Computational Analysis and Mathematical modeling of the implication of Micro RNA in the Transcriptional Regulation of Psoriasis

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Abstract

Psoriasis is an autoimmune disorder characterized by the activation of hyper proliferative keratinocytes in the subcutaneous region of human skin. In the era of post genomics, miRNA have been identified to play a significant role in the signaling pathways associated with the pathogenesis of in various autoimmune disorders. In this study, we focus on identifying a lead from computations to establish miRNA as a diagnostic and therapeutic biomarker for Psoriasis and develop novel insights into the pathophysiology of Psoriasis. In case of the identification of additional biomarkers with respect to miRNAs in signaling pathways associated with pathogenesis of Psoriasis, a path was created to enable the expansion of the disease profiling to identify the novel targets. Initially, the significant genes associated with Psoriasis were retrieved from Pubmed, OMIM and DisGeNET catalog and the significant miRNAs along with transcription factors were retrieved from miRTarBase and RegNetwork.

Key words: *Keratinocytes; Pathophysiology; Psoriasis; Cytokine and Chemokine signaling pathway; Pubmed; OMIM; DisGeNET; miRTarBase; RegNetwork*

I. INTRODUCTION

Psoriasis is considered as an autoimmune disease because both genetic and environmental factors play a significant role. The name “Psoriasis” was derived from Greek word “psora” which means “itch”. Psoriasis is a non-contagious and dry inflammatory disorder with ugly skin; it involves the entire system of a person [1]. In most cases, it is inherited and characterized by marginated scaly and erythematous plaques that develop in a relatively symmetrical distribution. The most affected sites were the scalp, palms, soles, umbilicus, gluteus, under the breasts and genitals, elbows, knees, tips of fingers and toes [2]. In nature, this disease is chronic with a tendency to relapse. In this disease, the skin keeps peeling to create a scaling as flakes and it is termed as “Psoriatic Plaques” due to excessive multiplication of epidermis cells to create fishy skin. Psoriasis can affect both male and female. It can occur at any age, though the common appearance for the first time is generally between ages 15-25 years. In western population, theprevalence of psoriasis was estimated to be around 2-3%. The prevalence of psoriasis in the United Kingdom was found to be 1.5% [3]. In Americans, the prevalence of psoriasis was found to be 2.1% among adults. The results from case studies found that around 25% of people with psoriasis could be classified from moderate to severe psoriasis [4]. Around one-third of people with psoriasis were reported with a family history and researchers have identified the association of genetic loci with the diseased condition [5].

Studies on monozygotic twins suggest 70% chance for a twin to develop psoriasis, if the other is affected. The rate of concordance was around 20% for dizygotic twins. The finding suggests that both predisposition in genetics and response in environment were involved in the development of psoriasis. Early onset before 4 years indicates a greater susceptibility in genetics of psoriasis [6]. Psoriasis is a non-communicable disease but it can be genetically transmitted to one another [7]. In most cases, Psoriasis occurs in the third decade of life and the higher levels of incidences were observed in females. Children were affected very rarely. Severities of Psoriasis were more among Whites than blacks. Nearly 30% of patients with Psoriasis have arthritis. The most common onset of the disease occurs at the age of 20 years and around 10 to 15 % of people have psoriatic arthritis. In USA, nearly 7 million people (2%-3% of people) were affected [8] by psoriasis (about 1, 50,000-2, 60,000 cases are being

diagnosed in each year). Patients who have psoriasis in nails may also have psoriasis in skin (cutaneous psoriasis). Only 5% of people do not have skin psoriasis, even if they have psoriasis in the nails. Patients who have psoriasis in skin may also have psoriasis in nails (around 10%-55%) and this condition is called "psoriatic nail disease".

People with skin psoriasis may also have psoriatic arthritis (about 10%-20%), a condition were people can have both the symptoms of psoriasis and arthritis. If a parent or a sibling is affected psoriasis, then the child may have a chance of 16%-25% to get affected by psoriasis. If both parents have psoriasis, then risk of child is 75% to get affected by psoriasis. Psoriasis occurs in people with diverse races [9].

Micro RNA is a family of non coding RNA (ncRNA) which was discovered in 1993, it consist of 19-25 nucleotides and regulates the expression of approximately 30% of protein-coding miRNAs in humans [10]. Base pairing at the position of 2-8 nucleotides were relative to the 5′ end of the small RNA to be termed as the "seed" region and it appears to be important for target recognition [11]. Maturation of miRNAs involves multiple steps and initially two intermediate forms of miRNAs, namely primary (pri-) and precursor (pre-) miRNAs, were produced sequentially. In this process, Drosha (RNase III enzyme) and the double-stranded RNA (dsRNA) binding protein Dgcr8 cleaves the pri-miRNAs to produce a hairpin-shaped pre-miRNAs that are recognized by Exportin5 and they are subsequently transported from the nucleus to cytoplasm. There is another RNase III enzyme called Dicer which cleaves the pre-miRNAs to release ~22-nt double-stranded RNA duplexes (namely miRNA/miRNA* duplexes) with ~2-nt 3' overhangs [12]. One strand of a RNA duplex is termed as a mature miRNA which is further loaded into an Argonaute protein in the RNA-induced silencing complex (RISC) to exert its regulatory function on the basis of its binding with the target transcripts [13].

A unique miRNA can regulate the expression of hundreds of proteins and the expression of a specific protein may be controlled by several miRNAs [14]. The sequence conservation of most miRNAs lies between the distantly related organisms to suggest the impact of a strong evolutionary pressure [15] and they have been shown to participate in many fundamental life processes like development, differentiation, organogenesis, growth control and apoptosis. Accordingly, deregulation of miRNA expression has been shown to contribute to cancer, heart diseases, infectious diseases, inflammatory diseases and other medical conditions, making them potential targets for medical diagnosis and therapy [16]. Initially, Lee had found lin-4 as a regulator of developmental timing in nematode Caenorhabditis elegans [17].

After several years, Reinhart had discovered lethal-7 (let-7) gene in Caenorhabditis elegans [18]. At present, 2500 miRNAs are in the human genome. Majority of miRNA are intragenic [19].Micro RNAs are initially transcribed as a part of an RNA stem-loop that in turn forms part of a several hundred nucleotides long miRNA precursor miRNA (pri-miRNA) [20].Mature miRNA is a part of an RNA-induced silencing complex (RISC) which contains Dicer and many associated proteins[21].Since, miRNA is involved in the functioning of eukaryotic cells, dysregulation of miRNA been associated with a disease and miR2Disease database contain documents with known relationships between miRNA dysregulation and human disease [22]. Micro RNAs can bind to the target messenger RNA (mRNA) transcripts of protein-coding genes and negatively control their translation or cause mRNA degradation and the key factor is to identify the importance of miRNA target with accuracy. A detailed review for the advances in the miRNA target identification methods and available resources has been published by Zheng. Next-Generation sequencing (NGS) is a sequencing technology with the incorporation of high-throughput methods for profiling the expression of various RNA in different species with a resolution of single-nucleotide on the basis of genome-wide scaling [23].

NGS technology sequences the RNA transcripts directly and hence it is able to facilitate the *de novo* discovery of genes with novel miRNA [24, 25]. Recent studies on NGS had discovered a pool of miRNAs and miRNA-like RNAs on the basis of their variants. These diverse miRNAs include canonical and noncanonical miRNAs [26-29], miRNA-like RNAs [30-32] and miRNA isoforms [33, 34]. Canonical miRNAs are generated from a biogenesis pathway that requires Drosha and Dicer. Noncanonical miRNAs are produced from alternative pathways in biogenesis where Drosha is not involved. The first example of noncanonical miRNA is the class of mitrons, which arise from a short nucleotide of sequence length ranges from 60 to 100 bases. Dicer dependent but

Dgcr8 independent miRNA-like RNAs can also arise from the formation of local hairpin within the region of larger noncoding RNA (ncRNA). Micro RNAs are typically defined as the most abundant small RNAs on pre-miRNA hairpins. Nevertheless, other less abundant but cognate small RNAs from the same pre-miRNAs, which differ by a few bases from miRNAs, have also been reported and the variants of these miRNA have been named as *isomiRs* [35].

IsomiRs can function as regular miRNAs [36] and they often share the mRNA target which is common with their companion miRNAs but in some cases they may also have their own exclusive genes as target [37]. The emergence of such diverse miRNAs as additional regulators of gene expression with potential functions is complementary to that of canonical miRNAs which reflects the robustness and plasticity of miRNA-mediated regulation of gene expression. There is an estimate that miRNAs regulate over the one-third portion of protein-encoding mRNAs in humans [38, 39].Recent studies on the development of mammalian skin have revealed the fact that that "Interaction between miRNAs and their target mRNAs is vital for the regulating signaling pathways during cell differentiation" [40-42]. There were also reports on Noncanonical miRNAs, miRNA-like RNAs and isomiRs in normal and psoriatic skin of human [43]. Dysregulation of miRNAs and their regulated targets has been implicated in the pathogenesis of psoriasis [44-46] as well as other forms of disorder of the skin, including malignant melanoma [47-49].

The aberrant expression of small noncoding RNAs in psoriatic skin has suggested functional roles of sncRNAs in psoriasis. This emerging theme on miRNAs suggests that these miRNAs have the potency to become a therapeutic target for treating psoriasis.

II. MATERIALS AND METHODS

In order to analyze the Pharmacogenomics and miRNA regulated networks in Psoriasis, a comparative analysis of a computational approach [50] was required to understand and predict the vital miRNAs and transcription factors involved in the disease pathology of Psoriasis. In this approach the associated genes were obtained from PubMed, DisGeNET and OMIM. The miRNAs were obtained from TargetScan and miRTarbase. Finally the transcription factors were obtained from RegNetwork.

PubMed

PubMed is an online search engine with open access facility to refer MEDLINE for identifying references and abstracts on topics in biomedical and life sciences. The United States National Library of Medicine (NLM) at the National Institutes of Health maintains the database as part of the Entrez system to retrieve information. Most of the records in PubMed contain links to the complete article, in PubMed Central [51]. Information regarding the indexed journals in MEDLINE can be found in the Catalog of NLM. In case of top-down approach, associated genes from pubmed references were collected and analyzed.

DisGeNET

DisGeNET [52] is a platform of pattern discovery, designed for addressing the queries regarding the genetic imprint of human diseases. DisGeNET is one of the largest repositories of gene-disease associations (GDAs) in humans (Piñero et al 2015). It offers a set of tools in bioinformatics to facilitate the data analysis by different users. It is maintained by the Integrative Biomedical Informatics (IBI) Group of the (GRIB)-IMIM/UPF at the Barcelona Biomedical Research Park (PRBB), Barcelona in Catalonia. In case of top-down approach, associated genes from DisGeNET database were collected and analyzed.

OMIM

Online Mendelian Inheritance in Man(OMIM) is a comprehensive compendium of human genes and phenotypes [53] that are available freely and updated daily. The complete text, referenced in the overviews of OMIM contains information on all known mendelian disorders for 15,000 genes. OMIM focuses on the relationship between genotype and phenotype. This database was initiated in 1960s by Dr. Victor A. McKusick as a catalog of mendelian traits and disorders and it was entitled Mendelian Inheritance in Man (MIM). Then, twelve book editions of MIM were published between 1966 and 1998. The online version, OMIM, was created in 1985.

It was made available on the internet in 1987. In 1995, OMIM was developed for the World Wide Web by NCBI, the National Center for Biotechnology Information. In
case of top-down approach, associated genes from OMIM database were collected and analyzed.

TargetScan

TargetScan [54] is a web server to predict the biological targets of miRNAs by searching for the presence of target sites that matches with the seed region of each miRNA. The target predictions of each miRNA are updated regularly. In case of top-down and bottom-up approach, the predicted miRNAs associated with Psoriasis genes were retrieved and analyzed.

miRTarBase

miRTarBase[55] is a curated database of miRNA based target interactions. At present, miRTarBase has accumulated more than fifty thousand interactions of miRNA with target (MTIs); the interactions were manually collected by surveying the literature after the processing of data mining of the text to filter research articles to functional studies of miRNAs in a systematic method. In general, the MTIs were also experimentally validated by a reporter assay, western blot, microarray and experiments on next-generation sequencing. The miRTarBase provides the most updated collection by comparing with the previously developed databases.In case of top-down and bottom-up approach, the validated miRNAs associated with Psoriasis genes were retrieved and analyzed.

RegNetwork

RegNetwork [56] is a data base that contains five types (Transcription Factor-Transcription factor, Transcription Factor-Gene, Transcription Factor-microRNA, microRNA-Transcription Factor) of transcriptional and posttranscriptional regulatory relationships for human and mouse. RegNetwork integrates the curated regulations from various databases and the potential regulations were inferred on the basis of transcription factor binding sites (TFBSs). Transcription factor (TF) and microRNA (miRNA) in gene regulations. Recently, more regulatory relationships in databases and literatures are available and it's valuable for studying the system of gene regulation by integrating the prior knowledge of the transcriptional regulations between TF and target genes along with the posttranscriptional regulations between miRNA and targets. The conservation of knowledge about the binding site of transcription factor (TFBS) can also be implemented to couple the potential regulation between regulators and their targets. In case of top-down, bottom-up, direct and indirect approaches, the transcription factors associated with Psoriasis genes were retrieved and analyzed.

Cytoscape

Cytoscape [57] software is used for network construction, visualization and analysis in bioinformatics with an open source platform for visualizing the interactions in molecular networks and integrating them with the profiles of gene expression. Additional features in cytoscape are available as plugins for network and molecular profiling. In case of top-down, bottom-up, direct and indirect approaches, the regulatory network of Psoriasis associated genes-miRNA-Transcription factors were constructed.

Cytohubba

Cytohubba [58] is a cytoscape plugin for performing the analyses of gene regulation & protein-protein interaction involved in the process of cellular pathways in the process of signal transduction. Cytohubba ranks the nodes of network by topological methods like Radiality, Betweenness, Closeness, Bottleneck, EcCentricity and etc.

miRmap

miRmap [59] software addresses the challenges in post transcriptional repression of miRNAs in human genome by evolutionary, probabilistic thermodynamic and sequence-based features. In case of top-down, bottom-up, direct and indirect approaches, implication of miRNAs in regulatory network was analyzed by miRmap.

Triplex RNA

Triplex RNA [60] is a database of cooperating miRNAs with their mutual targets. In this database miRNA target prediction is based on the analysis of predicted miRNA triplex with molecular dynamics simulations and differential modeling procedures in mathematics.

DAVID

The Database for Annotation, Visualiztion and Integerated Discovery (DAVID) contain information about the functional annotation of genes.The Current version of DAVID [61] is 6.8 and it provides a comprehensive set of tools to understand the biological meaning of genes. In case of top-down, bottom-up, direct and indirect approach, the associated pathways were retrieved and analyzed in DAVID.

Protocol (miRNA based associated regulation)

- Obtain the list of genes associated with psoriasis from Pubmed, DisGeNET and OMIM along with the analysis and implication of Protein-Protein interactions.
- Obtain the list of miRNA associated with psoriasis related genes from miRTarbase and TargetScan.
- Obtain the list of transcription factors associated with psoriasis related genes from RegNetwork.
- Construct and analyze the regulatory network in Cytoscape.
- Identify the top 10 nodes of a regulatory network from Cytohubba and check for the specificity of a gene regulatory network in the top 10 nodes.
- Identify the implication of miRNA in Regulatory network by miRmap & miRNA Triplex.
- Retrieve and analyze the associated pathways in DAVID.
- Identify the role of miRNA in translational repression in top-down approach.
- Illustrate a mathematical model of transcriptional regulation by miRNAs.

III. RESULTS AND DISCUSSION

Construction and analysis of Transcriptional Regulatory Network of Psoriasis (Cytoscape)

In case of top-down approach, the regulatory network was constructed with 92 genes, 437 miRNAs and 285 TFs. Network was initiated by the data mining of 722 regulators (i.e. 285 TFs & 437 miRNAs) to interact with the 92 target genes to form 822 nodes and 2119 edges (Fig 4.2.1).

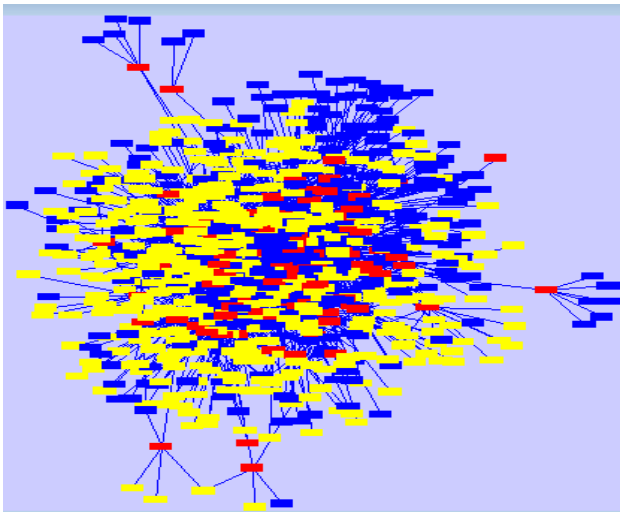

Fig. 1 Transcriptional Regulatory Network of Psoriasis (Top-down Approach: miRNAs-Yellow; Gene-Red; Transcription Factors-Blue)

Analysis ofTranscriptional Regulatory Network of Psoriasis in Top-down Approach in Cytoscape (miRNA based regulation of associated genes)

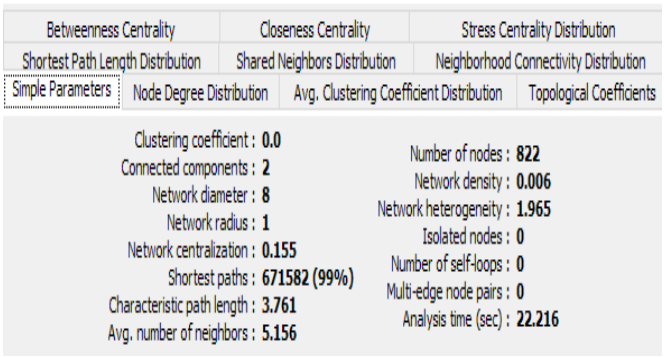

Fig.2 Statistical expression of Network Analysis with simple parameters (Top-down approach)

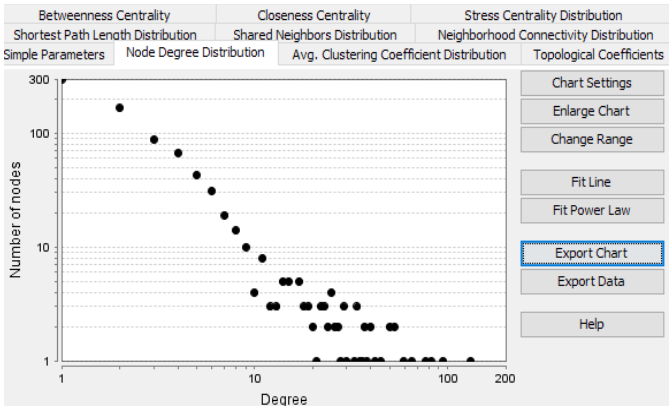

Fig.3 The above picture of node degree distribution shows the most of the nodes have a low degree of fewer than 5 whereas some key regulators have a degree nearing 300.

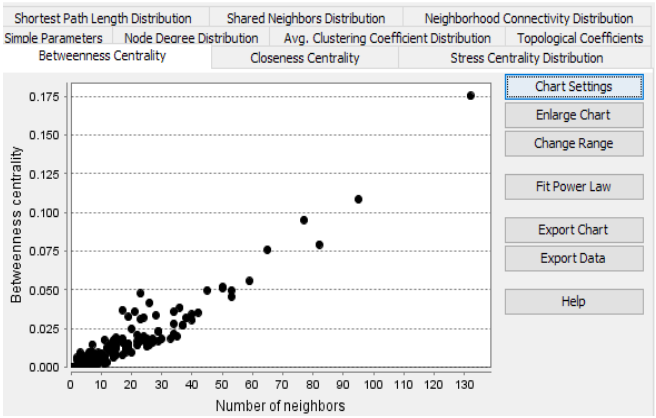

Fig.4 Betweenness Centrality is the calculation of the fraction of shortest path passing through the nodes. The Betweenness Centrality of nodes ranges from 0 to 0.175

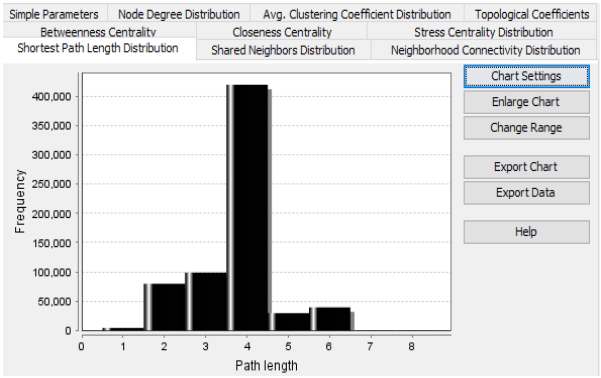

Fig.5 Shortest path length distribution of 4 indicates the fact that the small-world properties of analyzed network i.e. the length of network diameter are the maximum for the shortest path between the two nodes.

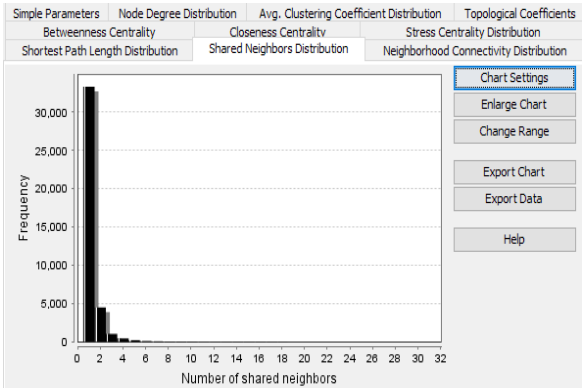

Fig.6 The high frequency of low numbers of shared neighbors indicates the fact that the connectivity among the average number of neighbors is high in the node of the network.

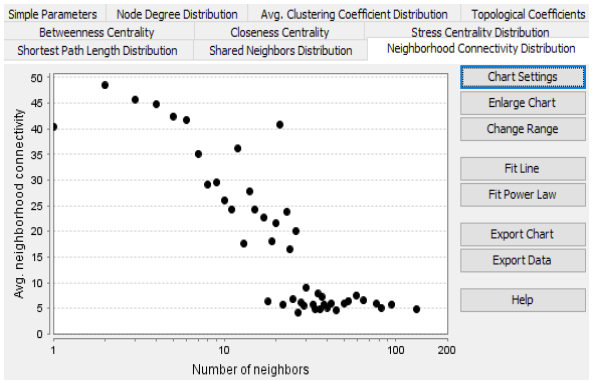

Fig.7 The connectivity of a node is the number of its neighbors. The neighborhood connectivity of a node is the average connectivity of all neighbors. Here the neighborhood connectivity distribution (*k*) is a decreasing in the curve and it is the indication of the nodes with less number of connection and the nodes with more number of connection prevail in this network.

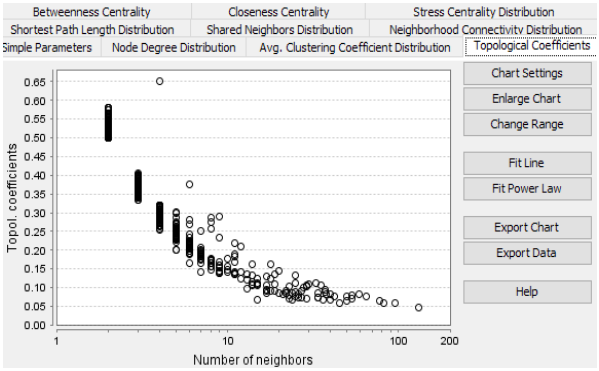

Fig. 8 Topological coefficients to estimate the tendency of the nodes in the network of shared neighbors. Nodes that have one or no neighbors are assigned a topological coefficient of 0.04 and all the nodes here share neighbors, but most of them (50 – 70 gene targets) have a low topological coefficient meaning the miRNAs control cooperatively a large number of their target genes, they affect the complexity of gene regulation.

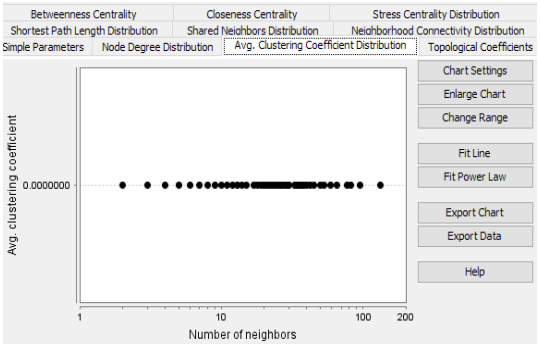

Fig.9 The clustering coefficient here is zero for all the neighbors. Clustering coefficient of a vertex indicates the depth of connection of the neighborhood of that vertex. The clustering coefficient is the ratio of the number of actual edges between neighbors to the number of potential edges between neighbors (all possible edges between the vertices). The cluster coefficient varies from 0 to 1 with 1 indicating that all the neighboring nodes are connected to one another.

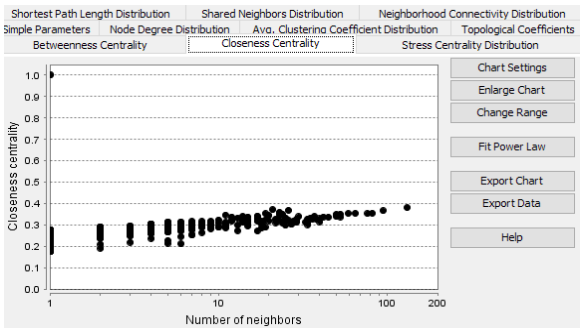

Fig.10 The closeness centrality of a node is the reciprocal of the average of shortest path length. The closeness centrality of each node is a number between 0 and 1. Network Analyzer computes the closeness centrality of all nodes and plots it against the number of neighbors. The closeness centrality of isolated nodes is equal to 0.

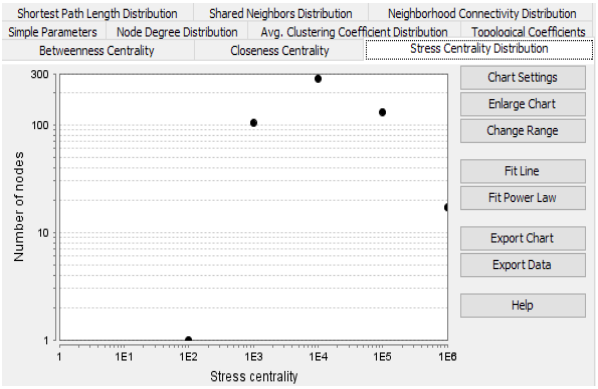

Fig.11 This attribute counts the number of shortest paths passing through a node. A node has a high stress if it is traversed by a high number of shortest paths. This parameter is defined only for networks without multiple edges. This network has a well distributed centrality of stress.

Identification of the Key Regulators in the Transcriptional Regulatory Network of Psoriasis (Cytohubba)

The genes and their regulators (Micro RNAs & Transcription Factors) in Top-down approach were subjected to the analysis in cytohubba by various global based statistical methods like Edge Percolated Component (A method to reduce the complexity of the network with the threshold 1. In mathematical terms, let Gk be the reduced network generated at the kth time in the reduced process, the nodes u and v are connected in Gk and the the threshold δk vt is set to be 1), Bottleneck (Let Ts be a shortest path of the tree rooted at node s. Then, BN (v) = s∈ V; ps(v), where ps(v) = 1 if more than |V(Ts)|/ 4 paths is from node s to other nodes in Ts in the vertex v), EcCentricity (EC(v) = |V(C(v))| |V| × 1 max{dist (v, w) : w ∈ C(v)}), Closeness (Clo (v) = w∈ V 1 dist(v,w)), Radiality (Rad(v) = |V(C(v))| |V| × w∈ C(v) (C(v) + 1 − dist(v,w)) max{dist(v,w) : w ∈ C(v)} , where ΔC(v) is the maximum distance between any two vertices of the component C(v).), Betweenness (BC(v) = s=t=v∈ C(v) σst(v) σst , where σst is the number of shortest paths from node s to node t.) and Stress (Str(v) = s=t=v∈ C(v) σst(v), where σst (v) is the number of shortest paths from node s to node t which use the node v) along with local based statistical methods like Maximal Clique Centrality (MCC(v) = C∈ S(v) (|C| − 1)!, where S(v) is the collection of maximal cliques which contain v, and (|C|-1)! is the product of all positive integers less than |C|. If there is no edge between the neighbors of the node v, then MCC(v) is equal to its degree), Density of Maximum Neighborhood Component (DMNC(v) = |E (MC(v))|/ |V(MC(v))|ε , where ε = 1.7), Maximum Neighborhood Component (MNC(v)=|V(MC(v))|, where MC(v) is a maximum connected component of the G[N(v)] and G[N(v)] is the induced subgraph of G by N(v)) and degree (Deg(v)=|N(v)|) to identify their connectivity. Among the various methods of analysis only a global based statistics of EcCentricity (EC(v) = |V(C(v))| |V| × 1 max{dist(v,w) : w ∈ C(v)}) method and the local based statistics of Maximal Clique Centrality (MCC(v) = C∈ S(v) (|C| − 1)!, where S(v) is the collection of maximal cliques which contain v, and (|C|-1)! is the product of all positive integers less than |C|. If there is no edge between the neighbors of the node v, then MCC(v) is equal to its degree) along with Density of Maximum Neighborhood Component (DMNC(v) = |E (MC(v))|/ |V(MC(v))|ε , where ε = 1.7) and Clustering coefficient methods in cytohubba resulted in obtaining a regulatory network of gene-miRNA-TFs within the top 10 nodes in top-down approach. The details of the first stage nodes, shortest path and the extended subnetworks of transcriptional regulation were given in Figures 4.4.1 - 4.4.3.

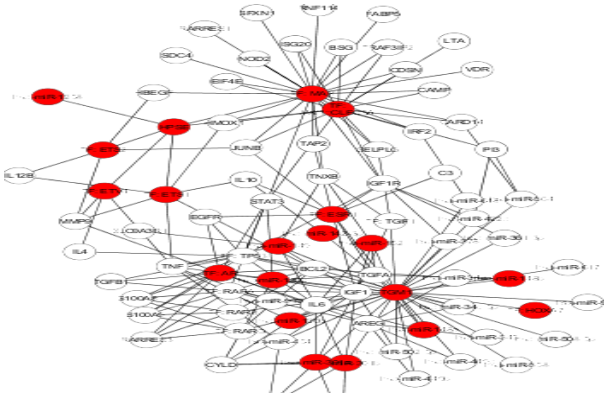

Fig.12 First stage node of the transcriptional regulatory network of Psoriasis in Top-down Approach (DMNC, MNC and Clustering coefficient).In case of first stage nodes, the nodes indicated in Red are highly essential on the basis of their connection with other nodes.

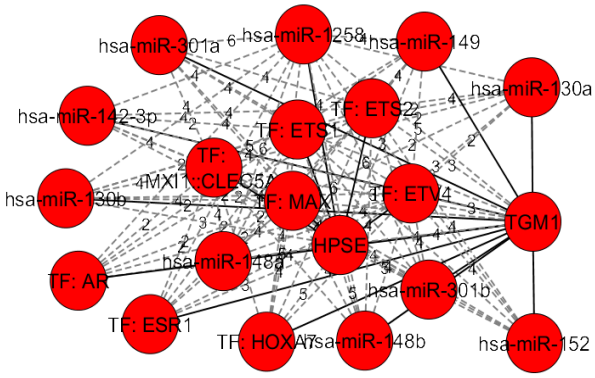

Fig.13shortest path of top 20 nodes in thetranscriptionalregulatory network of Psoriasis in the Top-down Approach (DMNC, MNC & Clustering coefficient methods)

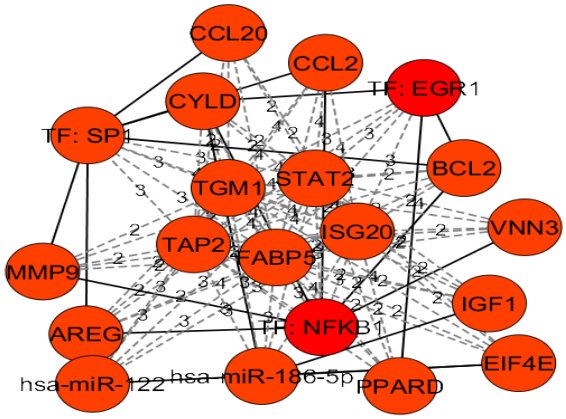

Fig. 14shortest path of the top 20 nodes of thetranscriptionalregulatory network of Psoriasis in the Top-down Approach (Eccentricity method)

Implication of miRNAs in the transcriptional regulatory network of Psoriasis

The implication of miRNAs in the transcriptional regulatory network of Psoriasis in Top-down approach was analyzed on the basis of compatibility with respect to gene-miRNA seed pairing and gene-miRNA-miRNA triplex with respect to nature of binding and the details were given in Table 4.5.1

Table. 1 Implication of MicroRNA in the transcriptional regulatory Network of Top-down Approach

S.No	Genes	Micro RNAs	Binding Score in % (miRmap)	Paired miRNA (Triplex RNA)	Binding Energy in Kcal/mol. (Triplex RNA)	Nature of Binding (Triplex RNA)
1	HPSE	hsa-miR-1258	NIL	NIL	NIL	NIL
2	TGM 1	hsa-miR-1258	NIL	NIL	NIL	NIL
		hsa-miR-186	NIL	NIL	NIL	NIL
		hsa-miR-122	NIL	NIL	NIL	NIL
3	CCL2	hsa-miR-186	NIL	NIL	NIL	NIL
		hsa-miR-122	NIL	NIL	NIL	NIL
4	CCL20	hsa-miR-186	44.69	NIL	NIL	NIL
		hsa-miR-122	NIL	NIL	NIL	NIL
5	EIF4E	hsa-miR-186	38.84	hsa-miR-495	-19.06	miRNA self-complementarity
		hsa-miR-122	NIL	NIL	NIL	NIL
6	STAT 2	hsa-miR-186-5p	47.13	NIL	NIL	NIL
		hsa-miR-122	NIL	NIL	NIL	NIL

In case of the implication of miRNAs in top-down approach, hsa-miR-186-5p is highly compatible on the basis of seed pairing. In the above mentioned table (Table .1) the term “NIL” indicates the absence of seed pairing, and thermodynamical stability.

Scope & Significance of miRNA in the transcriptional regulatory network of Psoriasis

Combinatorial Analysis of miRNA based regulatory network of Psoriasis in Top-down approach indicate the fact that miRNA has-miR-186-5p is involved in the repression of transcription factors EGR1 and SP1 along with the activation of gene STAT2 and the probable miRNA based transcriptional regulatory networks [62] are (i) Gene: STAT2, miRNA: hsa-miR-186-5p& TF: EGR1 and (ii) Gene: STAT2, miRNA: hsa-miR-186-5p & TF: SP1. The details of transcriptional activation were given in Fig 15 and 16.

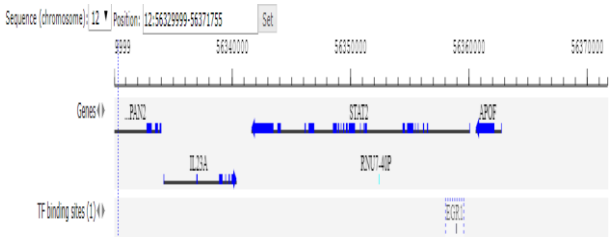

Fig.15 Binding of transcription factor EGR1 in gene STAT2

(i) Transcriptional activation of STAT2 by EGR1

In the human genome, the gene STAT2 is present in the position of Chr. 12: 56341597 – 56360155 (Yevshin et al 2017). The Gene STAT2 contains a binding site for the transcription factor EGFR1 and the detail is illustrated in Fig 4.6.1.

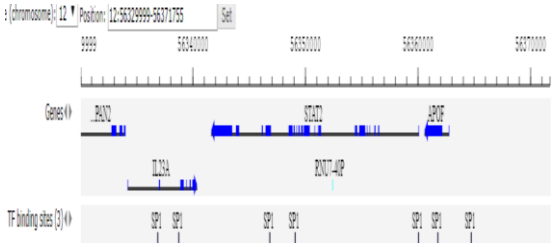

Fig.16. Binding of transcription factor SP1 in gene STAT2

(ii) Transcriptional activation of STAT2 by SP1

In the human genome, the gene STAT2 gene is present in the position of Chr. 12: 56341597 – 56360155 (Yevshin et al 2017). The Gene STAT2 contains three sites for the binding of the transcription factor SP1 and the detail is illustrated in Fig 4.6.2.

Implication of Transcriptional Regulatory network in Pathogenesis of Psoriasis

STAT2 is involved in the pathogenesis of psoriasis by promoting the production of CCL5 and CXCL11 in keratinocytes [63]. The miRNA, hsa-miR-186 was differentially expressed in the lesional skin of Psortatic patients [64]. Egr-1 is regulator for the upregulation of IL-17A-induced psoriasin in psoriasis [65]. The miRNA, hsa-miR-186-5pwas identified as a potential regulator in the subunits of NF-κB [66]. SP1 promotes angiogenesis on VEGFR-2 receptors to decrease the VEGF production in psoriasis [67]. A genetic variant of NFKB1 is associated with the clinical features of Psoriasis vulgaris [68]. According to the orthology analysis of KEGG the main module of STAT2 is associated with the signaling of JAK-STAT pathway [69] but association was also inferred in the signaling pathway of chemokines. In case of EGR1 the orthology analysis resulted in the alperin signaling pathway of neurotrophic receptors [70].The implication of the transcription factor SP1 was not illustrated in the analysis of the modules and the orthology of KEGG pathways. The protein EGR1 act as a transcriptional regulator [71] to

recognize the sequence of DNA (EGR site- 5'-GCG(T/G)GGGCG-3')in the promoter region of target genes to regulate the process of transcription. EGR1 regulates the expression of CXCL2 [72]. The transcription factor SP1 binds to the GC-rich motifs and regulates the expression of genes involved in the responses of the immune system and play an important role in the expression of genes involved in a differentiation, cell growth, immune responses and apoptosis [73].

Pathway Analysis

The obtained genes from Pubmed/DisGeNET/OMIM were subjected to pathway analysis in DAVID on the basis of P value and Benjamini statistic and the result is given in Table.2.

Table.2 Annotation of Kegg Pathways

S. No.	Pathway	P value	Benjamini
1.	Cytokine-cytokine receptor interaction	8.60E-16	1.20E-13
2.	Jak-STAT signaling pathway	2.40E-11	1.10E-09
3.	TNF signaling pathway	1.00E-07	2.70E-06
4.	HIF-1 signaling pathway	1.10E-04	1.10E-03
5.	FoxO signaling pathway	5.00E-04	3.60E-03
6.	Osteoclast differentiation	5.00E-04	3.60E-03
7.	Chemokine signaling pathway	2.00E-03	1.20E-02
8.	Adipocytokine signaling pathway	2.10E-03	1.20E-02
9.	Pathways in cancer	2.50E-03	1.30E-02
10	Allograft rejection	2.80E-03	1.40E-02
11	NOD-like receptor signaling pathway	6.50E-03	2.80E-02
12	mTOR signaling pathway	9.80E-03	4.00E-02
13	PI3K-Akt signaling pathway	1.20E-02	4.30E-02

14	RIG-I-like receptor signaling pathway	1.50E-02	5.00E-02
15	ErbB signaling pathway	2.70E-02	8.20E-02
16	NF-kappa B signaling pathway	3.10E-02	9.00E-02
17	T cell receptor signaling pathway	4.70E-02	1.30E-01
18	Sphingolipid signaling pathway	5.40E-02	1.40E-01
19	Autoimmune thyroid disease	5.60E-02	1.50E-01
20	AMPK signaling pathway	6.00E-02	1.50E-01
21	Rap1 signaling pathway	7.30E-02	1.80E-01

In case of Pathway Analysis of Psoriasis associated genes, the Cytokine-cytokine receptor interaction is statistically significant than the Jak-STAT signaling pathway and the Cytokine-cytokine receptor interaction pathway needs to be reconstructed with Gene: STAT2, miRNA: hsa-miR-186-5p, Transcription Factors: EGR1 and SP1 along with Proteins: LAPTM4A, EIF2C2, ACTL8 and ARL5B.

Mathematical Modeling of Micro RNA Based Transcriptional Repression in the Regulatory Network of Psoriasis

The purpose of Mathematical Modeling is to understand the network dynamics of miRNAs [74] in the feed forward (Coherent and incoherent regulation) and feedback mechanism (Activation and Inhibition) of transcriptional regulation in Psoriasis. The dynamic feature of feedback mechanism of miRNA is to obtain stability in repression of transcription factors and in case of feed forward mechanism the dynamic feature detects the fold change of miRNAs. The network motifs (Genes) mediated by miRNAs regulates the tuning of expression in genes along with transcription factors. The mutual inhibition of transcription factors and miRNAs in feedback mechanism regulate the level of transition from normal to the diseased condition. The feed forward mechanism of miRNA, illustrate the robust nature of a gene regulatory networks. The feedback mechanism of miRNAs initiates the repression of proteins in cell cycle along with the factors associated with transcription.

In case of the mathematical modeling of the miRNA mediated transcriptional regulatory network of psoriasis, the involved factors are gene:STAT2, miRNA: hsa-miR-186-5p and the transcription factors: EGR1 and SP1.The factors associated with transcriptional regulation varies with respect to time and the rate of change of transcription factors (EGR1 and SP1) with respect to miRNA (hsa-miR-186-5p) is illustrated by differential equations as [d (EGR1)/dt = k (Synthesis of EGR1) + STAT2 + k (Up regulation of EGR1) (hsa-miR-186-5p) - k (Degradation of EGR1) (EGR1)] and Similarly [d (SP1)/dt = k (Synthesis of SP1) + STAT2 + k (Up regulation of SP1) (hsa-miR-186-5p) - k (Degradation of SP1) (SP1)].In the above mentioned differential equation the gene STAT2 is an independent constant and the micro RNA (hsa-miR-186-5p) along with transcription factors EGR1 and SP1 are variables. According to the associative property of transcriptional regulatory networks the above

mentioned differential equations explaining the rate of change of transcription factor with respect to time for the parameters gene and miRNA can also be modified for explaining the rate of change of miRNA for the parameters gene and transcription factors. The modified equation is [d (hsa-miR-186-5p)/dt = k (Activation of hsa-miR-186-5p) ($EGR1^{STAT2}$ / k + $EGR1^{STAT2}$ – k (Degradation of hsa-miR-186-5p) hsa-miR-186-5p] and similarly in case of SP1 the differential equation explaining the rate of change of hsa-miR-186-5p with respect to time is modified as [d (hsa-miR-186-5p)/dt = k (Activation of hsa-miR-186-5p) ($SP1^{STAT2}$ / k + $SP1^{STAT2}$ – k (Degradation of hsa-miR-186-5p) hsa-miR-186-5p].

IV. CONCLUSION

Seed pairing of studies of gene-miRNA were analyzed using miRmap and thus the most significant regulatory networks were obtained and a further downstream pipeline was proposed. Further the signaling pathways associated with the progression of Psoriasis were identified by performing the Enrichment analysis of genes associated with Psoriasis. In case of Psoriasis, the transcriptional regulatory network was constructed with 92 Genes, 437 MicroRNAs (miRNAs) and 285 Transcription Factors (TFs). Network was initiated by the interaction of 722 regulators (i.e. 285 TFs & 437 miRNAs) with the 92 target genes to form 822 nodes and 2119 edges. Combinatorial Analysis of miRNA based regulatory transcriptional network of Psoriasis indicate the fact that the miRNA has-miR-186-5p is involved in the repression of transcription factors EGR1 and SP1 and activation of gene STAT2 and the probable miRNA based transcriptional regulatory networks are (i) Gene: STAT2, miRNA: hsa-miR-186-5p & TF: EGR1 and (ii) Gene: STAT2, miRNA: hsa-miR-186-5p & TF: SP1. Enrichment analysis of Psoriasis associated genes resulted in the identification of the pathway involved in the signaling of cytokine and chemokine receptor as a significant pathway to trigger the disease pathology of Psoriasis on the basis of statistical measures. Based on the analysis of text mining, graph theory and statistical measures, it was expected that the probable transcriptional regulatory network to initiate the pathophysiology of Psoriasis needs validation by reconstruction and simulation of Cytokine and Chemokine signaling pathway with hsa-miR-186-5p along with EGR1 and SP1.

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