long chain, having total 37 identical positions with 6.446% identity and consists of variety of secondary structures such as alpha-helices (inner, outer and trans-membrane domain), turns and beta sheets that impart structural diversity, dictating the functional diversity of this protein. In current scenario, sufficient knowledge about functional description of IFNAR-2 protein is not available, mainly due to the limitations of relevant sources including study models. Comprehensive information about the functions and molecular associations of IFNAR-2 protein is an important aspect to understand the potential role of protein in biological systems. Functional associations of human interferon alpha receptor 2 (IFNAR-2) protein based upon protein-protein interactions can be important for development of targeted drug therapy where we need to consider these multiple

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interactions of respective receptor based targeted therapeutics[6].

The database, STRING (Search Tool for the Retrieval of Interacting Genes/Proteins), is a fully pre-computed exploratory resource that contains a much larger number of associations than primary interaction database, with varying confidence scores. It basically provides three types of protein-protein interactions/associations evidenced under one common framework with an integrated approach. This approach offers various advantages such as (1) comparative analysis based upon single and stable set of proteins (2) more coverage for the protein of interest, based upon known and predicted information that complements its relevant associations (3) independent evidence based scoring providing confidence about the role and importance of specific protein (4) association mapping and transformation onto other kingdom systems setting the basis for evolutionary studies[7]. By far, this is best tool to provide a quick initial over-view of the functional partners of a query protein, especially for proteins that are still poorly characterized such as human IFNAR-2 protein. Hence, the present study was conducted to determine some interaction or association features of human IFNAR-2 protein with other proteins by employing the STRING server tool.

METHODOLOGY

The present study was conducted from December 2017 till June 2018, after approval from Institutional Review Board (IBR), at Department of Biochemistry, HITEC - Institute of Medical Sciences, Taxila Cantt, Pakistan. In this study STRING version 10.0, has been employed to find multiple protein interactions of human IFNAR-2 protein with other proteins by using coding sequence, NCBI Accession: P48551.1. Analysis was conducted by applying specific parameters. Initially, network edges were analysed based upon evidence. Following this, selective interactions were evaluated on the basis of experimental source. Protein-protein analysis consisted of active interaction parameters including (1) Text mining (2) Experiments (3) Databases (4) Co-expression (5) Neighbourhood (6) Gene fusion and (7) Co-occurrence. Maximum twenty proteins (interactors) were selected and minimum required interaction was set at score 0.900 as the highest confidence level. Analysis of molecular interactions comprised the same parameters except text mining source. STRING consortium 2017 consists of SIB (Swiss Institute of Bioinformatics), CPR-NNF (Centre for Protein Research) and EMBL (European Molecular Biology Laboratory) databases[8].

RESULTS

The fasta protein sequence of human IFNAR-2 protein used in this study is given below:

Uni Prot KB / Swiss-Prot: P48551.1 >sp|P48551.1 | INAR2_HUMAN Rec Name: Full = Interferon alpha / beta receptor 2; Short = IFN-R-2; Short = IFN-alpha binding protein; Short = IFN-alpha/beta receptor 2; Alt Name: Full = Interferon alpha binding protein; Alt Name: Full = Type I interferon receptor 2; Flags: Precursor

MLLSQNAFIFRSLNLVLMVYISLVFGISYDS
PDYTDESCTFKISLRNFRSILSWELKNHSIVPTH
YTLLYTIMSKPEDLKVVKNCANTTRSFCDLTDE
WRSTHEAYVTVLEGFSGNTTLFSCSHNFWLAI
DMSFEPPEFEIVGFTNHINVMVKFPSIVEEELQF
DLSLVIEEQSEGIVKKHKPEIKGNMSGNFTYIID
KLIPNTNYCVSVYLEHSDEQAVIKSPLKCTLLP
PGQESESAESAKIGGIITVFLIALVLTSTIVTLKWI
GYICLRNSLPKVLNFHNFLAWPFPNLPPLEAM
DMVEVIYINRKKKVWDYNYDDESDSDTEAAP
RTSGGGYTMHGLTVRPLGQASATSTESQLIDPE
SEEEPDLPEVDVELPTMPKDSPQQLELLSGPCE
RRKSPLQDPFPEEDYSSTEGSGGRITFNVDLNSV
FLRVLDDEDSDDLEAPLMLSSHLEEMVDPEDP
DNVQSNHLLASGEGTQPTFPSPSSEGLWSEDA
PSDQSDTSESDVDLGDGYIMR

Protein-protein interactions of human IFNAR-2 protein were studied by STRING platform. We used fasta protein sequence of human IFNAR-2 protein (query protein) in STRING software with a total number of 20 proteins to study a network of associations

around the query protein. Enrichment was tested statistically and validated. The average local clustering coefficient was 0.928 for total 21 nodes (one query and set of proteins) and 165 edges. Network nodes represented proteins, produced by a single, coding gene locus. Small size of node indicated the protein of unknown 3D structure, whereas large size of node represented the

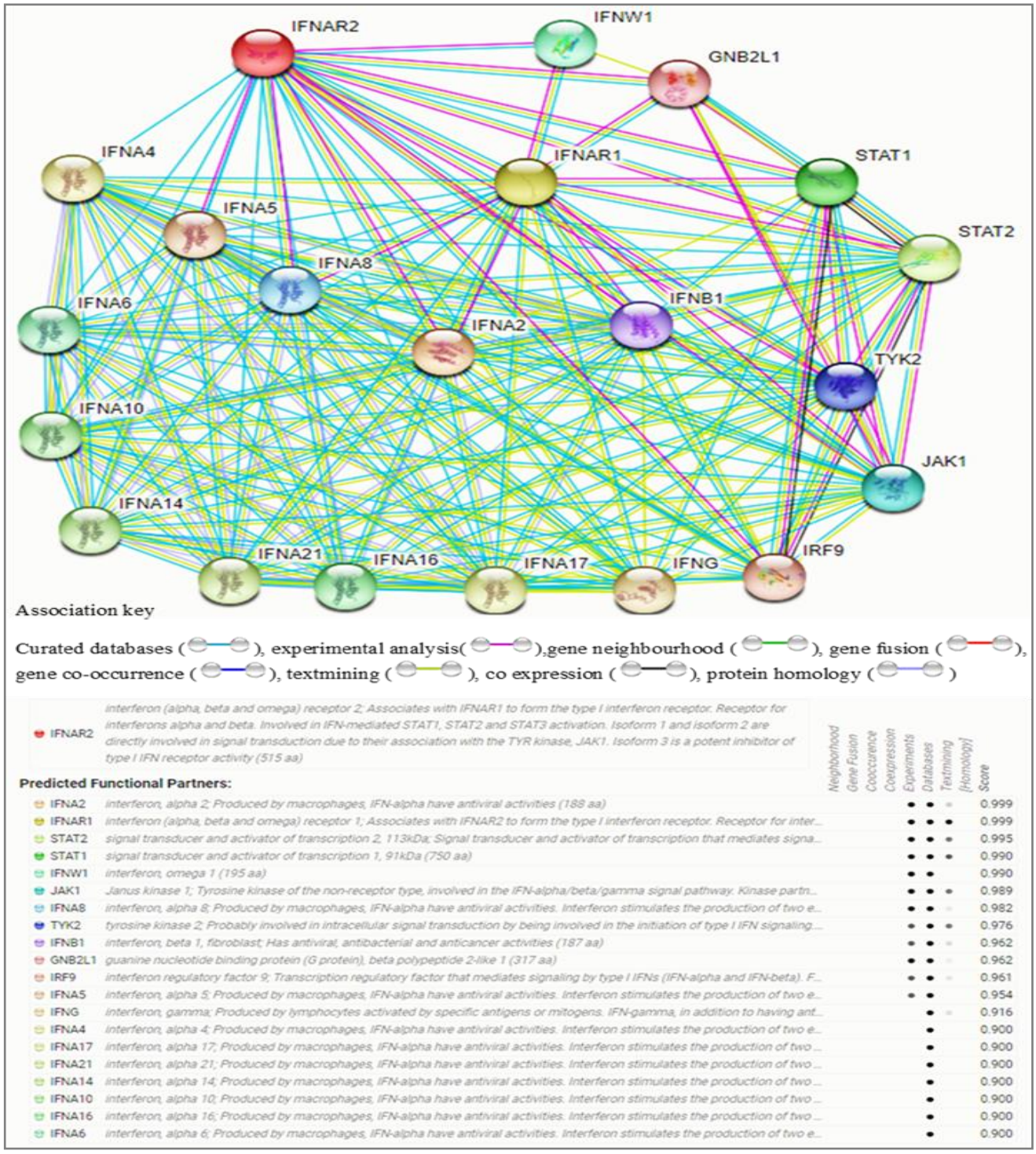

Figure-1: Predicted protein - protein associations of human IFNAR-2 protein.

known or predicted 3D protein structures. Figure 1 consisted of only large nodes, which showed that our analysis only included proteins of known 3D structures. Red coloured node represented the human IFNAR-2 protein (query protein) and the first shell of interactions with other proteins, whereas white nodes symbolized the second shell of interactions. The edges highlighted the protein-protein associations. Known interactions were extracted from curated databases and experimental analysis, whereas predicted interactions were based upon gene neighbourhood, gene fusion and gene co-occurrence. Results also showed some other interactions based upon textmining, co-exp-ression and protein homology. Analysis revealed that human IFNAR-2 protein interacts with many other proteins including groups of interferon (IFNA2, IFNA4, IFNA5, IFNA6, IFNA8, IFNA10, IFNA14, IFNA16, IFNA21, IFNA17, IFNB1, IFNW1, IFNG and IRF9), interferon receptor 1 (IFNAR1) (alpha, beta and omega) and signalling pathway proteins (STAT1, STAT 2, JAK1, TYK2 and GNB2L1) (fig-1). These associations showed that human IFNAR-2 protein has more interactions with a random set of proteins of similar size, drawn from the genome. Such an enrichment revealed that these proteins are biologically connected, at least partially, as a group. These associations interpreted functional enrichment of human IFNAR-2 protein, that includes biological process, molecular function, cellular component, KEGG

Table: Functional enrichment for human IFNAR-2 protein interaction.

Biological Process (GO)			
Pathway ID	Pathway description	Count in gene set	False discovery rate
GO:0060338	Regulation of type I interferon-mediated signaling pathway	17	1.36e-40
GO:0060337	Type I interferon signaling pathway	18	6.82e-40
GO:0071357	Cellular response to type I interferon	18	6.82e-40
GO:0001959	Regulation of cytokine-mediated signaling pathway	18	1.18e-35
GO:0033141	Positive regulation of peptidyl-serine phosphorylation of STAT protein	13	5.4e-34
Molecular Function (GO)			
Pathway ID	Pathway description	Count in gene set	False discovery rate
GO:0005132	Type I interferon receptor binding	12	6.68e-31
GO:0005126	Cytokine receptor binding	16	2.29e-25
GO:0005125	Cytokine activity	13	2.1e-20
GO:0005102	Receptor binding	17	4.39e-16
GO:0005515	Protein binding	19	1.1e-08
Cellular Component (GO)			
Pathway ID	Pathway description	Count in gene set	False discovery rate
GO:0005615	Extracellular space	14	6.4e-10
GO:0044421	Extracellular region part	15	4.16e-05
GO:0005576	Extracellular region	15	0.000291
KEGG Pathways			
Pathway ID	Pathway description	Count in gene set	False discovery rate
05162	Measles	20	1.27e-41
04630	Jak-STAT signaling pathway	20	2.84e-40
05164	Influenza A	19	1.29e-36
05168	Herpes simplex Infection	19	2.49e-36
05160	Hepatitis C	18	4.67e-36
PFAM Protein Domains			
Pathway ID	Pathway description	Count in gene set	False discovery rate
PF01017	STAT protein, all-alpha domain	2	0.00599
PF02864	STAT protein, DNA binding domain	2	0.00599
PF02865	STAT protein, protein interaction domain	2	0.00599
INTERPRO Protein Domains and Features			
Pathway ID	Pathway description	Count in gene set	False discovery rate
IPR001217	Trancription fator STAT	2	0.00641
IPR012345	STAT transcription factor, DNA-binding, subdomain	2	0.00641
IPR013799	STAT transcription factor, protein interaction	2	0.00641
IPR013800	STAT transcription factor, all-alpha domain	2	0.00641
IPR013801	STAT transcription factor, DNA-binding	2	0.00641

(Kyoto Encyclopedia of Genes and Genomes) pathways, PFAM (Protein Families) protein domains, INTERPRO (Inter Pro Protein sequence analysis & classification) protein domains and GO (Gene ontology) features (table).

Molecular interaction enrichment of human IFNAR-2 protein with 21 interactors was also tested statistically and results showed that interaction network was significant, having a PPI enrichment *p*-value 3.61x10-8 (fig-2). Network

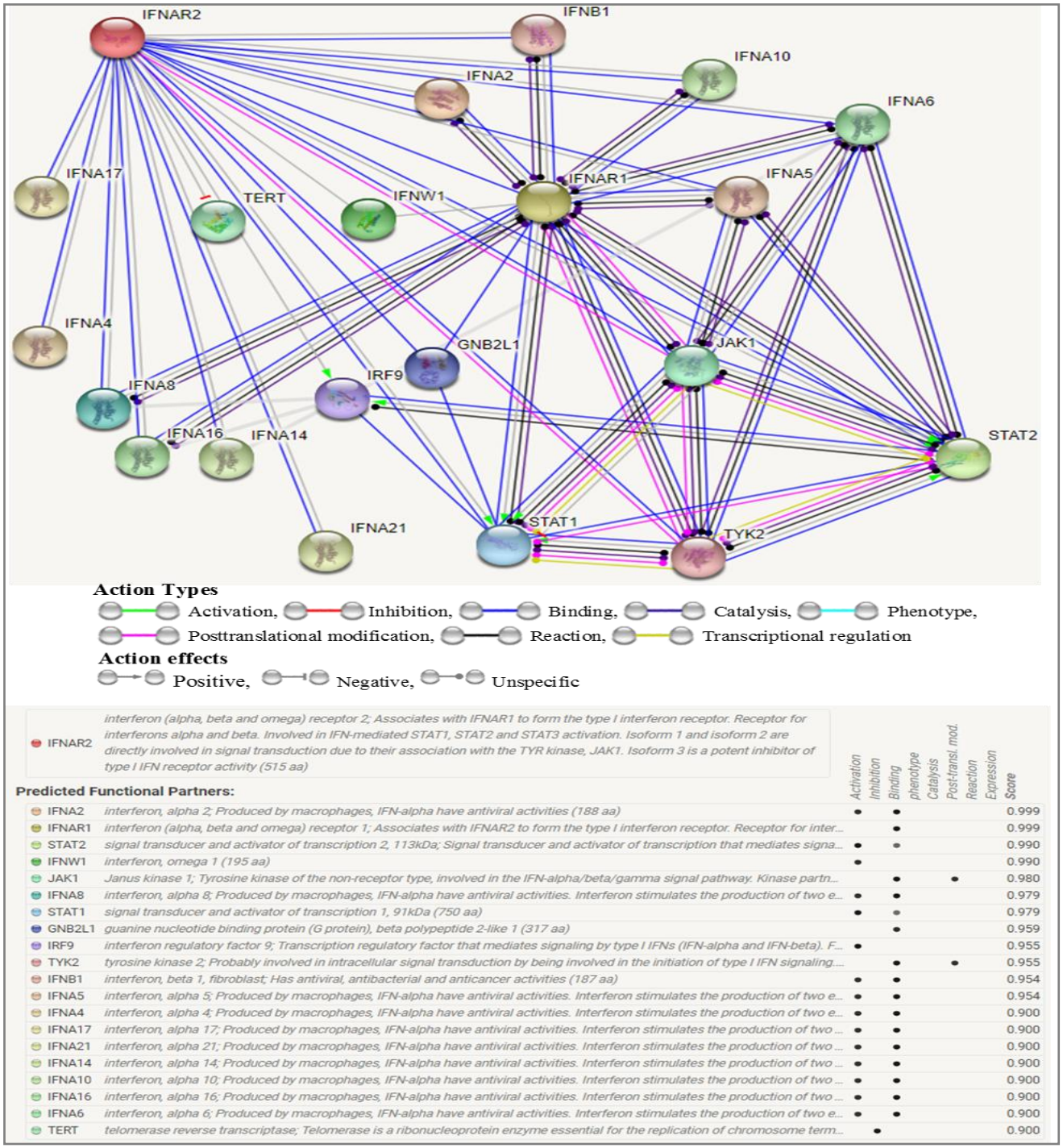

Figure-2: Predicted molecular interaction profile of human IFNAR-2 protein.

analysis of human IFNAR-2 protein molecular association showed that the number of interactions is the highest for binding and post-translational modification activities. This analysis also revealed another important function of human IFNAR-2 protein that it inhibits the activity of binding, type I interferon receptor activity and growth hormone receptor binding (fig-3).

DISCUSSION

In this study STRING software has been used to identify the functional aspects of human IFNAR-2 protein. Detailed profile showed

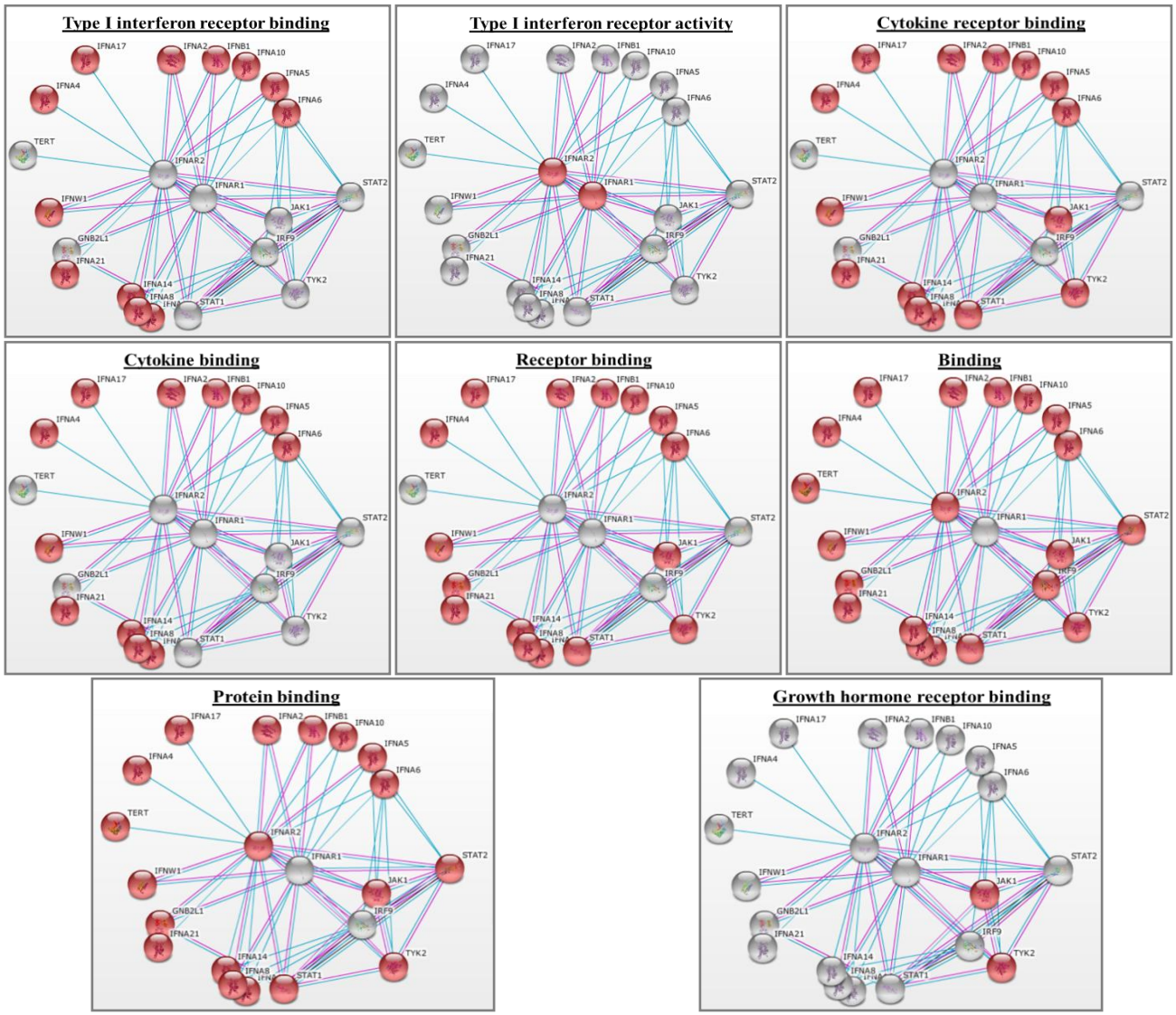

Figure-3: Molecular interaction partners of human IFNAR-2 protein, to impart specific effects through first (Red) and second shell (Grey) of interactions.

TERT (telomerase reverse transcriptase).

Human IFNAR-2 protein is involved in first and second shell of interactions to induce various molecular functions/effects such as type I interferon receptor binding, cytokine receptor binding, cytokine activity, receptor binding, protein comparative interactions of human IFNAR-2 protein with a set of interferons, interferon receptor and signalling molecules which translates its functional importance and vitality within the biological systems and regulatory networks at molecular and protein level. IFNAR-2 protein

interacts with IFNG (interferon, gamma) which is produced by lymphocytes and acts as potent antiviral and activator of macro-phages[9]. Activated macrophages produce various interferons like IFNA4, IFNA5, IFNA6, IFNA8, IFNA10, IFNA14, IFNA16, IFNA17 and IFNA21 which interact with IFNAR-2 protein and contribute in antiviral response particularly in HIV infection[10]. IFNAR-2 protein plays an important role in signal transduction and activation of transcription factors during respective response via interaction with STAT1, STAT2, TYK2 and GNB2L1[11,12]. Protein-protein analysis also reveals that most of these interacting partners share genetic neighbourhood and are involved in gene fusion and co-occurrence events. Second part of this study deals with molecular interactions and components of pathways involving IFNAR-2 protein that reveals interesting information regarding level of interactions and contributions of IFNAR-2 protein in respective pathways or its activity (fig-3). Results revealed that IFNAR-2 protein is directly involved in protein binding and interferon (alpha, beta and omega) receptor 1 activity[13], whereas it is indirectly involved in the production of various interferons from macrophages[14]. IFNAR-2 protein is also indirectly involved in growth hormone binding interactions. STRING molecular interaction analysis provides more functional coverage for IFNAR-2 protein, by revealing its role for inhibition of TERT (telomerase reverse transcriptase) activity, which is generally active in progenitor and cancer cells but is inactive, or very low activity in normal somatic cells[15-17]. This information also indicates the significance of IFNAR-2 protein and that it needs to be studied extensively. Predication analysis based results, through STRING provide strong evidence over independent predictions, because it combines the potential of machine learning with data mining source[18]. Therefore, taking into account this detailed functional enrichment profile of IFNAR-2 protein, we conclude that STRING platform provides extensive functional and molecular details and coverage about partially characterized proteins. Results of this study have potential to set the basis for further experimental investigations in more integrated and biologically linked pathway oriented perspective and approach which could provide more targeted outcomes.

CONCLUSION

Functional and molecular enrichment through STRING analysis revealed that IFNAR-2 protein has strong associations and serves as a key player in antiviral response of immune system. STRING software provides updated comprehensive molecular and functional associations of proteins.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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