

Analysis of IL-6 Interacting Proteins Using STRING Online Resource

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Protein-protein interaction (PPI) is one of the biological integrating communication systems. Also, biological feed-back mechanism is centred on PPI. It is furthermore important to be taken into consideration in viral diseases such as Covid-19 and consequent drug regimens leading to cytokine storm. STRING online resource (<https://string-db.org/cgi/network>) comprising of compiled data through machine learning tools, wide array of literature resources and embedded interactive platform aiming to explore associations among displayed proteins with combined score in predetermined parameters in the network setting box was adapted in the present study. The query protein viz., IL-6 was chosen to explore its interactions by choosing options in the setting box namely five interactors with zero and three clusters. The output indicated that the displayed network containing query IL-6 with participating five proteins shown to have significantly more interactions than predicted with a combined score of ~0.997. This study would appreciate intricacies involved in cytokine storm among Covid-19 contracted patients.

Keywords: IL-6; STRING Tool; Protein-protein Interaction; Covid-19; Cytokine Storm**Introduction**

Cytokines are vital for all physiological responses of cells particularly immune cells as they involve in the signal communication among them. They are small intercellular glycoproteins released by cells upon priming by a variety of influences classified as auto-crine, paracrine and endocrine. These cytokines are either pro- or anti- inflammatory and also promote to differentiate immune cells that involve in the elicitation of immune response in the host to a variety of challenges.

Excessive secretion of IL-6 was reported to cause an acute inflammatory response which is commonly referred to as 'cytokine storm', a hyper-inflammatory state characterised by the coagulation of blood cells and consequent clinical observations recorded inhibition of myocardial activity [1]. The regimen to block IL-6R was practiced through a monoclonal antibody therapy namely

tocilizumab [1]. In yet another study, it is reported among a few severely ill Covid-19 patients the elevated levels of IL-6 and IL-10 which led to a state of coagulopathy [2,3]. IL-6, IL-6R and STAT3 trans-signalling was reported in the bowel inflammatory diseases and consequent therapy targeting IL-6/STAT2 was suggested by Mitsuyama, *et al.* [4]. Both in mouse models and human patients the evidence to support the involvement of IL-6 in chronic lung diseases was authenticated but as a by-product [5]. These sporadic experimental and clinical reports will be more appreciated if such data are used to unfold the display of Protein-protein network of IL-6, which is being attempted in the present study. The STRING software resource v.11.0 that is adapted in the present investigation allows users to develop interaction networks of proteins with input of the user choice (<https://string-db.org/>) [6]. Therefore, IL-6 one of the most significant interleukins in the acute phase immune response was selected to explore its protein-protein network using STRING open resource.

Materials and Methods

In the present attempt, the query protein viz., IL-6 cytokine was chosen due to its importance as an agonist in inflammatory responses. The metadata on the interacting proteins with IL-6 either to promote or relegate inflammation was intended to develop in this article using STRING online software resource (<https://string-db.org/>) by choosing parameters defined in the network setting box indicated in figure 1. Upon presentation of the query protein namely IL-6 with set parameters, there resulted in five interacting proteins as depicted in figure 2. Protein-protein network (PPN) in zero and three clusters was shown in figure 2(A) and 2(B) respectively. The present study was limited to five interactors as the displayed participating and interacting proteins yielded PPI with significant combined scores. The resultant nodes, edges and PPI enrichment p-value score were all given in the legend along with figure 2. The scores for combined interactions among interactors in PPN were given in table 1. The PPN was downloaded using Cytoscape 3.8.2. The flowchart showing step-wise procedure followed in STRING software resource was generated using <https://lucid.app/documents> and downloaded in PNG format with Fresh Paint tool (Figure 1).

Results and Discussion

Cell to cell communication molecules are broadly categorized under cytokines. Both cell types namely leukocytes and lymphocytes are continuously under the surveillance of cytokines viz., interleukins and lymphokines starting from the blast cell stage up to the differentiated cells [7-9]. Most importantly, cytokines are

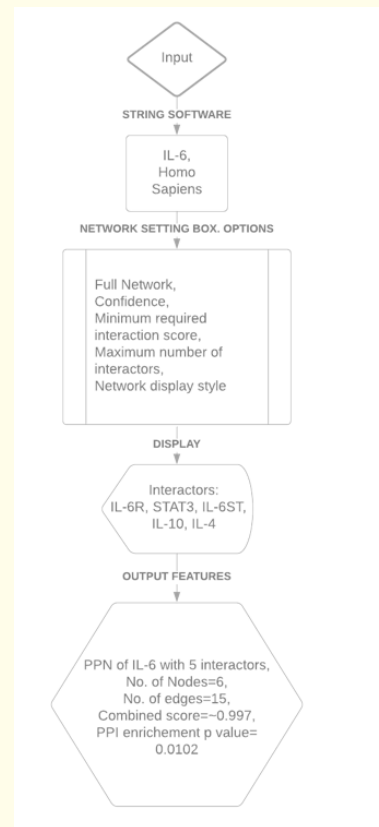


Figure 1: Flowchart showing the steps followed in STRING resource (<https://stringdb.org/cgi/network>). The flow chart is prepared using online tool <https://lucid.app/documents> and saved in PNG format using Fresh Paint tool.

Figure 2A: Protein-protein network (PPN) showing the predicted interaction of IL-6 with other cytokines and receptors in Homo sapiens. Basic settings to generate PPN chosen from network setting box in STRING (<https://string-db.org>) to derive the predicted network were as follows: (i) Full Network, (ii) Confidence (line thickness), (iii) Minimum required interaction score is set as 0.400, (iv) Maximum number of interactors chosen are five, (v) Network display style is set as interactive SVG (scalable vector graphics), (vi) Network display options: show input protein names, (vii) Each circle represents a node and labelled with the predicted interactive protein and (viii) Red coloured node is the query protein IL-6 in the first shell of interactions, (ix) Edges of lines between nodes represent the associations predicted with confidence. The scores of combined interaction are given in table 1. The number of nodes =6, Number of edges = 15, Average node degree = 5, Average local clustering coefficient = 1, Expected number of edges = 8 and PPI enrichment p-value was 0.0102.

Figure 2B: The three clustered protein-protein network showing the predicted interaction of IL-6 with other cytokines and receptors in Homo sapiens. The network shown in figure 2A is re-observed in a three specific cluster network in STRINGk option. IL-6 networked with IL-4 and IL-10 is shown as one within the three clustered PPN. The scores of combined interaction were given in table 1.

Interactors Interacting with IL-6	Combined Score
IL-6 R Interleukin-6 receptor subunit alpha	0.999
STAT3 Signal transducer and activator of transcription 3	0.997
IL6ST Interleukin-6 receptor subunit beta; Signaltransducing molecule.	0.997
IL10 Interleukin-10	0.997
IL4 Interleukin-4	0.996

Table 1: Combined score between the participating proteins with IL-6 obtained using STRING resource.

endowed with multiple attributes such as synergy, pleiotropy, antagonism etc., in influencing cell to cell communication and eliciting immune responses [1-3]. IL-6 is reported to be pleiotropic cytokine [4]. The compiled data on protein biology relating to their structure, gene sequence, homology, coexpression, association etc., processed through text mining, machine learning tools, phylogeny,

in-vitro, *in-vivo* and *in-situ* analyses were all embedded in STRING resource [6]. Hence, STRING was exploited in the present study to analyse IL-6 interacting proteins in *Homo sapiens* so as to provide clues on recruitment of WBCs and possible eruption of cytokine storm [1-3,6,8]. These interleukins are small molecular weight proteins and reported to be less than 30 - 40 kDa. They have corresponding receptors on leukocytes and lymphocytes to initiate a cascade of cell-mediated immune responses [7,8].

In the present investigation, the input query protein IL-6 and the host organism *Homo sapiens* fed to STRING online resource (<https://string-db.org/>) with the defined parameters in the network setting box yielded five protein interactors namely IL-6 R (Interleukin-6 receptor subunit alpha), STAT(Signal transducer and activator of transcription 3), IL-R ST (Interleukin-6 receptor subunit beta; Signal-transducing molecule), IL-10 (Interleukin-10) and IL-4 (Interleukin-4). STRING authenticated that the query protein IL-6 along with interactors have more interactions among themselves than what would be expected for a random set of proteins of similar size. Such enrichment indicates that these proteins (Figure 2A and 2B) are at least partially biologically connected, as a group (<https://stringdb.org/cgi/network>).

In the sequence of steps leading to elicitation of immune response there involves dendritic cell followed by Th or Tc and ultimately prime B-cell to secrete tailored immunoglobulins [8]. This chain invariably requires the mediation of a number of cytokines so as to differentiate the blast cells to primed cells [10,11]. One

of the prominent pro-inflammatory cytokine IL-6 chosen as the query protein in the present study was found to play a vital role in immune response in the host organism [1-3,11]. The STRING tool authenticated all interactors in the PPN as a group biologically connected (Figure 1 and 2). The three clustered PPN shown that IL-6 was found interacted with IL-4, IL-10 and IL-6 R (Figure 2B), whose involvement in Covid-19 subjects was noticed in clinical symptom namely cytokine storm indicating that IL-6 does promote acute phase immune response [2,3].

Furthermore, the significant individual roles of interactors made them as biologically connected are due to the fact that IL-6ST supplements IL-6R in transducing signals. STAT binds to IL-6 responsive elements in the promoters of a few acute-phase protein genes and thus promotes inflammation [8-10]. On the contrary, as a feed-back mechanism, IL-10 inhibits the synthesis of a number of cytokines and thus inhibits hematopoietic progenitor cells to differentiate. Both IL-6 and IL-4 influence B-cells for the synthesis of IgG and IgE respectively and thus induce hypersensitive immune response [11]. Hence, STRING resource authenticated that these participating interactors are connected *in situ* as a group in elicitation of immune response. Interestingly, the key proteins in the workflow containing text and data mining related to inflammation and immune responses of 46 targets for the diseases Covid-19 and HIV-1 are reported [12]. Recently, the machine learning tools are developed for the prediction of cytokine-receptor interactions [13-15] and viral-host protein-protein interactions [16] which paved the path towards the display of networking participating interactors in the metabolism.

Conclusion

The input query protein IL-6 and the host organism *Homo sapiens* fed to STRING online software resource (<https://string-db.org/cgi/network>) yielded five protein interactors namely IL-6 R, STAT, IL-R ST, IL-10 and IL-4. Further, STRING resource authenticated that the query protein IL-6 along with interactors are at least partially biologically connected as a group.

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