Symbiosis

www.symbiosisonline.org
www.symbiosisonlinepublishing.com

Short Communication | Journal of Biosensors, Biomarkers and Diagnostics | Open Access

A Computational Approach To Predict A Novel MicroRNA From The Associated Genes Of Cutaneous Lichen Planus: An Initiation Towards The Discovery Of Therapeutic Biomarkers

Harishchander Anandaram*

Department of Bioinformatics, Sathyabama University, Chennai.

Received: June 29, 2018; Accepted: July 31, 2018; Published: Aug 02, 2018

***Corresponding author:** Harishchander Anandaram, Department of Bioinformatics, Sathyabama University, Chennai. E-mail: harishchander.a@gmail.com*

Abstract

In the post genomic era, identifying a novel microRNA (miRNA) from the associated genes of an autoimmune disease and identifying the role of miRNA in the pathways associated with the disease pathology is a challenging task to execute. The challenge was approached by identifying the associated genes of Lichen Planus from DisGeNET followed by the identification of miRNAs from miRTarBase and transcription factors from RegNetwork. Then an association matrix was formed by the combination of genes, miRNAs and transcription factors to illustrate the regulatory network. The regulatory network was subjected to the analysis of graph theory in cytoscape followed by the identification of hub gene and its regulators by the "radiality" function in cytohubba. Finally the probable regulatory networks were identified on the basis of compatibility in the interaction between the gene and miRNA with respect to seed pairing in miRmap followed by the identification of associated pathways in Enrichr. In this study it was identified that the pathogenesis of cutaneous lichen planus was triggered by the regulatory network of BCL2 and the miRNA hsa-miR-143-3p regulates BCL2 with transcription factors RELA and NFKB1 and the enrichment analysis of associated genes resulted in the identification of AGE-RAGE signaling pathway on the basis of p value. In future, the detailed mechanism of has-miR-143-3p in cutaneous lichen planus will be studied by reconstructing the AGE-RAGE signaling pathway with miRNA and transcription factors.

Key words: Computational Approach; MicroRNA; Lichen Planus; Therapeutic Biomarkers;

Introduction

Lichen Planus (LP) is an inflammatory mucocutaneous disease with the pathogenesis of autoimmune disorders [1, 2]. Molecular studies on LP suggest that the result of lesions were raised from a cell-mediated autoimmunity and it was directed against the keratinocytes of the basal layer of skin to the form an infiltrate with CD4 and CD8 lympocytes [3]. MicroRNAs (miRNAs) are short noncoding RNAs and it play a vital role in the physiological and pathophysiological states of cellular processes including the development, differentiation, proliferation and apoptosis [4, 5]. Micro RNAs were identified as a key player in certain inflammatory disorders [6–9]. Most of the published studies on miRNA were from the pathogenesis of Oral Lichen Planus (OLP) but a few studies investigated the pathogenesis of cutaneous LP [10].

Material & Methods

DisGeNET

DisGeNET is one of the largest available web portals with the collection of genes and variants in human diseases [11]. It integrates data from the curated repositories of Genome Wide Association Studies (GWAS) catalogue, animal models and scientific literature. Data in DisGeNET are homogeneously annotated with the original metrics provided in the relationship of genotype to phenotype. The information is accessible through a web interface or a Cytoscape App or an R package. DisGeNET is a versatile platform to investigate the comorbidities and the molecular underpinnings of specific human diseases. In this study, DisGeNET was used to obtain the top 10 genes associated with cutaneous lichen planus on the basis of statistical score.

miRTarBase

The updated version of miRTarBase contain the target sites which were validated by the reporter assay can be downloaded [12]. The sequence of the target site can extract additional features for analysis by a machine learning approach to evaluate the performance of the target prediction of miRNA. In this study, miRTarBase was used to obtain the miRNAs of the genes associated with cutaneous lichen planus.

Regnetwork

RegNetwork is a database of regulatory interactions between miRNAs, transcription factors and genes [13]. RegNetwork contain a comprehensive set of experimentally validated relationship of transcriptional regulation. In this study, Regnetwork was used to obtain the transcription factors of the genes associated with cutaneous lichen planus.

Cytoscape

Cytoscape is an open source software project for studying the networks of high throughput biomolecular interaction [14].

Cytoscape is used in conjunction protein-protein, protein-DNA, and genetic interactions for humans. In this study, Cytoscape was used to construct the regulatory network with genes, miRNAs and transcription factors.

Cytohubba

CytoHubba provide a user-friendly interface to explore the important nodes in biological networks [15]. It computes all eleven methods (Degree, Edge Percolated Component, Maximum Neighborhood Component, Density of Maximum Neighborhood Component, Maximal Clique Centrality) and six centralities (Bottleneck, EcCentricity, Closeness, Radiality, Betweenness and Stress) for identifying the shortest path. In this study, Cytohubba was used to identify the top 10 nodes of a regulatory network.

miRmap

miRmap is a web portal to rank the potential targets of miRNA with a biologically meaningful criterion to combine the thermodynamic, evolutionary, probabilistic and sequence-based features from Target Scan, PITA, PACMIT and miRanda [16]. It offers a user-friendly resource for browsing the precomputed target prediction of miRNA for model organisms. In this study, miRmap was used to identify the seed pairing between the miRNA and the mRNA (gene).

Enrichr

Enrichment analysis is a popular method for analyzing gene sets generated from experiments and Enrichr is a domain to perform analysis [17]. Enrichr including the ability to analyze genes on the principle of fuzzy logics and the improved level of application in programming interface is resulted as a cluster gram and it can be represented as a Table or a network. In this study, EnrichR was used to identify the specific pathway associated with genes of cutaneous lichen planus on the basis of statistical significance.

Methodology: Text and Network mining

- Identification of disease associated genes, microRNAs and transcription factors from databases.
- Identification of connectivity between them in nodes and edges through cytoscape.
- Identification of connectivity in hub genes through radiality functions in cytohubba.
- Identification of gene-miRNA seed pairing in miRmap
- Identification of associated pathways in Enrichr
- Identification of a probable regulatory network from the outcome of text and network mining.

Results and Discussion

Identification of Associated Genes

The top 10 genes associated with cutaneous lichen planus on the basis of DisGeNET score were obtained from the disease id C0023646 in DisGeNET and the details are given in Table.1

Table 1: Top 10 genes associated with lichen planus

Gene	Gene Name	Score	PMIDs
TNF	tumor necrosis factor	0.007	5
HLA-DRB1	Major histocompatibility complex, class II	0.005	1
BCL2	BCL2, apoptosis regulator	0.003	2
MMP9	matrix metallopeptidase 9	0.003	1
MMP2	matrix metallopeptidase 2	0.003	1
MMP3	matrix metallopeptidase 3	0.003	1
IDO1	indoleamine 2,3-dioxygenase 1	0.003	1
IL6	interleukin 6	< 0.001	2
CXCR3	C-X-C motif chemokine receptor 3	< 0.001	2
CXCL9	C-X-C motif chemokine ligand 9	< 0.001	2

Identification of Associated miRNAs and Transcription Factors

MicroRNAs (miRNAs) and Transcription factors (TFs) associated with the associated genes of cutaneous lichen planus were identified from miRTarBase and Regnetworks respectively and the details are given in Table.2

Construction of regulatory network

Regulatory network was constructed in cytoscape with the 6 genes (targets) 100 miRNAs and 84 TFs (regulators) to form 192 nodes and 292 edges.

Table.2 Associated genes, miRNAs and Transcription Factors

Genes	MicroRNAs(miRNAs)	Transcription Factors (TFs)
TNF	hsa-miR-19a-3p, hsa-miR-203a-3p, hsa-miR-187-3p, hsa-miR-130a-3p, hsa-miR-143-3p, hsa-miR-125b-5p, hsa-miR-24-3p, hsa-miR-34a-5p, hsa-miR-17-5p	AHR, ARNT, ATF1, ATF2 CEBPB, CEBPD, CREB1, EBF1, EGR1, EGR4, ELK1 ETS1, ETV4, FOS, IKBKB IRF5, JUN, NFAT5, NFATC1 NFATC2, NFATC3, NFATC4 NFE2L1, NFKB1, NFKB2 POU2F1, RELA, SMAD6 SMAD7, SP1, SP3, STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B, STAT6, TBP, TFAP2A and TP53
BCL2	hsa-miR-34b-5p, hsa-miR-21-5p, hsa-miR-204-5p, hsa-miR-153-3p, hsa-let-7a-5p, hsa-miR-15a-5p, hsa-miR-15b-5p hsa-miR-16-5p, hsa-miR-34a-5p, hsa-miR-20a-5p, hsa-miR-17-5p, hsa-miR-29c-3p, hsa-miR-29b-3p, hsa-miR-29a-3p hsa-miR-34b-3p, hsa-miR-181d-5p, hsa-miR-181c-5p hsa-miR-181b-5p, hsa-miR-181a-5p, hsa-miR-34c-5p hsa-miR-192-5p, hsa-miR-195-5p, hsa-miR-630, hsa-miR-451a, hsa-miR-365a-3p hsa-miR-125b-5p, hsa-miR-449a, hsa-miR-34c-5p, hsa-miR-200b-3p, hsa-miR-200c-3p, hsa-miR-429, hsa-miR-136-5p, hsa-miR-7-5p, hsa-miR-148a-3p, hsa-miR-24-2-5p, hsa-miR-182-5p, hsa-miR-143-3p, hsa-miR-375, hsa-miR-205-5p, hsa-miR-126-3p hsa-miR-18a-5p, hsa-miR-497-5p, hsa-miR-1915-3p hsa-miR-206, hsa-miR-34a-3p hsa-miR-448, hsa-miR-125a-5p, hsa-miR-708-5p, hsa-miR-184, hsa-miR-30b-5p, hsa-miR-135a-5p hsa-miR-224-5p, hsa-miR-503-5p, hsa-miR-494-3p, hsa-miR-211-5p, hsa-miR-9-5p hsa-miR-139-5p, hsa-miR-26a-1-3p, hsa-miR-1284, hsa-miR-376c-3p, hsa-miR-190b hsa-miR-15a-3p, hsa-miR-16-1-3p, hsa-miR-98-5p	AR, ATF1, BCLAF1, BRCA1 CEBPA, CREB1, CTCF, CUX1, DDIT3, EGR1, ETS1 GLI1, GLI2, MYB, MYBL1 MYC, NFKB1, NFKB2, NR4A1, PARP1, PML, PPARG, RARA, RARB, RARG, RELA, SF1, SP1, STAT3, STAT5A, TP53 and WT1
MMP9	hsa-miR-451a, hsa-miR-491-5p, hsa-miR-338-3p, hsa-miR-204-5p, hsa-miR-21-5p, hsa-miR-9-5p, hsa-miR-211-5p, hsa-let-7e-5p, hsa-miR-133b, hsa-miR-29b-3p, hsa-miR-9-3p, hsa-miR-524-5p, hsa-miR-302a-5p, hsa-miR-132-3p, hsa-miR-15b-5p, hsa-miR-942-3p, hsa-miR-203a-5p, hsa-miR-133a-5p, hsa-miR-143-3p	AR, BACH1, BACH2, ERG ETS1, ETS2, ETV4, FLI1, FOS, FOSB, FOSL1, JUN, JUNB, JUND, MYC, NFE2, NFE2L1, NFKB1, NFKB2, PPARA, PPARG, RELA, RELB, SMAD3, SP1 and SPI1
MMP2	hsa-miR-29b-3p, hsa-miR-451a, hsa-miR-338-3p, hsa-miR-21-5p, hsa-miR-17-5p, hsa-miR-29c-3p, hsa-miR-491-5p, hsa-miR-9-5p, hsa-miR-29a-3p, hsa-miR-452-5p, hsa-miR-708-5p, hsa-miR-767-5p, hsa-miR-106b-5p, hsa-miR-221-3p, hsa-miR-130b-3p, hsa-miR-125b-5p, hsa-miR-520g-3p, hsa-miR-524-5p, hsa-miR-302a-5p, hsa-miR-218-5p, hsa-miR-203a-5p, hsa-miR-143-3p and hsa-miR-519d-3p	ERG, ETS1, ETV4, FLI1, FOS, JUN, MYC, NR2F2, SP1, SPI1, TFAP2A and TP53
MMP3	hsa-miR-138-5p, hsa-miR-93-3p and hsa-miR-93-5p	BACH1, ERG, ETS1, ETS2 ETV4, FLI1, FOS, FOSB JUN, JUNB, JUND, NFKB1 RELA and TBP
IDO1	hsa-miR-153-3p	STAT1 and STAT2
IL6	hsa-let-7a-5p, hsa-miR-203a-3p, hsa-miR-142-3p, hsa-miR-26a-5p, hsa-miR-365a-3p, hsa-miR-98-5p, hsa-miR-107 hsa-let-7c-5p, hsa-miR-223-3p hsa-miR-149-5p, hsa-let-7f-5p hsa-miR-146b-5p, hsa-miR-9-5p, hsa-miR-146a-5p, hsa-miR-125a-3p, hsa-miR-106a-5p, hsa-miR-136-5p and hsa-miR-451a	AR, ATF1, CEBPA, CEBPB, CEBPD, CREB1, CTCF, EGR1, FOS, IRF1, IRF5, JUN, MYC, NFE2, NFIC, NFKB1, NFKB2, PBX1, PPARG, RARA, REL, RELA RREB1, STAT3, STAT5A TP53, USF1 and ZBTB16

Identification of a hub gene and regulators in network

A specific hub gene from a network is identified by the radiality function in cytohubba. Among the regulatory network of 6 genes and 184 targets, BCL2 was identified as a hub gene. The regulatory network of BCL2 is given in Figure.1

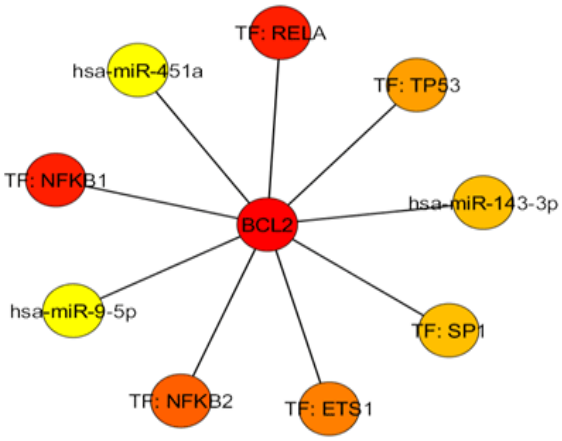

Figure 1: Regulatory network of BCL2

Identification of gene-miRNA seed pairing

Mimap was used to identify the seed pairing of BCL2 with hsa-miR-143-3p, has-miR-451a and hsa-miR-9-5p and it was identified that the mirmap score of BCL2 with hsa-miR-143-3p > BCL2 with has-miR-451a > BCL2 with hsa-miR-9-5p.

Identification of associated pathways

Associated genes of cutaneous lichen planus were subjected to enrichment analysis to identify the significance of pathways. The result of enrichment analysis is given in Figure.2 and it was identified that the AGE-RAGE signaling pathway is highly significant in the disease pathology of cutaneous lichen planus.

Identification of Probable Regulatory networks

In the identified network of hub, the nodes with red color are considered as highly essential nodes and the rest of the nodes were essential nodes and the seed pairing of hsa-miR-143-3p with BCL2 is highly compatible than the seed pairing of BCL2 with has-miR-451a and BCL2 with hsa-miR-9-5p and hence the most probable regulatory networks are

(i) Gene:BCL2, miRNA: has-miR-143-3p, TF: RELA and

(ii) Gene:BCL2, miRNA: has-miR-143-3p, TF: NFKB1.

Index	Name	P-value	Adjusted p-value	Z-score	Combined score
1	AGE-RAGE signaling pathway in diabetic complications_Homo sapiens_hsa04933	1.257e-7	0.000004788	-2.03	32.31
2	Rheumatoid arthritis_Homo sapiens_hsa05323	7.887e-8	0.000004788	-1.77	29.03
3	TNF signaling pathway_Homo sapiens_hsa04668	1.773e-7	0.000004788	-1.86	28.93
4	Hepatitis B_Homo sapiens_hsa05161	5.531e-7	0.000009464	-1.86	26.80
5	African trypanosomiasis_Homo sapiens_hsa05143	5.842e-7	0.000009464	-1.66	23.83
6	Tuberculosis_Homo sapiens_hsa05152	0.000001222	0.00001414	-1.66	22.58
7	Graft-versus-host disease_Homo sapiens_hsa05332	9.500e-7	0.00001283	-1.60	22.25
8	Inflammatory bowel disease (IBD)_Homo sapiens_hsa05321	0.000003868	0.00003917	-1.70	21.22
9	Cytokine-cytokine receptor interaction_Homo sapiens_hsa04060	0.000005943	0.00005349	-1.68	20.27
10	Pathways in cancer_Homo sapiens_hsa05200	0.00002921	0.0001820	-1.79	18.68

Figure 2: Pathways associated with cutaneous lichen planus

Conclusion

Pathophysiology of cutaneous lichen planus is poorly understood. This manuscript is an initiation to understand the pathophysiology of cutaneous lichen planus with respect to the principles of insilico methodologies in Bioinformatics and Systems Biology. In this manuscript an attempt was made to understand the pathophysiology of cutaneous lichen planus through the regulation of miRNAs with genes and transcription factors to lead to a pathway. The future work in this area involves the reconstruction of RAGE signaling pathway with BCL2, hsa-miR-143-3p, RELA and NFKB1 to identify the novel biomarkers to diagnose and treat the cutaneous lichen planus.

References

1. Parihar A, Sharma S and Bhattacharya SN . Clinicopathological study of cutaneous lichen planus. J Dermatol Dermatol Surg. 2015; 19(1):21-26.
2. Arora SK, Chhabra S, Uma SN, Sunil D and Minz RW. Lichen planus: a clinical and immune-histological analysis. Indian J Dermatol. 2014;59(3):257–261.
3. Lehman JS, Tollefson MM and Gibson LE. Lichen planus. Int. J. Dermatol. 2009;48(7):682–694. Doi: 10.1111/j.1365-4632.2009.04062.x.
4. Friedman RC, Farh KK, Burge CB and Bartel DP. Most mammalian mRNAs are conserved targets of miRNAs. Genome Res. 2009; 19(1):92–105. Doi: 10.1101/gr.082701.108.
5. Pasquinelli AE, Reinhart BJ, Slack F, Martindale MQ, Kuroda ML and Maller B. et al. Conservation of the sequence and temporal expression of let-7 heterochronic regulatory RNA. Nature 2000;408(6808):86-89.
6. Sonkoly E and Pivarcsi A. Advances in microRNAs: implications for immunity and inflammatory diseases. J Cell Mol Med. 2009; 13(1):24-38.
7. Danielsson K, Wahlin YB, Gu X, Boldrup L and Nylender K. Altered expression of miR-21, miR-125b, and miR-203 indicates a role for these microRNAs in oral lichen planus. J Oral Pathol Med. 2012;41(1):90-95.
8. Sonkoly E, Wei T, Janson PCJ, S€aaf A, Lundeburg L and Tengvall LM. et al. MicroRNAs: novel regulators involved in the pathogenesis of psoriasis. PLoS ONE. 2007;2:e610. Doi:10.1371/journal.pone.0000610
9. Zhao X, Tang Y, Qu B, Cui H, Wang S and Wang L. et al. MicroRNA-125a contributes to elevated inflammatory chemokine RANTES levels via targeting KLF13 in systemic lupus erythematosus. Arthritis Rheum. 2010;62(11):3425-3435. Doi: 10.1002/art.27632.
10. El-Rifaie AA, Rashed LA, Doss RW and Osman ST. MicroRNAs in cutaneous lichen planus. Clinical and Experimental Dermatology. 2017;1:4.
11. Janet P, Àlex B, Núria QR, Alba GS, Jordi DP and Centeno E. et al. DisGeNET: a comprehensive platform integrating information on human disease-associated genes and variants. Nucleic Acids Research. 2017;45(D1):D833-D839. Doi: 10.1093/nar/gkw943.
12. Chou CH, Shrestha S, Yang CD, Chang NW, Lin YL and Liao KW et al miRTarBase update 2018: a resource for experimentally validated microRNA-target interactions. Nucleic Acids Res. 2018;46(D1):D296-D302. Doi: 10.1093/nar/gkx1067.
13. Zhi-Ping L, Canglin W, Hongyu M, Hulin W. RegNetwork: an integrated database of transcriptional and post-transcriptional regulatory networks in human and mouse. Database (Oxford). 2015;bav095:1-12. Doi: 10.1093/database/bav095.
14. Paul S, Andrew M, Owen O, Nitin SB, Jonathan TW and Ramage D. et al. Cytoscape: A Software Environment for Integrated Models of Biomolecular Interaction Networks. Genome Res. 2003;13(11):2498-2504.
15. Chia-Hao C, Shu-Hwa C, Hsin-Hung Wu, Chin-Wen Ho, Ming-Tat K and Chung-Yen L. cytoHubba: identifying hub objects and subnetworks from complex interactome. BMC Systems Biology. 2014;8(Suppl. 4):S11. Doi: 10.1186/1752-0509-8-S4-S11.
16. Charles EV, Matthias B and Evgeny MZ. miRmap web: comprehensive microRNA target prediction online. Nucleic Acids Res. 2013;41:W165–W168. Doi: 10.1093/nar/gkt430.
17. Maxim VK, Matthew RJ, Andrew DR, Nicolas FF, Qiaonan D. Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. Nucleic Acids Res. 2016;44(W1):W90-97. Doi: 10.1093/nar/gkw377.