Differential genes expression analysis of invasive aspergillosis: a bioinformatics study based on mRNA/microRNA

Maryam Hosseinipour¹, Shirin Shahbazi²*, Shahla Roudbar-Mohammadi¹, Maryam Khorasani³, Majid Marjani⁴

1) Department of Medical Mycology, Faculty of Medical Science, Tarbiat Modares University, Tehran Iran
2) Department of Medical Genetics, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
3) Molecular Medicine Department, Pasteur Institute of Iran, Tehran, Iran
4) Clinical Tuberculosis and Epidemiology Research Center, National Research Institute of Tuberculosis and Lung Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran

ABSTRACT

Invasive aspergillosis is a severe opportunistic infection with high mortality in immunocompromised patients. Recently, the roles of microRNAs have been taken into consideration in the immune system and inflammatory responses. Using bioinformatics approaches, we aimed to study the microRNAs related to invasive aspergillosis to understand the molecular pathways involved in the disease pathogenesis. Data were extracted from the gene expression omnibus (GEO) database. We proposed 3 differentially expressed genes; S100B, TDRD9 and TMTC1 related to pathogenesis of invasive aspergillosis. Using miRWalk 2.0 predictive tool, microRNAs that targeted the selected genes were identified. The roles of microRNAs were investigated by microRNA target prediction and molecular pathways analysis. The significance of combined expression changes in selected genes was analyzed by ROC curves study. Thirty-three microRNAs were identified as the common regulator of S100B, TDRD9 and TMTC1 genes. Several of them were previously reported in the pathogenesis of fungal infections including miR-132. Predicted microRNAs were involved in innate immune response as well as toll-like receptor signaling. Most of the microRNAs were also linked to platelet activation. The ROC chart in the combination mode of S100B/TMTC1, showed the sensitivity of 95.65 percent and the specificity of 69.23 percent. New approaches are needed for rapid and accurate detection of invasive aspergillosis. Given the pivotal signaling pathways involved, predicted microRNAs can be considered as the potential candidates of the disease diagnosis. Further investigation of the microRNAs expression changes and related pathways would lead to identifying the effective biomarkers for IA detection.

Keywords: Fungal infection; Gene expression; MicroRNAs; Signaling pathways

INTRODUCTION

Invasive aspergillosis (IA) exhibits more than 80 percent mortality rate in individuals with immunodeficiency, including patients with blood malignancies and bone marrow transplant
recipients. The incidence of IA has not been well elucidated yet, however it was considered responsible for 30-50 percent of invasive fungal diseases among immunocompromised patients [1]. *Aspergillus fumigatus* and *Aspergillus flavus* are the most common cause of IA [2]. The diagnosis is mainly based on clinical examinations and serological tests. The gold standard methods are histopathological tests and tissue culture following the lung biopsy or bronchoalveolar lavage (BAL). However, this invasive approach is contraindicated in severe conditions such as thrombocytopenia [3]. Since IA progresses rapidly, the high mortality rate is a great challenge due to the lack of prompt standard diagnostic test.

Recently, the role of microRNAs has been taken into consideration as small molecules that are involved in the immune system and inflammatory response [4]. MicroRNAs regulate the gene expression following the external stimuli. Expression and function of microRNAs are essential for numerous physiological functions and cellular homeostasis. Changes in microRNAs can affect the expression of several target genes and subsequent proteins [5]. Evaluation of the mRNAs/microRNAs levels would lead to the identification of the key factors in pathways that are involved in the disease pathogenesis [6]. Active cells produce microRNAs that can be detected and traced in body fluids. As a result, circulating microRNAs are potential biomarkers in a variety of diseases, such as cancer, metabolic disorders, and cardiovascular diseases [7].

Following infection, significant changes occur in the profiles of circulating microRNAs [8]. It has been shown that the expression of miR-455, miR-125a, miR-146 and miR-155 were increased in rat macrophages in response to *Candida albicans* infection [9]. The expression of miR-204 and miR-211 were decreased in kidney tissue of rats with candidemia-induced kidney injuries [10]. In monocytes and dendritic cells contaminated with *Aspergillus fumigatus*, miR-132 and miR-155 showed higher expression levels [11]. Serum analysis of the patients infected with *P. brasiliensis* revealed increased expression of 8 microRNAs linked to apoptosis and immune response [12].

Validation of clinical biomarkers is a pivotal aspect in bioinformatics and biostatistics. With the development of high-power technologies, profiling the multiple gene expression is a useful approach to find differentially expressed genes correlated to the disease pathogenesis. Since microRNAs are quite stable in different ranges of clinical specimens, they could serve as biomarkers [13].

Based on this knowledge, we aimed to investigate the microRNAs that could be applied as the disease biomarkers. Given the vitality of early diagnosis of IA, we analyzed the available datasets using various bioinformatics tools to find the microRNAs most connected to the pathogenesis of the disease.

**MATERIALS AND METHODS**

**Microarray and published data used for gene selection:** In the present study, the gene expression dataset GSE78000 with the platform of Affymetrix Human Genome 19 (GPL21464) was extracted from the gene expression omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/gds). GSE78000 included 23 samples obtained from haematological patients with IA and 13 samples from non-IA haematological patients. Two of the non-IA samples were reported as a possible invasive fungal disease (IFD). Nine control samples from healthy donors were also included in the dataset. The S100 calcium-binding protein B (*S100B*) was suggested as a potential new biomarker for the diagnosis of IA on the GSE78000 [14]. Recently, using the same dataset, transmembrane O-mannosyltransferase targeting cadherins (*TMTC1*) gene was introduced as a new biomarker of IA [15]. Since IA shares many in commons with severe inflammatory response syndrome we also included tudor domain containing 9 (*TDRD9*) gene in our study. Using microarray analysis *TDRD9* was previously identified related to the pathogenesis of the SIRS [16].
Identification of gene targeting microRNAs: The predicted microRNAs that target S100B, TDRD9 and TMTC1 were identified using the predictive tool, miRWalk 2.0 (http://zmf.umm.uni-heidelberg.de/apps/zmf/mirwalk2/) [17]. To confirm the obtained results additional bioinformatics algorithms were applied including, miRNAmap, RNA22, MicroT4, miRanda, RNAMap, and Targetscan.

In-silico pathway analysis: The roles of microRNAs in molecular pathways were evaluated based on the Kyoto encyclopedia of genes and genomes (KEGG). Analysis of gene ontology (GO) was examined using the DIANA TOOLS-mirPath v.3 database (http://snf-515788.vm.okeanos.grnet.gr/).

Analysis of the ROC curve: MedCalc V.12.1.4 software was applied to analyze the significance of expression change in selected gene by drawing the ROC curves. The gene expression data were extracted from GSE78000 dataset. A logistic regression model was used to check the combination modes of gene expressions. The area under the curve, sensitivity and one minus its specificity were calculated to compare the predictive values of the genes.

RESULTS

According to the analysis of microRNAs, predicted by the miRWalk, 33 microRNAs were able to target S100B, TDRD9 and TMTC1 (Table 1). To this end, microRNAs approved by at least three different algorithms were considered significant. The sequences of the microRNAs have been indicated in Table 1. One of the predicted microRNAs, miR-132, was previously shown related to Aspergillus infection. Our list also comprised microRNAs with a known function in fungal infection such as miR-155. However, we also found microRNAs that had not been previously reported to be associated with infection or inflammation.

Table 1: The microRNAs predicted by miRWalk 2.0 with ability to target S100B, TMTC1 and TDRD9

| ID       | Accession | Sequence                  |
|----------|-----------|---------------------------|
| hsa-miR-516a-3p | MIMAT0006778 | UGCUUCUUUCGAGGAGGU        |
| hsa-miR-516b-3p | MIMAT0002580 | UGCUUCUUUCGAGGAGGU        |
| hsa-miR-1287-5p | MIMAT0005878 | UGCUUGCGAGAGUGGUAGGUC     |
| hsa-miR-583 | MIMAT0003248 | CAAAGAGGAGGCUCCCAUAUC     |
| hsa-miR-3978 | MIMAT0019363 | GUGGAAAGCAUCAGCAGGAGGU   |
| hsa-miR-186-5p | MIMAT0000456 | CAAAGGAAUCUCUUUCUUGGCU   |
| hsa-miR-400-5p | MIMAT0004764 | CCAUGGACUCUGCAGGAGGU     |
| hsa-miR-155-5p | MIMAT0006064 | UUAUGCCUAUCGCGAGAGGU     |
| hsa-miR-4717-5p | MIMAT0019829 | UAGGGCACAGCCCACCAUGGU   |
| hsa-miR-650 | MIMAT0003230 | AGGAAGCGAGCGUCUCUGAGAC   |
| hsa-miR-345-5p | MIMAT0000772 | CGUCAGCCUUAGCAGGAGGU     |
| hsa-miR-551b-3p | MIMAT0004794 | GAAACGACUGGCGAGGAGGU     |
| hsa-miR-875-3p | MIMAT0004923 | CUGGAAACACUGAGGAGGU     |
| hsa-miR-576-5p | MIMAT0003241 | ACUCCAAAUCCUGCCAGU     |
| hsa-miR-593-3p | MIMAT0004802 | UGCUUCUGCAGGAGGAGGU     |
| hsa-miR-3928-3p | MIMAT0018205 | GGAAGACCCUUCAGGAGGAGGU   |
| hsa-miR-346 | MIMAT0000773 | UGCUUGCGCCGCAGUCCGUGCU   |
| hsa-miR-7856-5p | MIMAT0034031 | UUUAAGGACACUGGAGGAGGU   |
| hsa-miR-7162-5p | MIMAT0028234 | UGCUCCUUUCUGCAGGAGGU   |
| hsa-miR-222-3p | MIMAT0002799 | AGCUACAGCGUCUCCGAGGAGGU   |
| hsa-miR-1276 | MIMAT0005930 | UAAAGAGCCUGUGGAGGAGCA   |
| hsa-miR-383-5p | MIMAT0000738 | AGAUCAGAAAGUGAUUGGUGCU   |
| hsa-miR-1289 | MIMAT0005879 | UGGAUGCCAGAAUCUGCAUU   |
| hsa-miR-4311 | MIMAT0016863 | GAAAGAGAGCUGAGUGAGGU   |
| hsa-miR-34c-3p | MIMAT0004677 | AACUCUAAACCAGCGGCGAGAG   |
| hsa-miR-4652-3p | MIMAT0019717 | GUCUGUUAACACCACUCCCUA   |
| hsa-miR-384 | MIMAT0001075 | AGCUCGAGAAUCUGCAUA   |
| hsa-miR-4743-3p | MIMAT0002978 | UUGCUAACGUGUUGGU     |
| hsa-miR-887-3p | MIMAT0004951 | GUGAACGCGCGCCAUCCCGAGG |
| hsa-miR-132-3p | MIMAT0000426 | UAAACUGUCACAGCAGGUGCU   |
| hsa-miR-642a-5p | MIMAT000312 | GUCUCUCUAACAGUUGUGGU     |
| hsa-miR-2115-5p | MIMAT0011158 | AGCUUCAGACUCUCUGGUGGA   |
| hsa-miR-34b-3p | MIMAT0004676 | CAAUCACUAACUCUCUGGCAUC    |

Hosseinipour et al., / Mol Biol Res Commun 2020;9(4):173-180 DOI: 10.22099/mbrc.2020.37432.1509 MBRC

http://mbrc.shirazu.ac.ir
We investigated statistical significant roles of microRNAs in KEGG pathways which is a reference database for pathway mapping. The results revealed regulatory roles of the microRNAs in several signaling pathways with the highest significance related to mucin type O-Glycan biosynthesis (P<0.05) (Table 2). Several other important pathways also explored including proteoglycans in cancer. It should be noted that many pathogens recruit proteoglycans to invade host cells.

Table 2: Results of examining KEGG of microRNAs predicted by mirParth v.3

| KEGG pathway                                      | P-Value | #genes | #miRNAs |
|---------------------------------------------------|---------|--------|---------|
| Mucin type O-Glycan biosynthesis                  | 1.22E-06| 15     | 14      |
| Proteoglycans in cancer                           | 2.50E-06| 110    | 28      |
| GABAergic synapse                                 | 2.76E-06| 45     | 26      |
| Signaling pathways regulating pluripotency of stem cells | 3.15E-06| 79     | 28      |
| Hippo signaling pathway                           | 1.18E-05| 83     | 29      |
| Renal cell carcinoma                              | 1.86E-05| 43     | 28      |
| Prion diseases                                    | 3.44E-05| 12     | 12      |
| Glialoma                                          | 0.0001508| 38    | 28      |
| Pathways in cancer                                | 0.0001508| 200  | 32      |
| Circadian rhythm                                  | 0.0001865| 23   | 24      |
| Wnt signaling pathway                             | 0.0003638| 73    | 28      |
| FoxO signaling pathway                            | 0.0005044| 74    | 26      |
| Adrenergic signaling in cardiomyocytes            | 0.0009892| 76    | 31      |
| Long-term potentiation                            | 0.001245| 42     | 28      |
| AMPK signaling pathway                            | 0.0019863| 69    | 30      |
| Glutamatergic synapse                             | 0.0023066| 59    | 30      |
| cAMP signaling pathway                            | 0.0023066| 104  | 31      |
| Gap junction                                      | 0.0025114| 45   | 29      |
| Nicotine addiction                                | 0.0026905| 25   | 22      |
| Prostate cancer                                   | 0.0026905| 50   | 31      |
| Estrogen signaling pathway                        | 0.0028283| 50    | 29      |
| cGMP/PKG signaling pathway                        | 0.0028422| 85    | 31      |
| Rap1 signaling pathway                            | 0.0029022| 105   | 30      |
| Thyroid hormone synthesis                         | 0.0037497| 36    | 28      |
| Axon guidance                                     | 0.0039922| 63    | 25      |
| Alanine, aspartate and glutamate metabolism       | 0.0042956| 22    | 21      |
| Long-term depression                              | 0.0046957| 32    | 28      |
| ErbB signaling pathway                            | 0.0046957| 49    | 29      |
| MAPK signaling pathway                            | 0.0046957| 126   | 30      |
| PI3K-Akt signaling pathway                        | 0.0046957| 161   | 32      |
| Gastric acid secretion                            | 0.004872| 43    | 25      |
| Ubiquitin mediated proteolysis                    | 0.0052406| 74    | 29      |
| Insulin secretion                                 | 0.0052406| 47    | 29      |
| Oocyte meiosis                                    | 0.0055651| 62    | 30      |
| Oxytocin signaling pathway                        | 0.0057047| 80    | 29      |
| Amphetamine addiction                             | 0.0063306| 36    | 29      |
| SNARE interactions in vesicular transport         | 0.0089967| 20    | 24      |
| Protein processing in endoplasmic reticulum       | 0.0112118| 81    | 29      |

As indicated in Figure 1, the results of the study on GO of microRNAs using mirPath v.3 revealed that the all 33 predicted microRNAs were involved in the innate immune response. Thirty of them were linked to the toll-like receptor (TLR) signaling. Furthermore, most of the microRNAs play role in platelet activation. These fundamental functions contribute in the pathophysiological process of IA.

The ROC curve analyses were shown in Figure 2. The combined panel of three genes, S100B/TDRD/TMTC1 could detect the IA with AUC: 0.69, sensitivity: 78.26, and specificity: 69.23. Statistical analysis showed that 95 percent confidence interval (CI) of combined 3 genes was 0.520 to 0.837 with the significance P value of 0.04. Meanwhile, the ROC chart had robust results in the combination mode of S100B/TMTC1 with AUC: 0.9, sensitivity: 95.65, and specificity: 69.23. The reported CI was 0.757 to 0.976 and P value was calculated <0.0001 (Fig. 2).
Figure 1: Pie chart the biological processes analysis of predicted microRNAs. The first number in parentheses indicates the number of microRNAs, and the second number in parentheses indicates the number of genes involved.

Figure 2: ROC curve analysis to evaluate the diagnostic value of S100B, TMTC1 and TDRD9 expression in the IA. A: Analysis of the ROC curve for S100B/TMTC1/TDRD9 combination. B: Analysis of the ROC curve for S100B/TMTC1 gene expression data combination.

DISCUSSION

Aspergillus fumigatus and Aspergillus flavus are saprophyte fungi, widespread in the environment. Exposure to fungal spores leads to IA in immunocompromised patients, with a high mortality rate [18]. Studies to find new biomarkers for rapid and accurate detection of IA are ongoing. Recently, triacetylfusarinine C which is an Aspergillus fumigatus siderophore was introduced as a urine biomarker for early diagnosis of IA [19]. Furthermore, high-throughput screening and bioinformatics studies have been conducted to identify diagnostic biomarkers in various diseases including IA [20]. Comparing gene expression profiles of IA with non-IA patients, it has been shown that S100B could be served as a diagnostic biomarker of IA [14].

TMTC1 was up-regulated 2.6 folds in IA comparing to non-IA patients with the 78.3 percent sensitivity and 81.8 percent specificity [15]. TMTC1 is located on the membrane of endoplasmic
reticulum and play a role in calcium homeostasis. It is also involved in the protein glycosylation by mannosyl transfer to the hydroxyl group of serine or threonine residues [21].

On the other hand, TDRD9 is a DEXH-box RNA helicase and is involved in PIWI-interacting RNAs (piRNAs) formation [22]. Besides the male reproductive system, it mainly expressed in blood cells including monocytes and dendritic cells which play important roles in the innate immune response against IA. Monocytes express a variety of receptors for the identification of fungal cells, such as TLRs, c-type lectin receptors (CLRs) and dectin-1. These receptors detect fungal pathogen molecules such as beta-d-glucan that are located in the cell wall of *Aspergillus* species.

In our study, we identified 33 microRNAs as the regulator of S100B, TDRD9 and TMTC1. Based on our finding, predicted microRNAs were involved in key cellular functions including TLR signaling [23]. It has been shown that innate immune detection of *Aspergillus fumigatus* is facilitated by TLRs [24].

Our results also revealed that all 33 microRNAs were involved in innate immune response. Among them, miR-132 was previously recognized related to the *Aspergillus* infection. Gupta *et al.* showed that miR-132 is differentially expressed in monocytes and dendritic cells following contamination by *Aspergillus fumigatus* [11]. As mentioned earlier, monocyte and dendritic cells are among the main expression sites of TDRD9 which is a conserved target of miR-132. New roles for TDRD9 have also been identified in lung cancer and was suggested as a potential therapeutic target [25]. Furthermore, miR-132 was reported to be increased in dendritic cells and natural killer cells following the exposure to *Aspergillus fumigatus* [26].

The microRNAs predicted in our study also included miR-155 which is a negative regulator of TLRs [27]. It has been shown that miR-155 is an essential factor in the innate immune response to fungal infection [28].

We also observed that the predicted microRNAs were related to platelet activation process. The activation of Platelets is an important component of hemostasis and *Aspergillus fumigatus* is a well-known platelets activator [29]. On the other hand, it has been elucidated that platelets are important factors in tissue integrity following pulmonary infection of *Aspergillus fumigatus* [30].

Previous studies have indicated that microRNAs regulate the host response in viral, fungal, and bacterial infections [31]. Although the pathogenesis of IA is not well known, many factors such as microRNAs may contribute to the disease development. Also, understanding molecular pathways involved in the disease pathogenesis could lead to the finding of new biomarkers [32]. According to the result of the present study, further evaluation of 33 predicted microRNAs can lead to the design of a diagnostic panel for IA. Analyses of differentially expressed microRNAs are a promising approach to improve the proper diagnosis of the condition and could lead to a better understanding of the mechanisms underlying the association between human host cells and IA.

**Acknowledgements:** The authors declare that they have no conflict of interest. The study was approved by the Ethics Committee of the School of Medicine, Tarbiat Modares University, Tehran, Iran.

**Conflict of Interest:** The authors declare that they have no conflict of interest.

**REFERENCES**

1. Pagano L, Caira M, Candoni A, Offidani M, Fianchi L, Martino B, Pastore D, Picardi M, Bonini A, Chierichini A, Fanci R, Caramatti C, Invernizzi R, Mattei D, Mitra ME, Melillo L, Aversa F, Van Lint MT, Falcucci P, Giovanna Valentini C, Girmenia C, Nosari A. The
epidemiology of fungal infections in patients with hematologic malignancies: the SEIFEM-2004 study. Haematologica 2006;91:1068-1075.
2. Latge JP, Chamilos G. Aspergillus fumigatus and Aspergillosis in 2019. Clin Microbiol Rev 2019;33.
3. Pfaller MA, Diekema DJ. Epidemiology of invasive mycoses in North America. Crit Rev Microbiol 2010;36:1-53.
4. Reid G, Kirschner MB, van Zandwijk N. Circulating microRNAs: Association with disease and potential use as biomarkers. Crit Rev Oncol Hematol 2011;80:193-208.
5. Khan AA, Betel D, Miller ML, Sander C, Leslie CS, Marks DS. Transfection of small RNAs globally perturbs gene regulation by endogenous microRNAs. Nat Biotechnol 2009;27:549-555.
6. Li X, Gill R, Cooper NG, Yoo JK, Datta S. Modeling microRNA-mRNA interactions using PLS regression in human colon cancer. BMC Med Genomics 2011;4:44.
7. Cai P, Gobert GN, You H, Duke M, McManus DP. Circulating miRNAs: Potential novel biomarkers for hepatopathology progression and diagnosis of schistosomiasis Japonica in two murine models. PLoS Negl Trop Dis 2015;9:e0003965.
8. Drury RE, O'Connor D, Pollard AJ. The clinical application of microRNAs in infectious disease. Front Immunol 2017;8:1182.
9. Monk CE, Hutvagner G, Arthur JS. Regulation of miRNA transcription in macrophages in response to Candida albicans. PLoS One 2010;5:e13669.
10. Li XY, Zhang K, Jiang ZY, Cai LH. MiR-204/miR-211 downregulation contributes to candidemia-induced kidney injuries via derepression of Hmx1 expression. Life Sci 2014;102:139-144.
11. Das Gupta M, Fliesser M, Springer J, Breitschopf T, Schlossnagel H, Schmitt AL, Kurzai O, Hunninger K, Einsele H, Loffler J. Aspergillus fumigatus induces microRNA-132 in human monocytes and dendritic cells. Int J Med Microbiol 2014;304:592-596.
12. De Lacorte Singulani J, De Fatima Da Silva J, Guilo FP, Costa MC, Fusco-Almeida AM, Enguita FJ, José Soares Mendes-Giannmini M. Preliminary evaluation of circulating micro-RNAs as potential biomarkers in paracoccidioidomycosis. Biomed Rep 2017;6:353-357.
13. Kruth S, Hubner M, Hinske LC. MicroRNAs as Clinical Biomarkers and Therapeutic Tools in Perioperative Medicine. Anesth Analg 2018;126:670-681.
14. Dix A, Czakai K, Springer J, Fliesser M, Bonin M, Guthke R, Schmitt AL, Einsele H, Linde J, Loffler J. Genome-wide expression profiling reveals S100B as biomarker for invasive Aspergillosis. Front Microbiol 2016;7:320.
15. Deng W, Ma Y, Liang P, Huang C, Zhang Y, Zhang K, Liu X, Shao J, Chen J, Li R. Diagnostic biomarkers for invasive aspergillosis utilizing weighted gene co-expression network analysis 2020. PREPRINT (Version 1) available at Research Square. DOI:10.21203/rs.3.rs-27305/v1
16. Tong DL, Kempsell KE, Szakmany T, Ball G. Development of a bioinformatics framework for identification and validation of genomic biomarkers and key immunopathology processes and controllers in infectious and non-infectious severe inflammatory response syndrome. Front Immunol 2020;11:380.
17. Dweep H, Sticht C, Pandey P, Gretz N. miRWalk--database: prediction of possible miRNA binding sites by "walking" the genes of three genomes. J Biomed Inform 2011;44:839-847.
18. Romani L. Immunity to fungal infections. Nat Rev Immunol 2011;11:275-288.
19. Hoenigl M, Orusch T, Faserl K, Prattes J, Loeffler J, Springer J, Gsaller F, Reischies F, Duettmann W, Raggam RB, Lindner H, Haas H. Triacetylfusarinine C: A urine biomarker for diagnosis of invasive aspergillosis. J Infect 2019;78:150-157.
20. Lin Y, Qian F, Shen L, Chen F, Chen J, Shen B. Computer-aided biomarker discovery for precision medicine: data resources, models and applications. Brief Bioinform 2019;20:952-975.
21. Sunryd JC, Cheon B, Graham JB, Giorda KM, Fissore RA, Hebert DN. TMTC1 and TMTC2 are novel endoplasmic reticulum tetratricopeptide repeat-containing adapter proteins involved in calcium homeostasis. J Biol Chem 2014;289:16085-16099.

22. Shoji M, Tanaka T, Hosokawa M, Reuter M, Stark A, Kato Y, Kondoh G, Okawa K, Chujo T, Suzuki T, Hata K, Martin SL, Noce T, Miyagawa SK, Nakano T, Sasaki H, RS Pillai, Nakatsuji N, Chuma Sh. The TDRD9-MIWI2 complex is essential for piRNA-mediated retrotransposon silencing in the mouse male germline. Dev Cell 2009;17:775-787.

23. Garth JM, Steele C. Innate lung defense during invasive aspergillosis: New mechanisms. J Innate Immun 2017;9:271-280.

24. Rubino I, Coste A, Le Roy D, Roger T, Jaton K, Boeckh M, Monod M, Paul Latgé J, Calandra T, Yves Bochud P. Species-specific recognition of Aspergillus fumigatus by Toll-like receptor 1 and Toll-like receptor 6. J Infect Dis 2012;205:944-954.

25. Guijo M, Ceballos-Chávez M, Gómez-Marín E, Basurto-Cayuela L, Reyes JC. Expression of TDRD9 in a subset of lung carcinomas by CpG island hypomethylation protects from DNA damage. Oncotarget 2017;9:9618-9631.

26. Dix A, Czakai K, Leonhardt I, Schäferhoff K, Bonin M, Guthke R, Einsele H, Kurzai O, Löffler J, Linde J. Specific and novel microRNAs are regulated as response to fungal infection in human dendritic cells. Front Microbiol 2017;8:270.

27. Taganov KD, Boldin MP, Chang KJ, Baltimore D. NF-kappaB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. Proc Natl Acad Sci USA 2006;103:12481-12486.

28. Croston TL, Lemons AR, Beezhold DH, Green BJ. MicroRNA regulation of host immune responses following fungal exposure. Front immunol 2018;9:170.

29. Rodland EK, Ueland T, Pedersen TM, Halvorsen B, Müller F, Aukrust P, Frøland SS. Activation of platelets by Aspergillus fumigatus and potential role of platelets in the immunopathogenesis of Aspergillosis. Infect Immun 2010;78:1269-1275.

30. Tischler BY, Tosini NL, Cramer RA, Hohl TM. Platelets are critical for survival and tissue integrity during murine pulmonary Aspergillus fumigatus infection. PLoS Pathog 2020;16: e1008544.

31. Shwetha S, Gouthamchandra K, Chandra M, Ravishankar B, Khaja MN, Das S. Circulating miRNA profile in HCV infected serum: novel insight into pathogenesis. Sci Rep 2013;3:1555.

32. Weiland M, Gao XH, Zhou L, Mi QS. Small RNAs have a large impact: circulating microRNAs as biomarkers for human diseases. RNA Biol 2012;9:850-859.