Article

Identification of potential microRNAs-mediated from sialic acid to MMP-9 pathway through integrative analysis

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Abstract
Sialic acids and MMPs play critical roles in inflammatory diseases. Furthermore, Interaction between Sialic acid and receptors such as siglecs leads phosphorylation of ITIM domains and promote downstream inhibitory signaling through SHP-1 phosphatases. SHP1 could positively regulate TNF-α and by control, the production of TNF-α could play a crucial role in inflammation. Besides, TNF-α could mediate the signaling pathway leading to MMP-9 gene expression. MMP-9 also is recognized as therapeutic targets in a variety of diseases including vascular pathologies, cancers, and auto-immuned diseases. The present *in-silico* study aims to identify the most potent micro-RNAs could control the signaling pathway from siglec to MMP-9. To this end, with review some articles and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis21 genes involved in this pathway have been selected. Then TARGET SCAN, DIANA-TarBase8, and miRDB database were utilized to predict the miRNAs which have the most effective to target genes. Finally, using bio-studying Software Cytoscape, three microRNA-mRNA networks were constructed for existing banks. We found shared micro-RNAs that in the three networks. Eventually, using miRTarBase database microRNAs that were linked to more genes in this path were assigned a higher privilege. The twenty-one selected micro-RNAs could be the proper options for experimental studies from sialic acid receptors (siglecs) to MMP-9. Among them miR-34a-5p could be the most interesting target.

Keywords miRNA-mRNA network; Cytoscape;DIANA-TarBase8; TARGET SCAN; miRDB; miRTarBase.

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1 Introduction

Sialic acids are nine-carbon sugar, which could be attached to the end of glycan chains in animals and could mediate a variety of pathological and physiological processes. The evidence also suggested that MMPs and sialic acids play key roles in inflammatory neurodegenerative diseases. Interaction between sialic acid and their receptors such as siglecs plays an important role in inflammation and neurodegenerative diseases. This leads to phosphorylation of immune-receptor tyrosine-based inhibitory motif (ITIM) and siglec signaling through the recruitment of SHP-1 phosphates (Bochner et al., 2015).

SHP1 could positively regulate TNF-α and by control, the production of TNF-α could play a crucial role in inflammation (Xie et al., 2000). TNF-α, a multifunctional cytokine implicated in the signaling pathways which leading to MMP-9 expression. Involvement of glycan such as sialic acid in the signaling pathways that provoke MMP-9 gene expression in glial cells is not clear. Therefore, this study tries to shed some light on the relationship between sialic acid and MMP-9 and the effects of this ligand on the signaling processes of the neurodegeneration and inflammatory demyelination (Cho et al., 2012).

MMP-9 is one of the members of metalloproteinase family moreover, hasvarious biological functions due to its tissue-damaging role. MMP-9 also is a prototypical target in inflammatory diseases. MMP-9 is also a recognized therapeutic target in a variety of diseases like vascular pathologies, autoimmune diseases, and cancer (Rosenblum et al., 2007).

MiRNAs could have multiple targets that each protein-coding genecould be targeted by multiple miRNAs leading complex regulatory network. Identifying miRNA-target interaction network is a challenging task. Therefore, construction of networks facilitates modeling of biological systems. Since miRNAs play important roles in many biological processes and pathways, it is crucial to have tools that can integrate miRNA-related data into networks (Akhtar et al., 2015).

These network data extend and complement a great deal of other information available in the biomedical sciences. Cytoscape is a popular bioinformatics package for biological network visualization and data integration (Smoot et al., 2010). Cytoscape software was used to visualize the regulatory co-expression network by using these co-expression relationships and potential miRNA-target pairs (Wang 2016). miRDB is an online database for prediction and functional annotations of miRNA target. All the targets in miRDB were predicted by a bioinformatics tool, MirTarget, which was developed by analyzing thousands of miRNA-target interactions from high-throughput sequencing experiments (Wong and Wang, 2014). DIANA-TarBase8 (Karagkouni et al. 2017) is a manually curated target database. The latest version (v8.0) contains more than one million miRNA-target interactions (MTIs) entries, curated from published experiments performed with 356 different cell types from 24 species. DIANA-TarBase8 is the most frequently updated and is associated with the latest miRBase version (v21), which offers High-Confidence miRNA sets (Smoot et al., 2010).

2 Materials and Methods

This bioinformatics study was accomplished during three stages (Fig. 1). In this study, signaling pathway from sialic acid to MMP-9 has been drawn based on review articles and information from KEGG. Then, 21 genes involved in this pathway have been indicated. In the next step, DIANA-TarBase8 (http://www.microrna.gr/tarbase), TARGET SCAN (http://www.targetscan.org/vert_72/) and miRDB database (http://mirdb.org) have been used to predict the miRNAs which can target these genes and find the microRNAs those effects in all components in this pathway.

Then with using bio-studying software such as Cytoscape_v3.6.1, three microRNA-mRNA networks have been created for existing banks and with statistical studies conducted on 2384 micro-RNAs that potentially intervened in this pathway (Fig. 2-3). After integrating obtained data, using advance network merge 49 iRNAs have selected. Finally, these selected microRNAs have surveyed by miRTarBase database (http://mirtarbase.mbc.nctu.edu.tw/php/index.php) for finding the experimentally validated microRNAs link to genes in the pathway (Chou et al., 2017).

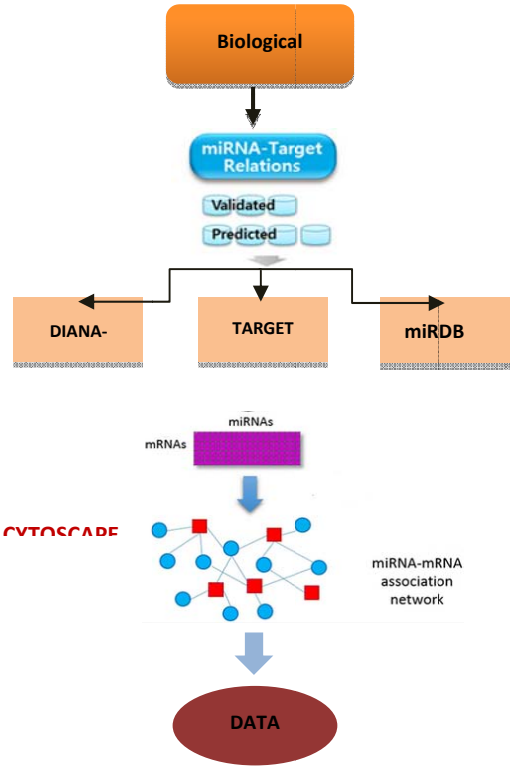


Fig. 1 Schematic overview of the path of the process. DIANA-TarBase8, TARGET SCAN, and miRDB database have used to predict the miRNAs and then in the next step with the use of Cytoscape Software, three microRNA-mRNA networks have created.

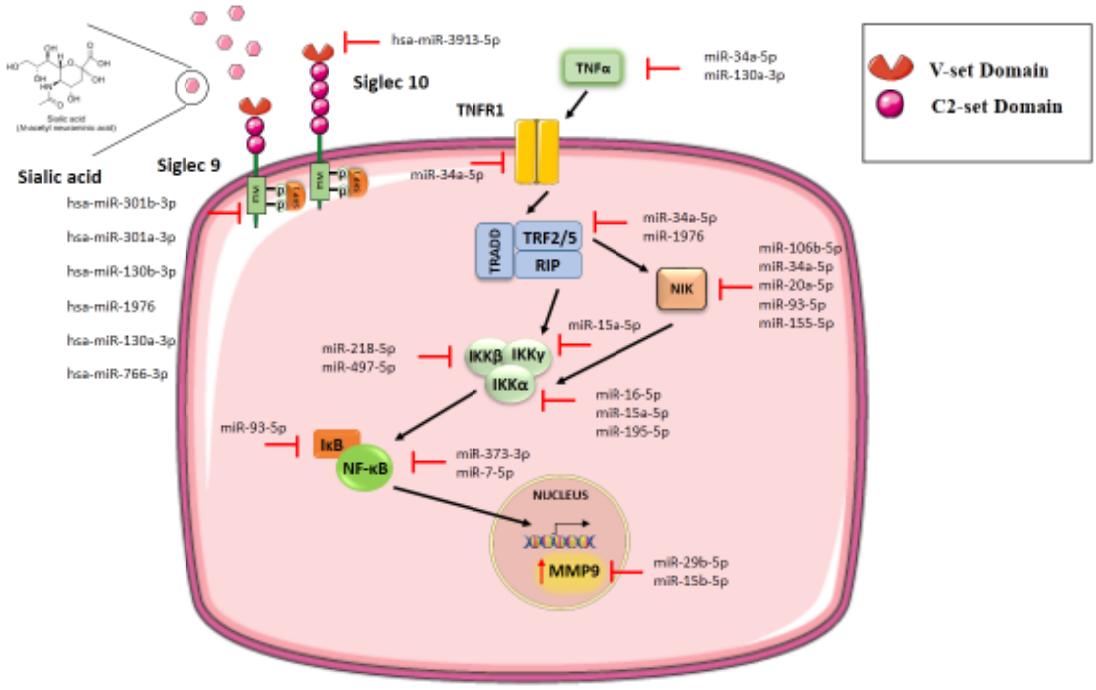
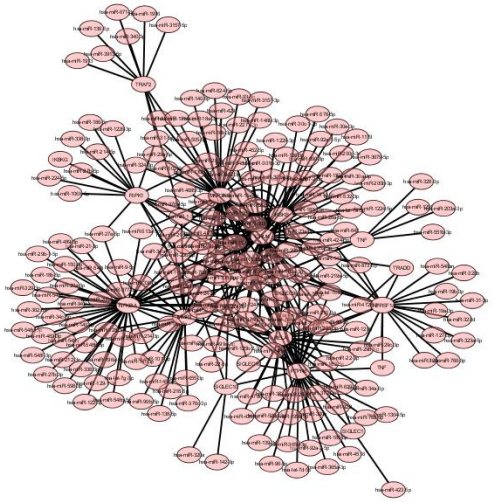


Fig. 2 An illustration representing microRNAs (miRNAs) and their targets involved in siglec to MMP-9 pathway. The depicted miRNAs are potential miRNAs in this signaling pathway.

a.



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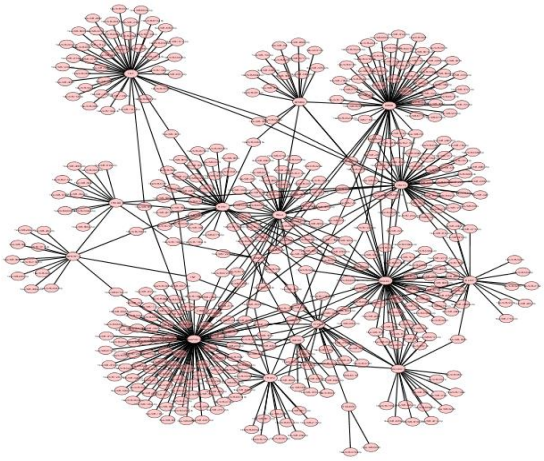

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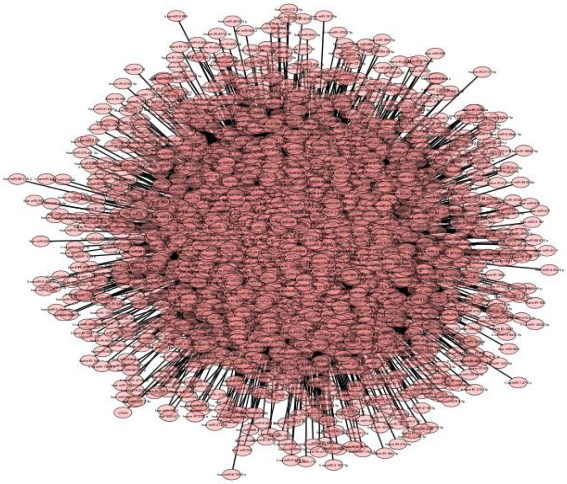

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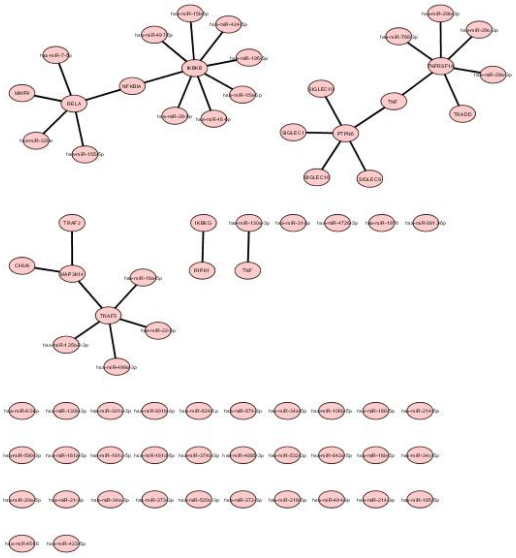

Fig. 3 (a) View of a Cytoscape network from DIANA. (b) View of a Cytoscape network from MIRDB. (c) Force based view on a very large network. View of a Cytoscape network from TARGET SCAN. (d) View of a Cytoscape network using advanced network merge.

3 Results

Validation of a possible miRNA target in the laboratory is time-consuming and expensive since each miRNA has a large number of potential target sites, computational approaches could help reduce their number for experimental validation (Smoot, Ono et al. 2010).The goal of this study is to identify miRNAs involved in pathway from sialic acid to MMP-9. We found forty-nine micro-RNAs(miR-7-5p,miR-15a-5p,miR-15b-5p, miR-16-5p, miR-18a-5p,miR-20a-5p, miR-21-3p, miR-22-5p,miR-28-5p,miR-29a-3p,miR-29b-3p,miR-29c-3p, miR-31-3p,miR-34a-3p,miR-34a-5p,miR-93-5p, miR-106b-5p,miR-125b-2-3p, miR-130a-3p,miR-130b-3p,miR-155-5p,miR-181a-5p,miR-181c-5p,miR-181d-5p,miR-195-5p,miR-218-5p, miR-301a-3p, miR-301b-3p,miR-320e,miR-372-3p,miR-373-3p,miR-374b-3p,miR-423-5p,miR-424-5p,miR-494-3p,miR-497-5p, miR-499a-3p,miR-520c-3p,miR-532-3p,miR-590-3p,miR-624-5p,miR-642a-5p,miR-766-3p,miR-874-3p,miR-4516, miR-1976,miR-3913-5p,miR-4685-3p,miR-4726-3p) were shared in the three networks.

In the next step, these 49 selected microRNAs have surveyed by miRTarBase database for finding the experimentally validated microRNAs linked to genes with role in the pathway. Then21 miRNAs were selected (Table 1).

Finally, microRNAs that were related to more genes in this path have been assigned a higher privilege; furthermore, the selected micro-RNAs could be potential proper options for experimental studies on the path from siglec receptors to MMP-9.

Table 1 MiRTarBase the experimentally validated microRNA-target interaction database found microRNAs finding from Cytoscape that links to genes in pathway.

Human gene name	Sequence accession ID	Cell Type/Tissue	miRNAs	References
NIK (MAP3K14)	ENSG00000006062	B cells endothelial cells B cells B cells B cells	miR-106b-5p miR-34a-5p miR-20a-5p miR-93-5p mir-155-5p	(Riley et al. 2012) (Wang et al. 2015) (Riley et al. 2012) (Riley et al. 2012) (Xu et al. 2010; Riley et al. 2012)
IKBKB	ENSG00000104365	glioma cells Prostate cancer cell	miR-218-5p miR-497-5p	(Song et al. 2010) (Kong et al. 2015)
TNFα	ENSG00000232810	hepatocellular carcinoma cervical cancer cells	miR-34a-5p miR-130a-3p	(Yacoub et al. 2016) (Zhang et al. 2014)
TNFαR1 (TNFRSF1A)	ENSG00000067182	hepatocellular carcinoma	miR-34a-5p	(Yacoub et al. 2016)
TRAF2	ENSG00000127191	hepatocellular carcinoma -	miR-34a-5p miR-1976	(Yacoub et al. 2016) (Chi et al. 2009)
NFkB (RELA)	ENSG00000173039	- -	miR-373-3p miR-7-5p	(Lim et al. 2005) (Grimson et al. 2007; Webster, Giles et al. 2009; Hafner et al. 2010; Choi et al. 2014; Chaudhuri et al. 2015; Zhao et al. 2015)
SIGLEC9	ENSG00000129450	Prostate cancer cell Prostate cancer cell Prostate cancer cell brain Prostate cancer cell -	miR-301b-3p miR-301a-3p miR-130b-3p miR-1976 miR-130a-3p miR-766-3p	(Hamilton et al. 2016) (Hamilton et al. 2016) (Hamilton et al. 2016) (Karginov et al. 2013, Xue et al. 2013, Hamilton et al. 2016) (Hamilton et al. 2016) (Karginov et al. 2013)
IKBα (NFKBIA)	ENSG00000100906	peripheral blood nuclear cells	miR-93-5p	(Dang et al. 2017)
SIGLEC10	ENSG00000142512	-	miR-3913-5p	(Karginov et al. 2013)
MMP9	ENSG00000100985	breast cancer cell glioma cells	miR-29b-3p miR-15b-5p	(Chou et al. 2013) (Xiao et al. 2015)
IKKα (CHUK)	ENSG00000213341	Macrophage cells Macrophage cells hepatocellular carcinoma	miR-16-5p miR-15a-5p miR-195-5p	(Li et al. 2010) (Li et al. 2010) (Ding et al. 2013)
IKBKG	ENSG00000269335	-	miR-15a-5p	(Helwak et al. 2013)

4 Conclusions

MicroRNAs could have multiple targets and each protein-coding gene could be targeted by multiple miRNAs result in a complex regulatory network. Hence, achieving biological network would be a step towards determination of the relationship between pathways and the ability to identify intermediate miRNAs (Brown et al., 2019). The investigation of the biological importance of the miRNA-target interaction network is a challenging task. Furthermore, construction of networks also enables modeling of complex biological systems.

Since miRNAs play a key role in many processes and pathways, it is crucial to have tools that can integrate miRNA-related data into networks(Smoot et al., 2010; Mensi et al., 2019).

Pathway models have proven themselves as powerful tools for biologists to describe and analyze the biological processes. Identification of miRNAs targets is critical for characterization of their functions (Russo et al., 2019). This model could be helpful to clinical and laboratory research to target factors play main role in disease development without spending too much time.

In a nutshell, our study demonstrates 21 microRNAs (miR-7-5p, miR-15a-5p, miR-15b-5p, miR-16-5p, miR-20a-5p, miR-29b-3p, miR-34a-5p, miR-93-5p, miR-106b-5p, miR-130a-3p, miR-130b-3p, miR-155-5p, miR-195-5p, miR-218-5p, miR-301a-3p, miR-301b-3p, miR-373-3p, miR-497-5p, miR-766-3p, miR-1976, miR-3913-5p) that could be more effective in the sialic acid to MMP-9 pathway (Fig. 2).

MiR-34a-5p could be potentially a significant candidate for the future experimental validation among these miRNAs. This miR could affect more targets in the studied pathway; therefore it is privilege for future inflammatory related studies. Moreover, more experimental studies need to indicate validation of these results.

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