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Original Research Article

MicroRNA and gene signature of severe cutaneous drug hypersensitivity reactions reveal the role of miR-483-5p/miR-28-5p in inflammation by targeting Granulysin gene

Abdallah Ahmed Elbakkoush[1], Anas Khaleel[2] and Chien-Tsai Liu[1*]
[1]Graduate Institute of Biomedical Informatics, College of Medical Science and Technology, Taipei Medical University, Taipei 11031, Taiwan. [2]Institute of Biological Chemistry, Academia Sinica, 128 Academia Road, Section 2, Nankang District, Taipei 11529, Taiwan

For correspondence: **Email: ctliu@tmu.edu.tw*

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Abstract

Purpose: *To build a microRNA and gene signature of severe cutaneous adverse drug reactions (SCAR), including Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN).*
Methods: *MicroRNA expression profiles were downloaded from miRNA expression profile of patients' skin suffering from TEN using an array comprising of 372 miRNAs; download site: www.jacionline.org. The patient samples were eight TEN, ten SJS patients and twenty-two healthy individuals. A total of 192 microRNAs were found with unique expression patterns (overexpressed) in contrast with healthy skin controls and patients. Thereafter, the following databases were used for downstream analysis: geneMANIA, DIANA-miRPath version 3, DIANA-TarBase version 7.0, Ingenuity Pathway Analysis (IPA) as well as DAVID, STRING and GENECODIS online tools.*
Results: *Granulysin (GNLY) geneMANIA database search yielded 21 interacting genes that were 64.6 % in physical interaction, 17 % in co-expression pattern. miRBD potential microRNAs that target the 21 genes were 79 miRs. Eighteen miRs overlap between the overexpressed miRs from SJS/TEN samples and the miRs targeting the 21 genes. Moreover, Ingenuity pathway analysis IPA revealed that the microRNAs were involved in inflammation.*
Conclusion: *Analysis of differential microRNA expressions reveals two significant DE miRs that target Granulysin (483-5p/miR-28-5p). MiR-GNLY loop interactions in hypersensitivity reactions may function as biomarkers for SCAR including SJS and TEN.*

Keywords: *Severe cutaneous adverse drug reactions (SCAR) Steven-Johnson Syndrome, Toxic epidermal necrolysis, Granulysin, Biomarkers, MicroRNA signature*

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INTRODUCTION

The clinical appearances of Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN range from mild severe cutaneous adverse drug reactions (SCAR), both are rare but seldom fatal. Yet, morbidity and mortality is in rapid increase worldwide [1]. The pathogenesis of SCAR is a compound and multifactorial. It is known that drug antigen interactions, genetic background, and environmental factors might be involved [2]. SCAR are among the most significant serious skin conditions amid cutaneous drug-provoked hypersensitivity adverse conditions [3]. SJS and TEN rely on immune mediators in their hypersensitivity reactions that involve the cutaneous tissues and layers [4]. Mortality still occurs in over 5 % of

hypersensitivity adverse patients with SJS and greater than 30 % of the same patients [5]. Nevertheless, the mechanisms of SJS and TEN are not fully elucidated. MicroRNAs or miRs are single stranded RNAs that are capable of posttranscriptional gene regulation via targeting their Mrna [6]. MicroRNAs are very important regulators in many human diseases, for instance, cancer [7]. Here, microRNAs are imposed as new regulators in adverse cutaneous reactions [8]. T-cell lymphocyte association with SJS and TEN was recently observed to be linked with skin reactions, in addition to the recently reported Granulysin and natural killer cells inhibitory receptors [9]. Currently, Granulysin acts as the substantial player and responsible for the dispersed keratinocyte death [10] and it has a role in cytotoxic T lymphocyte conditions [11].

In addition to Granulysin, multiple reports have revealed that numerous cytokines contributing an essential role in the apoptosis (programmed cell death) and/or the cytokine inflammations. In other reports levels of SJS and TEN cytokines were considerably greater in patient serum than healthy individuals' [12].

In the current study, Granulysin is deemed to establish core regulator and mediator along with his related/co-expressed genes and microRNAs for SJS and TEN pathogenesis. The databases and software used in this work not only attain information for DEG miRs in severe cutaneous SJS and TEN reactions, but also facilitate the approach towards discovering substantial roles in the pathogenesis of SJS and TEN.

METHODS

Database and topological investigations

MicroRNAs array: differentially expressed in SCAR

MicroRNA expression profile were downloaded from the inclusive miRNA expression profile of patients skin suffering from TEN using an array comprising of 372 miRNAs; Ichihara *et al* article's Online Repository at www.jacionline.org [13]. The samples were achieved from eight TEN patients, ten SJS patients with and 22 healthy volunteers. A total of 192 microRNAs were found with distinctive expressions patterns (overexpressed) in contrast between healthy skin controls and patients. Then we upload the overexpressed miRs to DIANA-miRPath v3. to obtain the significant gene list and pathways [14].

Granulsyin core gene

We loaded Granulsyin gene (*GNLY*) to geneMANIA database [15]; this search was important to reveal the intern actions between this core gene in SJS and TEN with other genes and microRNAs. This search yielded 21 genes that most interacting with *GNLY.*

MicroRNAs targeting Granulsyin gene

Characterization of the *GNLY* expression profiles using, DIANA-TarBase v7.0 to find the potential microRNAs targeting these genes [16].

Functional annotation by DAVID web tool

DAVID stands for: Database for Annotation, Visualization and Integrated Discovery. DAVID tool was used to annotate/confirm the function of the genes list related to Granulysin. Selection of Gene Ontology GO standings with adjusted *p*-value less than 0.05.

Network and Pathway analysis by Ingenuity Pathway Analysis IPA

The twenty-one genes related to Granulysin were then investigated and characterized in KEGG (Kyoto Encyclopedia of Genes and Genomes) [17], Ingenuity Pathway Analysis (IPA) tools by the GENECODIS software [18]. REACTOME, is a tools for the visualization, interpretation and analysis of pathway knowledge to support basic research, genome analysis, modeling, systems biology and education [19].

RESULTS

1-DIANA-miRPath 70 pathway results for the microRNA

This search yielded more than 70 significant pathways; we chose the top ten according to p Value result and number of genes targeted by the miR list loaded to DIANA-miRPath tool. All summarized in Table 1.

2-GeneMANIA database results for Granulsyin gene

Granulsyin gene (*GNLY*)/geneMANIA database [15], search yielded 21 genes that most interacting with *GNLY.*

3-MicroRNAs is targeting Granulsyin gene in prediction database

DIANA-TarBase v7.0 to confirm the microRNAs targeting these genes [16]. This search yielded around 20 miRs that target GNLY gene. Using the original database DEG miRs (192 miRs).

Two miRs were overexpressed in SJS and TEN were among the newly identified from DIANA-TarBase Search. They are miR-483-5p/miR-28-5p. This confirm the role of both microRNAs-and their target granulysin protein.

MicroRNAs targeting 21 Granulsyin-related genes

In order to further characterize the 21 Granulsyin co-expressed genes, all genes were applied to DIANA-TarBase to find the potential microRNA targeting them.

The miRs found to be potential regulators are shown in Table 2.

After loading the whole gene list of 21 gene names. More accurate list with more genes and microRNA were acquired from MirDB database (The target prediction database, miRDB) [20].

Table 1: DIANA-miRPath pathway results for the microRNA list

KEGG pathway	*P*-value	#Genes	#miRNAs
Proteoglycans in cancer	4.34E-12	164	88
Mucin type O-Glycan biosynthesis	3.25E-11	26	51
Morphine addiction	1.90E-09	76	83
Pathways in cancer	7.86E-08	306	89
Axon guidance	1.01E-07	107	85
ErbB signaling pathway	3.16E-07	77	81
ECM-receptor interaction	5.84E-07	64	77
Rap1 signaling pathway	8.89E-07	171	87
N-Glycan biosynthesis	6.03E-06	38	62

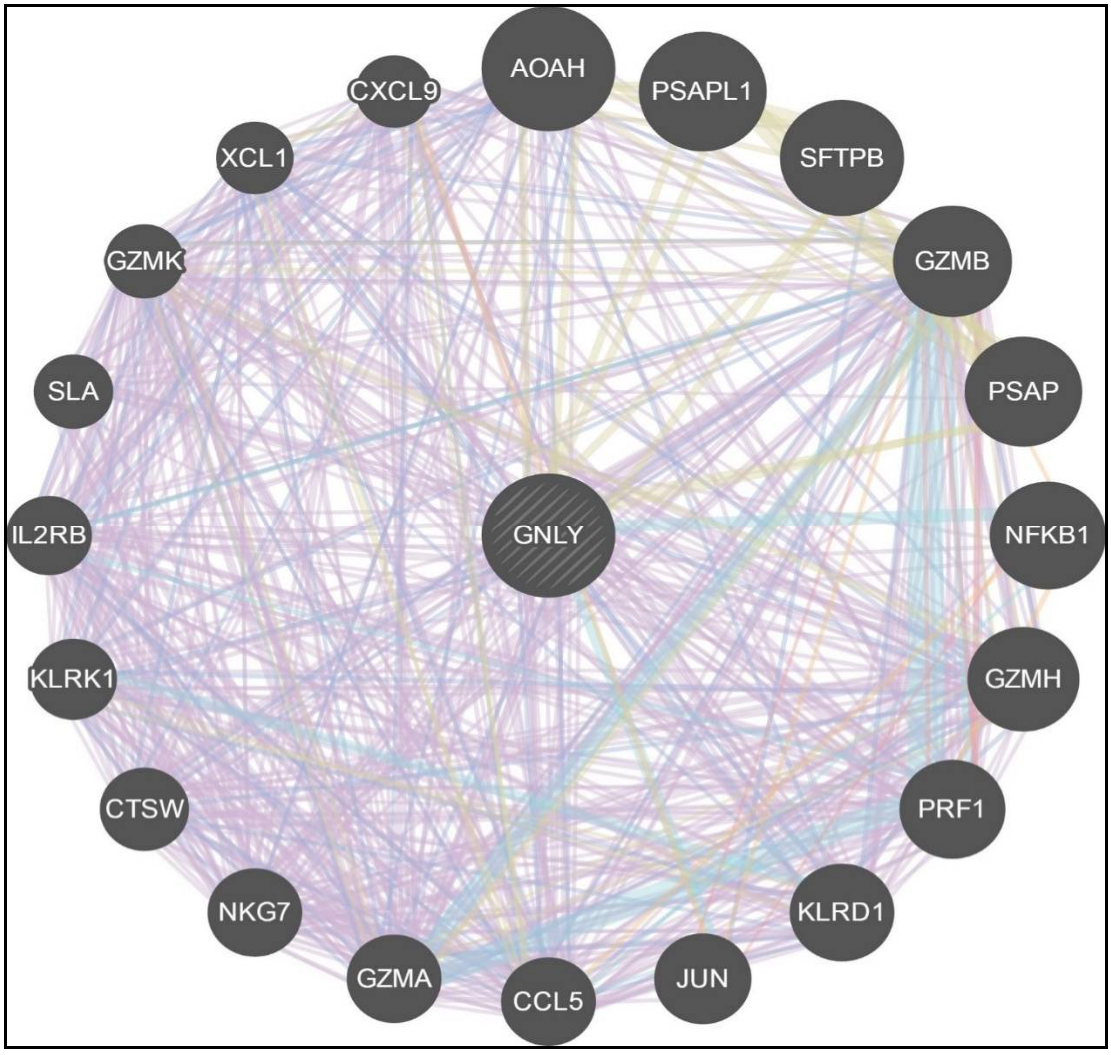

Figure 1: The twenty-one genes that interacted most with *GNLY*

Table 2: DIANA-TarBase for the 21 genes interacting with Granulysin gene/*GNLY*

Gene	miR	Score	Method
NFKB1	hsa-miR-9-5p	0.967	Immunoprecipitation (IP)
	hsa-miR-625-5p	0.954	
IL2RB (hsa)	hsa-miR-335-3p	0.908	Immunoprecipitation (IP)
SLA (hsa)	hsa-miR-155-5p	0.907	Immunoprecipitation (IP)
IL2RB	hsa-miR-424-5p	0.866	Immunoprecipitation (IP)
	hsa-miR-629-5p	0.861	
	hsa-miR-497-5p	0.825	
	hsa-miR-34a-5p	0.738	
	hsa-miR-449a	0.726	
	hsa-miR-186-3p	0.698	
	hsa-miR-449b-5p	0.672	
	hsa-miR-195-5p	0.636	
	hsa-miR-16-5p	0.631	
	hsa-miR-532-5p	0.628	
SLA	hsa-miR-23b-3p	0.621	Immunoprecipitation (IP)
	hsa-miR-23a-3p	0.619	
	hsa-miR-23c	0.618	
AOAH	hsa-miR-199a-5p	-	-
	hsa-miR-199b-5p	-	
CTSW	hsa-miR-335-5p	-	-
	hsa-miR-129-2-3p	-	
	hsa-miR-335-5p	-	
PSAP	hsa-miR-454-3p	0.996	Immunoprecipitation (IP)
	hsa-miR-301a-3p	0.980	
	hsa-miR-301b	0.975	
	hsa-miR-130a-3p	0.973	
	hsa-miR-130b-3p	0.967	
	hsa-miR-3127-5p	0.821	
	hsa-miR-19b-3p	0.790	
	hsa-miR-296-3p	0.774	
	hsa-miR-19a-3p	0.769	
	hsa-miR-1226-3p	0.739	
JUN	hsa-miR-200b-3p	0.988	Immunoprecipitation (IP)
	hsa-miR-1277-5p	0.967	
GZMK	hsa-miR-335-5p	-	-
SFTPB	hsa-miR-199a-5p	-	-
CCL5	hsa-miR-125a-5p	-	-

We scrutinized our search for microRNAs that are predicted to target the 21 genes and the overexpressed 193 miRs and found 18 miRs overlapping, summarized in Table 3.

DAVID Functional annotation results

The DAVID tool [21], yielded six important cluster enrichments score were as follows 5.26, 3.14, 3.04, 2.85, 1.87 and 0.06.

Most related to SJS/TEN are Chemokine signaling pathway, inflammatory response and Natural killer cell mediated cytotoxicity.

Network/pathway analysis by ingenuity pathway analysis

IPA for 21 genes related to Granulysin were then investigated and characterized in KEGG (Kyoto Encyclopedia of Genes and Genomes) [17], Ingenuity Pathway Analysis (IPA) tools by the GENECODIS software [18]. REACTOME, is a tools for the visualization, interpretation and analysis of pathway knowledge to support basic research, genome analysis, modeling, systems biology and education [19].

IPA results for Granulysin and the important 21 genes are shown in Figure 2.

Table 3: The 18 microRNAs validated by PCR to be overexpressed in SJS/TEN and targeting granulysin-related genes

Ranking	Target mark	miRNA Tag	Gene	Gene full name
5	97	miR-301b-3p	PSAP	prosaposin
7	96	miR-19b-3p	PSAP	prosaposin
8	95	miR-92a-1-5p	CXCL9	chemokine (C-X-C motif) ligand 9
12	94	miR-200c-3p	JUN	jun proto-oncogene
18	88	miR-661	KLRD1	killer cell lectin-like receptor subfamily D, member 1
27	80	miR-34a-5p	IL2RB	interleukin 2 receptor, beta
28	80	miR-449a	IL2RB	interleukin 2 receptor, beta
29	80	miR-34c-5p	IL2RB	interleukin 2 receptor, beta
43	74	miR-411-5p	KLRD1	killer cell lectin-like receptor subfamily D, member 1
55	66	miR-125b-5p	PSAPL1	prosaposin-like 1 (gene/pseudogene)
62	65	miR-342-5p	PSAPL1	prosaposin-like 1 (gene/pseudogene)
63	64	miR-145-3p	GZMK	granzyme K (granzyme 3; tryptase II)
65	63	miR-454-3p	GZMK	granzyme K (granzyme 3; tryptase II)
69	63	miR-301a-3p	GZMK	granzyme K (granzyme 3; tryptase II)
70	62	miR-92b-3p	AOAH	acyloxyacyl hydrolase (neutrophil)
72	62	miR-491-5p	SFTPB	surfactant protein B
24	83	miR-185-5p	CXCL9	chemokine (C-X-C motif) ligand 9
10	94	miR-429	JUN	jun proto-oncogene

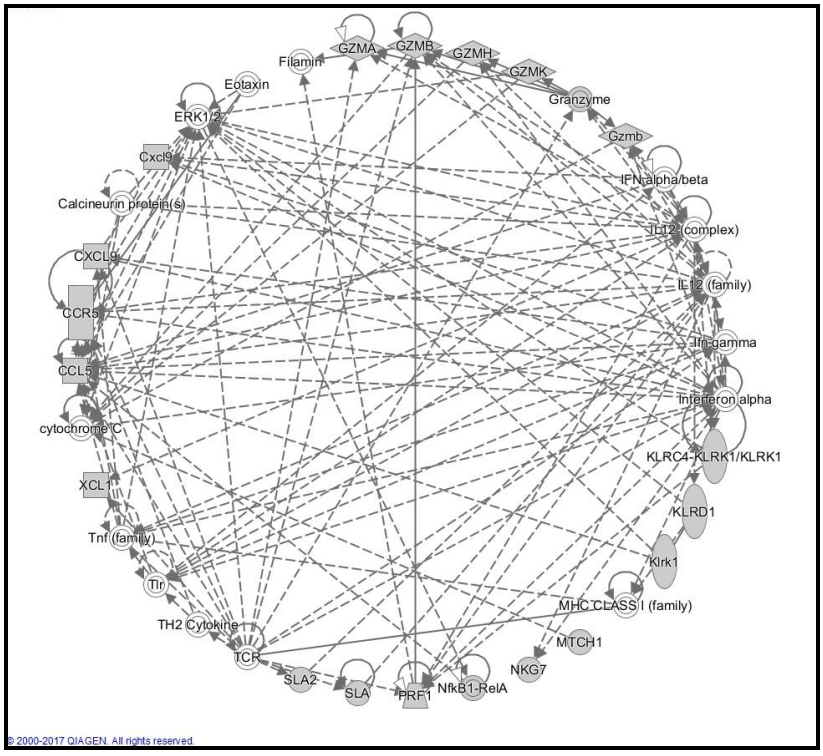

Figure 2: The confirmed 21 genes interacting with *GNLY* in IPA database

In this report, several databases and web tools analyses were approached to pinpoint pathways/ network and microRNAs targeting important proteins i.e. Granulysin contributing to pathogenesis of SJS/TEN. Predominantly, Granulysin were validated to be involved in SJS and TEN before. However, never were studied from microRNA perspective. Moreover, here we prove the potential microRNA regulators of Granulysin; miR-483-5p/miR-28-5p.

REACTOME pathways [19] results for the 21 gene clusters related to Granulysin indicated that the main pathways were associated with the following:

1-Toll-like Receptor 1/2 TLR1:TLR2 Cascade
2-Toll-like Receptor 2 (TLR2) Cascade
3-Chemokine receptors bind chemokines
4-Toll-Like Receptor 4 (TLR4) Cascade
5-Toll-Like Receptors Cascades.

DAVID tool [21] results were agreeing with the other pathways analyses; IPA.

DAVID results for the 21 gene were as following:

1- Chemokine signaling pathway
2- inflammatory response i.e. SJS/TEN
3- and Natural killer cell mediated cytotoxicity

These data were generated through the use of Ingenuity Pathways Analysis, a web-delivered application that enables biologists to discover, visualize and explore therapeutically relevant networks significant to their experimental results, such as gene expression array data sets. For a detailed description of Ingenuity Pathways Analysis, visit www.Ingenuity.com.

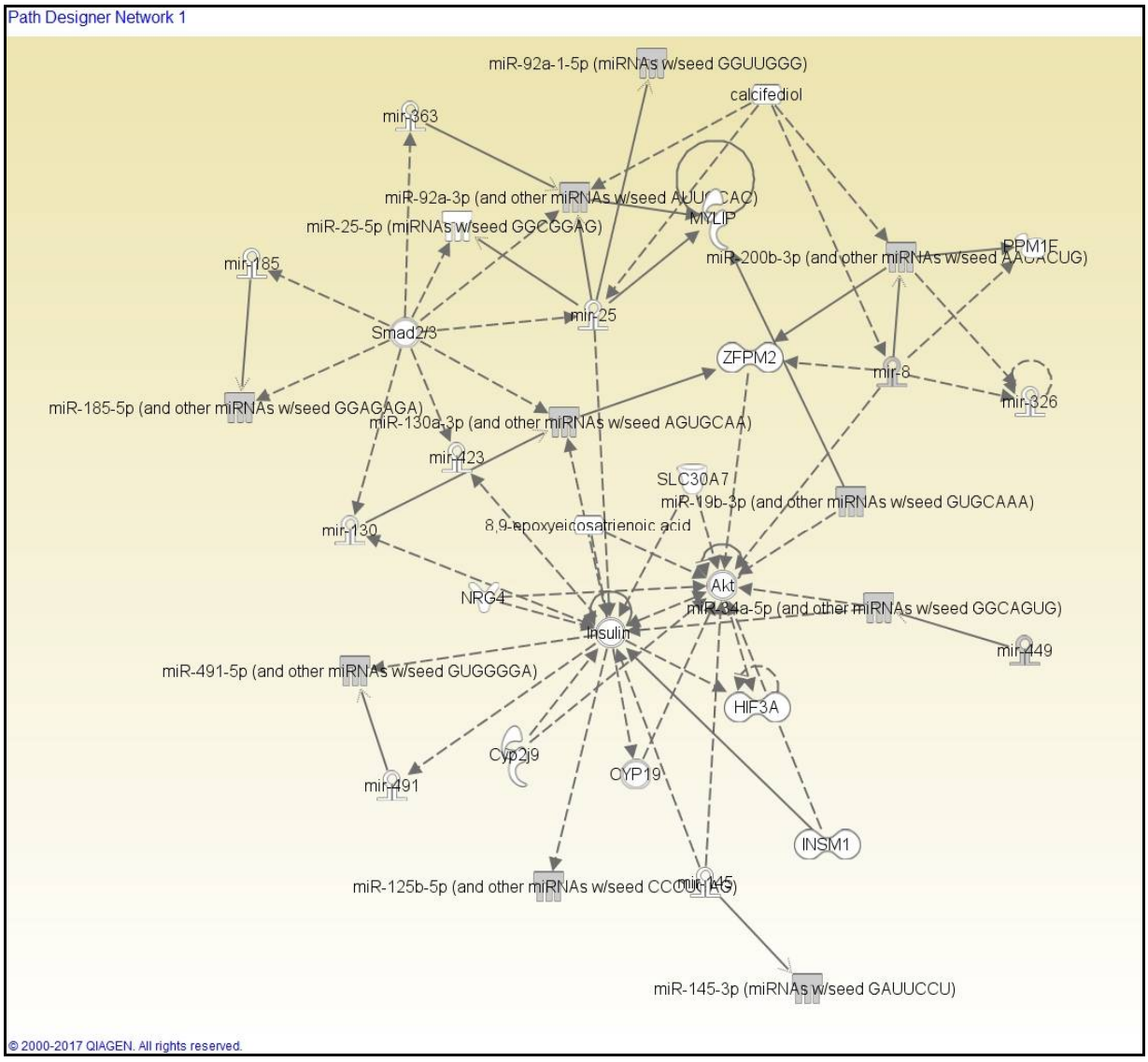

Figure 3: Genes targeted by the 18 miRs analyzed by IPA database

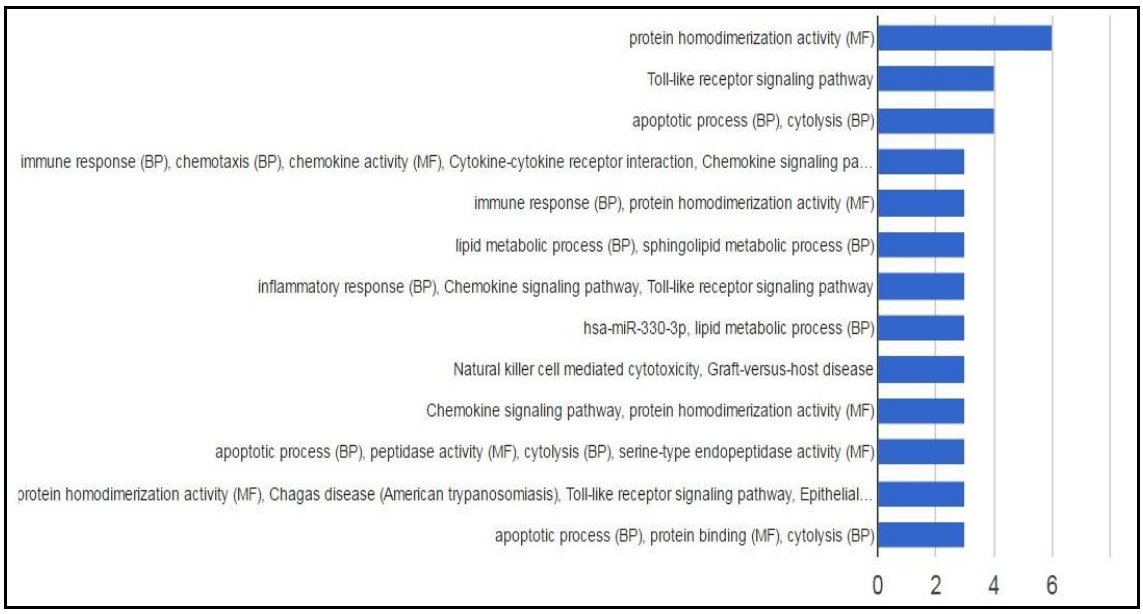

Figure 4: The twenty-on genes major pathways in IPA database

DISCUSSION

The complete genetics behind SCAR pathogenesis remains to be identified. STS and TEN, the major clinical manifestation of SCAR, continue to cause significant morbidity and mortality. In this report, databases and tools were used to investigate the differentially expressed microRNAs in SCAR.

Obtained from miRDB were 79 miRNAs predicted to target the 21 genes that interact with the Granulysin gene, the core causal gene in SCAR. Then, by overlapping the over-expressed miRNAs and potential miRNAs that target the 21 genes, a cluster of 18 miRNAs was obtained and found by Ingenuity pathway analysis (IPA) to be related to inflammation. Two significant DE miRNAs targeting Granylysin (miR-483-5p/miR-28-5p) were found. MiR-GNLY loop interaction in hypersensitivity may be useful biomarkers for SCAR, including SJS and TEN.

After the pathway-related microRNA clusters were identified, their genetic signature was generated to understand the pathogenesis of SJS and TEN. Our hypothesis that gene/miR signature can be applied to such a complex disease agrees with the genetic signature of HLA–B, –C, –A and –DRB1 alleles and cytochrome-P450 in a previous study [22]. Many significant pathways were also identified as hallmarks of SCAR, such as the necroptosis pathway that is mediated by annexin1 gene which ligates to FPR1 receptor [23]. Pathways are also important signatures for SCAR. In the current report, toll-like receptor was found to be the second most important in IPA and this pathway was proved to be involved in SJS and TEN [24]. There are many reports of other pathways in SCAR-related inflammatory response [25].

In terms of drugs causing SCAR, most candidates were, Allopurinol, Carbamazepine, furosemide Erythromycin and paracetamol [26]. While, in this study, specific Granulysin gene/miR cluster could potentially serve as a biomarker for purposes of diagnosis and prognosis.

CONCLUSION

Highlighted in this report are 18 Granulysin gene-targeting microRNAs that can provide insight into the pathogenesis of SCAR. Specifically, 483-5p/miR-28-5p, which are over-expressed in SCAR and validated to directly target the *GNLY* gene, are novel regulators of miRs that can modulate Granulysin gene expression. It is evident that the MiR-*GNLY* loop signature/control in hypersensitivity reactions can function as biomarkers for SCAR, including SJS and TEN. From the identified pathway-related microRNA clusters, a genetic signature is presented to understand the pathogenesis of SJS and TEN and will be highly valuable in the diagnosis and prognosis of SCAR.

DECLARATIONS

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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