Exosomes Derived From Heat Stroke Cases Carry miRNAs Associated With Inflammation and Coagulation Cascade

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Research

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Abstract

Background: The pathological mechanism of HS is associated with the dysbalanced inflammation and coagulation cascade. The cells-derived circulating extracellular vesicles (EVs) as a novel pathway mediating intercellular communication were evidenced to be associated with immune response and inflammation in critical inflammatory syndromes such as sepsis. Despite previous studies demonstrating that these vesicles contain genetic material related to their biological function, their molecular cargo during heat stroke is relatively unknown. In this study, we evaluated the presence of microRNAs (miRNAs) and messenger RNAs (mRNAs) related to inflammatory response and coagulation cascade in exosomes of patients with heat stroke.

Methods: Blood samples were collected from 3 patients with heat stroke at ICU admission. Three healthy volunteers were used as control subjects. Exosomes were isolated by ultracentrifugation, their miRNA content was profiled by next generation sequencing and mRNA content was evaluated by qRT-PCR array.

Results: As compared with healthy volunteers, exosomes from patients with heat stroke had significant changes in 202 exosomal miRNAs (154 miRNAs upregulated and 48 downregulated). The most upregulated miRNAs included miR-511-3p, miR-122-5p, miR-155-3p, miR-1290 and let7-5p whereas the most downregulated ones included miR-150-3p, 146a-5p and 151a-3p. The GO enrichment by the miRNAs of patients with heat stroke compared with control subjects were related mostly to inflammatory response including T cell activation, B cell receptor signaling, DC chemotaxis and leukocyte migration, and platelet activation and blood coagulation. KEGG pathway analysis determined those identified miRNAs were mainly enriched to the signal transduction pathways namely, T cell receptor signaling pathway, Ras signaling pathway, Chemokine signaling pathway, Platelet activation, and Leukocyte transendothelial migration. These pathways were mainly related to inflammation and hemostasis. Multiple targeted mRNAs associated with the inflammatory response, blood coagulation and platelet activation were further verified in serum exosomes.

Conclusions: Exosomes from patients with heat stroke convey miRNAs and mRNAs related to pathogenic pathways, including inflammatory response and coagulation cascade. Exosomes may represent a novel mechanism for intercellular communication during heat stroke.

Background

Heat stroke (HS) is one of the warranted causes of death during intensified exercise and military training in summer. It appears to be a major challenge to the intensivist due to the lack of definitive evidence-based therapy. Recently, HS shows an increasing trend because of the growing frequency of heat wave attack, and the resultant mortality rates are quite high, particularly the HS-induced multiple organ failure (MOF) with the mortality rate of 40%-60% [1, 2].

The molecular mechanism of HS pathogenesis is complicated and remains poorly documented [3]. Evidences have verified that mechanism of HS is associated with the dysbalanced inflammation and
coagulation cascade [4]. In recent years, the cells-derived circulating extracellular vesicles (EVs) shed from different cell types in a critical inflammatory syndrome such as sepsis, together with their roles in disease pathophysiology, have been extensively investigated, especially those associated with immune response and inflammation [5, 6]. In addition, it has been revealed in our previous study that the EVs derived from hepatocytes of HS patients are correlated with liver dysfunction, which is mediated by a route associated with activation of EV-induced inflammatory NOD-like receptor pathway and necrosis (an inflammatory death form) in hepatocytes [7, 8].

EVs may mediate the communication between cells under normal physical conditions or in some disorders via carrying the functional microRNAs (miRNAs) and messenger RNA (mRNA). By adopting pathway enrichment analysis, a pilot study suggests that the miRNA profiling in serum EVs from septic patients might exert potentially vital biological roles in sepsis [9]. The authors revealed a differential expression pattern of EV-miRNA in sepsis which was involved in the modulation of immune system and cell cycle. However, no existing study has examined the genetic content of exosomes in the context of HS.

This article aimed to analyze the changes of miRNA profiles in serum EVs and their biological functions in HS patients. Findings in this work can possibly shed more lights on the pathophysiology of HS and offer the research windows for developing new treatments.

**Materials And Methods**

*Patient recruitment and clinical data collection*

From June 2019 to June 2020, 6 patients admitted within the first 24 h of severe HS onset into the intensive care unit (ICU) of the General Hospital of South Theatre of People's Liberation Army (PLA) of China, a tertiary hospital, were retrospectively enrolled in this study. HS was diagnosed according to the Expert Consensus on Diagnosis and Treatment of Heat Stroke in China (2019) (10) released by the Expert Group on Prevention and Treatment of Heat Stroke and Critical Care Committee of PLA of China. The patients with active malignant tumors, chronic liver and kidney diseases, chronic cardiac insufficiency (New York grade 3-4), chronic pulmonary insufficiency, underlying central nervous system (CNS) disease, metabolic disorders or those using heparin or any other medications, were eliminated from this study. Additionally, three healthy people from the Physical Examination Center were enrolled as the control group. Baseline demographic characteristics were recorded upon admission into ICU (Day 1). Blood samples and organ function data were collected on Day 1; besides, the sequential organ failure assessment (SOFA) and the age and chronic health evaluation (APACHE II) scores were determined to evaluate the disease severity. Patients or the corresponding family members provided the informed consent. Our study protocol gained approval from the Medical Ethics Review Committee of General Hospital of Southern Theater of PLA of China.

*Blood sample collection and exosome isolation*
Altogether 30 ml peripheral venous blood sample was obtained from each patient using the ethylenediaminetetraacetic acid (EDTA) anticoagulant tubes. Then, the blood collection tubes were left standing vertically for 30 min at 22–27 °C. Afterwards, plasma was obtained from whole blood through 10 min of centrifugation under 4 °C and 2,500 × g. After the addition of protease inhibitors (supplemented with 3 mM phenylmethylsulfonyl fluoride, 1 µg/ml pepstatin, as well as 1 µg/ml aprotinin), the plasma was stored at -80 °C. When processing, plasma was diluted with equivalent volume of phosphate-buffered saline (PBS) and centrifuged at 4°C (30 min at 2,500 × g, 45 min at 12,000 × g, along with 2 h at 110,000 × g, SW 28 Ti Rotor, Optima L-90K Ultracentrifuge, Beckman Coulter, Fullerton, USA). At last, 50–200 µl PBS was used to resuspend the precipitate (exosomes), followed by storage under -80 °C.

**Exosome morphology observed via transmission electron microscopy (TEM)**

Exosome samples were collected from a healthy individual, a patient with mild HS, and a patient with severe HS (randomly selected), respectively, and prepared for TEM evaluation. Firstly, exosomes were dehydrated in 2% formalin. After adding 5 ml exosome suspension into the copper grid coated with formvar (Mecalab, QC, Canada), the sample was subjected to 30 min of incubation, washing with 100 µl PBS, 10 min of 2% paraformaldehyde fixation, and 15 min of 2% uranyl acetate staining (dissolved into 50% ethanol). Thereafter, the Philips CM10 transmission electron microscope (JEM-2100F, Netherlands) was utilized for sample visualization.

**Nanoparticle tracking analysis (NTA)**

For detecting exosome content and size distribution, NTA was performed using the NanoSight (NS3000 (Malvern Instruments, Worcestershire, UK) in accordance with specific protocols. In brief, exosome sample was diluted with sterile PBS at 1:5,000, and the NanoSight LM10 and NTA software (NanoSight Ltd, Amesbury, UK) was employed for sample analysis for thrice (60 s each).

**Western blotting**

Protein was extracted from 1 ml ultracentrifuged plasma (120,000 g for 2.5 h) using the DE buffer (consisting of 20 mM Tris-HCL, 12 mM 2-mercaptoethanol, 1 mM EGTA, 1 mM EDTA, 1% Triton-X 100, together with 10% glycerol) (5) that contained the protease inhibitor mix (GE Healthcare, Uppsala, Sweden). Total protein was quantified by Bradford, then, 12% SDS-PAGE was applied in separating 5 µg protein from each sample. Later, the proteins were transferred onto the nitrocellulose membranes and blotted using polyclonal antibodies against CD9 (ABcam ab10895, Cambridge, UK), CD63 (ABcam ab92726, Cambridge, UK) and Tsg-101 (ABcam ab30871, Cambridge, UK). GADPH was used as a loading control for protein extracts. Target protein expression level was quantified by densitometry analysis using the ImageJ software.

**Exosomal RNA sequencing (next-generation sequencing, NGS)**

**Exosomal RNA extraction**
The Trizol reagent was employed to extract the RNA from exosomes.

**Library construction and sequencing**

The NEB SmallRNA Library kit was adopted for library construction, while illumina HiSeq was applied for sequencing, with a data volume of 20M reads per sample.

**Filtering and miRNA mapping**

Clean data were filtered from raw reads after sequencing in accordance with criteria below: a) 30% base quality <20, b) read length < 17bp, and c) adaptor sequence. Afterwards, clean data were mapped to the Danio Rerio miRNA database (miRBase v21.0) together with the Danio Rerio genome (Zv10, NCBI) using BWA software. Later, those unmapped reads were used to map the Rat, Mouse and Human miRNA database (miRBase v21.0) to achieve the expression profiles of miRNAs.

**New miRNA prediction**

The novel miRNA prediction was applied using mirdeep based on the reads mapped to Zv10 genome but unmapped against the Danio Rerio miRNA database (miRBase v21.0), so as to identify new miRNA. The BWA software mapping was applied in measuring the expression of new miRNA. (miRDeep*: an integrated application tool for miRNA identification from RNA sequencing data. An J, et al. Nucleic Acids Res. 2013 Jan;41(2):727-37. doi: 10.1093/nar/gks1187.)

**Mapping of RNA sequencing**

Pair-end read mapping. Prior to read mapping, adaptor sequences, low quality reads with over 20% bases that had <20 quality, and those having over 5% ambiguous bases (N) were eliminated from raw reads to obtain clean reads. The zebrafish genome (version: Zv10) was used to align clean reads by Tophat program. [Trapnell C, et al. Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks.Nat Protoc. 2012 Mar 1;7(3):562-78. doi: 10.1038/nprot.2012.016.]

**Differential-gene-finder**

The DESeq2 package was adopted to identify miRNA and mRNA with differential expression according to the numbers of miRNA and mRNA, FDR and P-value[Benjamini Y, et al. Controlling the false discovery rate in behavior genetics research. Behav Brain Res. 2001 Nov 1;125(1-2):279-84.], with the selection criteria of i) Fold Change (FC)>2 or <0.5; ii) P-value<0.05 and FDR<0.05.

**Target Analysis**

Miranda target analysis [John B, Enright A J, Aravin A, et al. Human MicroRNA targets.[J]. Plos Biology, 2004, 2(11):: e363.] was adopted for predicting the target of miRNA in the mRNA of zebra fish. For discovering the possible target of miRNA in the present work, the target of miRNA with no negative association of mRNA with miRNA was filtered.
Gene ontology (GO) functional annotations

GO analysis was carried out for elucidating the biological roles of specific genes in typical or significant differentially expressed gene (DEG) profiles [Ashburner M, et al. Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. Nat Genet. 2000 May;25(1):25-9.]. First of all, those GO annotations were downloaded from Gene Ontology (http://www.geneontology.org/), UniProt (http://www.uniprot.org/), along with NCBI (http://www.ncbi.nlm.nih.gov/) databases. Then, those significant GO categories were identified by Fisher’s exact test, while p-values were corrected by FDR.

Pathway enrichment analysis

Pathway enrichment analysis was also applied for discovering DEGs based on the KEGG database. Those significant pathways were selected by Fisher’s exact test, with FDR and P-value being the significance thresholds. [Draghici S, et al. A systems biology approach for pathway level analysis. Genome Res. 2007 Oct;17(10):1537-45. Epub 2007 Sep 4.]

Analysis of miRNA and inflammation/coagulation-related gene expression

30 ng total RNA was collected for the reverse transcription of miRNAs using specific stem-loop primers. The TaqMan Low Density Array (TLDA, TaqMan MicroRNA Array v3.0, Life Technologies, Grand Island, EUA), a micro fluidic card simultaneously detecting 754 mature miRNAs, was used to analyze the pre-amplification reaction products by qPCR. Immune study was performed in 21 septic patients and 12 controls. The High Capacity RNA-to-cDNA Kit (Life Technologies) was used for the reverse transcription of 100 ng RNA into cDNA following manufacturer’s instructions. cDNA was distributed in TLDA cards (TaqMan Low Density Array Immune Profiling, Life Technologies) designed to evaluate 90 genes related to immune response. The 7900HT thermocycler (Applied Biosystems) was used for real-time PCR using the SDS 2.4 software. All data collected were analyzed in the ExpressionSuite 1.0.3 software (Life Technologies), where both baseline and threshold were automatically set. Each gene was checked individually and the threshold was adjusted to the exponential scale as needed. In the analysis of gene expression associated with OS, the PCR array platform used in these experiments comprised 84 genes related to OS as well as the antioxidant defense (PAHS065-A, SABiosciences/Qiagen, Valencia, USA). A total of 21 patients and 10 healthy donors were evaluated. In line with specific protocols, 100 ng RNA was used in the reverse transcription into cDNA, and the specific primers were used in pre-amplification of cDNA. To avoid variation, the PCR plates were prepared in high precision robotic pipetting (QI Agility, Qiagen). Quantitative real time PCR (qRT-PCR) was completed in the 7300HT thermocycler (Life Technologies). Data were processed using the SDS 1.3 software, and the same baseline (3-15) and threshold (0.2) were considered for each gene.

qPCR data analysis

The Statminer v5 software (Integromics, Granada, Spain) was used to analyze the cycle threshold (Ct) data. According to specific manufacturer instructions, Ct values ≥ 32 were excluded from immune and
miRNAs studies. For OS studies, the cutoff value was set at 35 in line with manufacturer's protocols. With regard to miRNA analysis, the optimal reference was identified by geNorm approach, and the median miR17, miR20a and miR106a was applied in normalizing data. The 18S rRNA (7) was used to normalize the immune gene expression, whereas beta-actin was utilized to normalize OS gene expression. Besides, the $2^{\Delta\Delta Ct}$ method was adopted to calculate relative expression of genes.

**Statistical analysis**

The normality of data was assessed by Kurtosis test. Data with normal distribution were expressed in the manner of mean ± standard deviation (SD), whereas those with abnormal distribution were expressed in the manner of median ± interquartile range. The two-tailed Student’s t-test was applied in the statistical analysis between two groups, while the one-way analysis of variance (ANOVA) followed by post hoc test was adopted for comparison among multiple groups. Spearman’s rank correlation test was adopted to analyze the correlations. A difference of $p$-value <0.05 (two tailed) indicated statistical significance. The GraphPad version 7.0 (La Jolla, CA, USA) was employed for all test.

**Results**

*Baseline characteristics and outcomes of patients*

All patients and healthy controls were male with a relatively young age and few comorbidities. Age was comparable between healthy controls and HS patients (25 ± 6 vs. 21 ± 4 years, respectively). No participant previously used any medication. Clinical and laboratory data are summarized in Table 1. Core body temperatures on admission were higher in HS patients (39.00 ± 1.94 vs. 36.42 ± 0.46°C, $p<0.001$). In addition, 3 of the 6 HS cases died during the ICU hospitalization. The patients stayed at the ICU for 5-10 (median, 7) days.

All severe HS patients were complicated with multiple organ dysfunction. There was plenty of organ support when patients were enrolled, because 67% patients received mechanical ventilation while 50% received norepinephrine. The overall disease severity, as assessed by the APACHE II and SOFA scores, were 17.05 ± 4.35 and 10.95 ± 5.64, respectively (Table 2). None of the enrolled patients received prophylactic heparin, or transfusion of red blood cells/platelet/fresh frozen plasma, or antibiotics on admission before the collection of study samples.

*Characterization of plasma exosomes*

TEM showed that the separated preparation from the plasma of healthy controls and HS patients exhibited a double-membrane vesicle-like structure about 100 nm in diameter (Fig. 1A). Besides, the NTA results indicated that the plasma exosome concentration of HS group increased compared with that of control group (5.67 ± 2.34×10^9 particles/ml vs. 0.47 ± 0.15×10^9 particles/ml, respectively) (Fig. 1B). Finally, Western blotting analysis showed the positive expression of the membrane-associated proteins
related to endocytosis, as well as the exosomal markers (CD9, CD63 and Tsg-101) in both control and HS groups (Fig. 1C). Taken together, the extracted vesicles were mostly consistent with exosomes.  

**HS remarkably changed the expression patterns of circulating exosomal miRNAs**

For testing whether HS changed exosomal miRNAs expression, the miRNA profiling analysis was conducted firstly among HS cases admitted at the ICU; then, the results were compared against the healthy controls. As a result, HS made no difference to 90.72% miRNAs accumulation (Fig. 2A). It was also observed from Fig. 2B that, differences in most identified miRNAs were not significant, due to the close abundance ratio of HS versus control EVs. Nonetheless, in HS patients, 202 miRNAs (9.28%) were identified as differentially expressed compared with controls [154 up-regulated (7.08%) and 48 down-regulated (2.21%)] (Fig. 2A). Among them, the miRNAs expression FC in HS versus control EVs (ratio of HS to control group <0.6 or >1.5), along with statistically significant P-values are summarized in Table 1 and displayed in a volcano map (Fig. 2B).

Additionally, hierarchical cluster analysis was carried out on 202 differentially expressed miRNAs based on samples or stimulants, so as to examine the creditability of those selected target miRNAs (Fig. 3). There was significantly differential expression in HS patients versus controls, with low SD among 3 samples, which suggested that our results were highly repeatable. Some specific miRNAs with significant elevation within HS EVs relative to controls were identified, including miR-511-3p, miR-122-5p, miR-155-3p, miR-1290 and let7-5p. The most downregulated miRNAs included miR-150-3p, 146a-5p and 151a-3p. The ACC IDs of all the disregulated miRNAs and the corresponding FCs are presented in Table 3. The results suggested that, HS changed certain miRNAs levels within plasma EVs, and this was associated with the biological effect.

**The up-regulated miRNAs in HS EVs were related to the inflammation and coagulation signal transduction pathways**

Thereafter, each EV miRNAs discovered from NGS was classified for exploring the specific biological role by the use of GO functional annotation clustering approach. As suggested by the results of molecular functional analysis, most identified EV miRNAs participated in regulating the signaling and molecular functions. As a result, the targeted miRNAs were speculated to exert an important part in the EVs biological activity.

The 20 most significant biological terms related to the up-regulated or down-regulated miRNAs in HS EVs, as well as the miRNA enrichment significance determined for all clusters, are presented in Fig. 4. Particularly, inflammatory response including T cell activation, B cell receptor signaling, DC chemotaxis and leukocyte migration, and platelet activation and blood coagulation, as well as cell responses to various stimuli including cytokines, regulation of cell death and cell adhesion/migration and cell cycle were the most significantly up-regulated clusters and were all involved in the mechnism of HS.
KEGG enrichment helps to determine the miRNA enrichment significance of every pathway. In this study, KEGG analysis discovered that those identified miRNAs were mainly enriched to the signal transduction pathways below, namely, T cell receptor signaling pathway, Ras signaling pathway, Chemokine signaling pathway, Platelet activation, and Leukocyte transendothelial migration (Fig. 5). These pathways were mainly related to inflammation and hemostasis. Thus, it was speculated that the circulating EVs might be related to HS-induced injury via the activation of these signal transduction pathways within target cells.

Targeted mRNAs associated with the inflammatory response, blood coagulation and platelet activation

Table 4, 5 and 6 list the targeted mRNAs related to the top three up-regulated clusters, inflammatory response, blood coagulation and platelet activation.

For assessing the presence or absence of immune response-related mRNAs within exosomes, qPCR was performed to compare the inflammation/immune-related gene expression levels based on RNA extracted from HS and healthy individuals. It was observed that 18 genes, such as PRKD1, SP100, IL25, CRLF2, NLRP2, AOAN, and CXCR1, were significantly up-regulated in HS patients (p<0.05) (Fig. 6).

As illustrated from Fig. 7 that, the platelet activation-related mRNAs expression in exosomes like VWF, TXA2R, P2RY12, CD40LG, and THBS1 in HS patients were also up-regulated comparing to those in healthy controls.

We also investigated the expression of mRNAs related to blood coagulation and demonstrated an increased expression of exosomal mRNAs of PDE9A, LRRC16A, ITGAV, FCR, EGF, ITGA1, CD59, SERPING1, IRF2, etc (Fig. 8).

Putative pathways focusing the most enriched pathways, including chemokine signaling pathway, platelet activation pathway, T cell receptor signaling pathway and leukocyte transendothelial migration pathway were constructed based on KEGG mapping (Fig. 9).

**Discussion**

In the present work, the different circulating exosome-miRNAs expression profiles with corresponding targets involved in the immune and coagulation system, were firstly identified in HS patients.

The organ injury mechanism in HS remains unknown at present. The current opinion tends to believe that an inflammatory and coagulation response initiated by the hyperthermal injury may play a more important role than the direct physical damage from heat exposure [3]. The pathological findings of HS (core temperature attained 42.5~43°C) baboon models include massive transmural migration of leukocytes, widespread microthrombosis, microvascular endothelium injury, endothelial leukocyte-platelet interaction, and extensive apoptosis in multiple organs including spleen, gut, liver, kidney and lung [4].

As demonstrated in the present study, the enriched miRNAs conveyed in circulating exosomes might participate in the inflammatory response and coagulation cascade, suggesting that exosomal miRNAs
might potentially mediate the HS-associated damage. Little existing research demonstrates the presence of miRNAs in exosomes during HS. Our previous first relevant study is conducted in exosomes derived from human vascular endothelial cells exposed to 41°C hyperthermal stress [10]. The gene expression profiles in lung of a HS rat model reveals that the miRNAs related to inflammatory/immune responses, including leukocyte migration, response to lipopolysaccharide, NIK/NF-kappaB signaling, and response to reactive oxygen species, are up-regulated, which is consistent with our results [11]. Whole blood mRNA and microRNA was identified as biomarkers of tissue damage and immune function resulting from heat stroke exposure [12].

In other acute critical inflammatory disease, miRNAs exert vital parts in the immune/inflammatory responses in the context of trauma and sepsis [13]. In one work, investigators carry out miRNA profiling analysis on 10 subjects with polytrauma, sepsis and septic shock, and some differentially expressed miRNAs are identified [14]. In Real et al's study that enrolled 24 septic shock patients, the circulating exosomal miRNAs significantly altered and the same expression kinetics was maintained during the course of disease [9]. Such results possibly suggested that the body responded in a similar way to that of severe inflammatory injury.

MiR-150, one of the earliest miRNAs detected in critically ill patients or those with sepsis. Through microarray gene expression profiling, numerous reviewers have reported that miR-150 is a part of the miRNA panels with abnormal expression within leukocytes/PBMC obtained from septic patients in comparison with normal subjects [15-17]. Such alterations are reflected by the consistent changes in the miR-150 expression in serum. For instance, Vasilescu et al. discovered that miR-150 expression was down-regulated in 16 cases with abdominal sepsis [15]. The reduced miR-150 expression in serum was related to the increased SOFA score and sepsis severity. Furthermore, Ma et al. [18] discovered that miR-150 expression was down-regulated in septic patients in comparison with those having non-infectious SIRS or normal subjects.

MiRNA-122 was one of the most significantly enriched miRNAs in our profiling. Changes in its serum concentration have also been confirmed to significantly increase in systemic inflammatory diseases such as sepsis, which is related to the inflammatory response level and considered to be a reliable biomarker for early disease stratification and prognostic evaluation [19]. The level of miRNA-122 can predict the degrees of inflammation and organ injury such as ARDS [20] and coagulation disorders [21]. According to the longitudinal samples obtained from septic patients, the miR-122 level markedly increased on day 14 upon admission to ICU, which was strongly related to antithrombin III (R = 0.913, p < 0.001) [21]. For determining the direct or indirect impacts of miRNAs on coagulation, the crosstalk between cytokines, miRNAs dysregulation, and thrombocyte synthesis/apoptosis should be identified in more studies.

MiRNA-155 was also among the most upregulated miRNAs in our study. In a heatstress cell model, microRNA-155 promotes heat stress-induced inflammatory responses in microglia by facilitating inflammatory factors expression by increasing NF-κB pathway activation via targeting liver X receptor α [22].
Little is known about the interactions between exosomal miRNAs and the corresponding targets for exerting the specific activities. A majority of investigators propose that exosomes can serve as the effective carriers to carry genetic materials and proteins to the surrounding or the distant cells. Nonetheless, it is still largely unknown about the exosome-recipient cell interaction mechanism. Such interaction generally begins with the internalization of exosomes via a variety of proposed pathways, such as the phagocytosis, clathrin-mediated endocytosis, as well as macropinocytosis [23]. The exosomal miRNAs and mRNAs in recipient cells have certain functions and may have interactions with related targets for synthesizing novel proteins or modulating the gene level [24]. It is usually suggested in the laboratory cells; as a result, the interaction of exosomal genetic contents with the target cells in the case of HS is still speculative.

Certain limitations should be noted in this work. Firstly, the present exploratory study enrolled few patients, and the results obtained were interpreted as hypothesis-generating. Additionally, only cases at the the first HS episode were enrolled in this work, for the sake of preventing subjects who had immunosuppression or chronic inflammation possibly affecting our results. It might thus restrict the generalizability of results in this study. The plasma exosome counts were not determined in either HS patients or normal controls, as a result, it was impossible to estimate the impacts of HS on exosome number, and the contents of exosomal miRNAs and mRNAs were not normalized relative to the plasma exosome amount. Moreover, only mRNAs associated with coagulation activation and inflammatory response, since they have been suggested in previous studies to regulate exosomes in HS. Using such method, it was feasible to assess additional pathophysiological targets for exosomal mRNAs. For comparing the effects between infectious and noninfectious injuries, the control group constituted by infected ICU cases was not enrolled in this work.

**Conclusions**

Exosomes from patients with heat stroke convey genetic material that may be related to key pathways in the pathogenesis of heat stroke, including inflammatory response, blood coagulation, and platelet activation. Further functional studies are required to clarify the exact contribution of these vesicles to the exchange of genetic material and intercellular communication during heat stroke.

**Declarations**

**Ethics approval and consent to participate**

All animal experiments were conducted in compliance with the criteria outlined in the Guide for the Care and Use of Laboratory Animals (National Institutes of Health publication 86-23, 1985 revision) and were approved by the Animal Care and Use Committee of the General Hospital of Guangzhou Military Command. Blood sample collection from HS patients was approved by the Ethics Committee of the General Hospital of Guangzhou Military Command. Informed consent was obtained from the patients or their representatives.
Consent for publication

Not applicable.

Availability of data and material

All data generated or analysed during this study are included in this published article [and its supplementary information files].

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Huasheng Tong supervised the complete study. Yue Li designed the complete study and performed the NTA, western-blotting, statistical analysis as well as manuscript writing. Qiang Wen performed the isolation of exosomes and data collection. Xinghui Wu and Huasheng Chen performed the TEM. Lei Su performed the clinical data and blood sample collection.

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Tables

Table 1. Basic clinical characteristics and disease severity scores of the participants

| Characteristics                                      | Healthy controls (N=3) | Severe HS (N=6) | P value |
|------------------------------------------------------|------------------------|-----------------|---------|
| ICU length of stay (days), median (interquartile range) | 0 (0)                  | 10 (3–17.45)    | <0.001 |
| T (°C)                                               | 36.42 ± 0.46           | 39.00 ± 1.94    | <0.001 |
| APACHE II score                                      | 0.47 ± 0.83            | 17.05 ± 4.35    | <0.001 |
| SOFA score                                           | 0.6 ± 0.63             | 10.95 ± 5.64    | <0.001 |

APACHE, Acute Physiology and Chronic Health Evaluation; ICU, intensive care unit; SOFA, Sequential Organ Failure Assessment; T, body temperature

Table 2. Comparison of clinical and laboratory indexes of patients with heat stroke and healthy controls according to day of admission and outcome status
| Characteristics       | Healthy controls (N=3) | Severe HS (N=6) | P value |
|-----------------------|------------------------|----------------|---------|
| **Hemodynamic data**  |                        |                |         |
| HR (beats/min)        | 74.3 ± 7.74            | 96.85 ± 32.43  | <0.001  |
| MAP (mmHg)            | 77.6 ± 6.99            | 72.15 ± 20.95  | 0.782   |
| Vasoactive drug, n (%)| 0 (0)                  | 3 (50)         | <0.001  |
| Lactate (µmol/L)      | 1.07 ± 0.47            | 3.11 ± 2.55    | <0.001  |
| **Ventilatory data**  |                        |                |         |
| PaO₂/FiO₂             | 378.7 ± 72.25          | 312.3 ± 67.56  | 0.007   |
| MV, n (%)             | 0 (0)                  | 6 (67)         | <0.001  |
| **Inflammatory data** |                        |                |         |
| WBC (×10⁹ cells/L)    | 9.42 ± 3.14            | 10.81 ± 4.47   | <0.001  |
| PCT (ng/ml)           | 0.34 ± 0.34            | 4.06 ± 4.14    | <0.001  |
| **Hepatic data**      |                        |                |         |
| ALT (U/L)             | 25.51 ± 13.7           | 1448 ± 2360    | <0.001  |
| AST (U/L)             | 22.4 ± 13.8            | 2144 ± 3861    | 0.001   |
| TBil (µmol/L)         | 9.23 ± 4.45            | 62.83 ± 93.67  | <0.001  |
| ALB (g/L)             | 40.79 ± 5.36           | 37.56 ± 3.51   | 0.302   |
| **Renal data**        |                        |                |         |
| Cr (µmol/L)           | 95.4 ± 27.28           | 161.1 ± 84.24  | <0.001  |
| BUN (mmol/L)          | 5.51 ± 2.19            | 8.03 ± 6.73    | <0.001  |
| Urine output (ml/d)   | 2680 ± 727.2           | 2095 ± 1369    | <0.001  |
| **Coagulation data**  |                        |                |         |
| PT (s)                | 13.39 ± 0.93           | 25.67 ± 15.61  | <0.001  |
| INR                   | 13.39 ± 0.93           | 2.50 ± 2.21    | <0.001  |
| Fib (g/L)             | 3.53 ± 0.66            | 2.05 ± 0.76    | <0.001  |
| PLT (×10⁹/L)          | 219.6 ± 65.05          | 101.2±61.35    | <0.001  |
| D-dimer               | 1.46 ± 1.32            | 15.77 ± 5.96   | <0.001  |
| FDP                   | 6.83 ± 2.88            | 100.8 ± 184.3  | 0.001   |
| **Rhabdomyo data**    |                        |                |         |
| ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CK, creatine kinase; CK-MB, CK-myocardial band; CNS, central nervous system; Cr, creatinine; cTnI, cardiac troponin I; FDP, fibrin degradation product; Fib, fibrin; FiO₂, percentage of inspired oxygen; GCS, Glasgow Coma Scale; HR, heart rate; INR, international normalized ratio; MAP, mean arterial pressure; MV, mechanical ventilation; MYO, myoglobin; PaO₂, partial pressure of arterial oxygen; PCT, procalcitonin; PLT, platelet; PT, prothrombin time; TBil, total bilirubin; WBC, white blood cell |
|---|---|---|---|
| **Cardiac data** | | | |
| CK (μg/L) | 54.33 ± 23.52 | 5155 ± 5888 | <0.001 |
| MYO (μg/L) | 48.15 ± 24.91 | 1374 ± 964.4 | <0.001 |
| **CNS data** | | | |
| GCS score | 15 ± 0 | 8.3 ± 4.28 | <0.001 |

Table 3 miRNAs dysregulated in HS-hepatocyte EVs identified by NGS analysis
| AccID       | log2FC         | Pvalue          | FDR             | Style |
|------------|----------------|-----------------|-----------------|-------|
| hsa-miR-511-3p | 10.44555535 | 0.001051524 | 0.014788254 | up    |
| hsa-miR-122-5p | 8.276723708 | 0.005868702 | 0.046320826 | up    |
| hsa-miR-155-3p | 7.658481446 | 5.18604E-05  | 0.002694095 | up    |
| hsa-miR-1290 | 7.535022672 | 1.23381E-07  | 3.42525E-05  | up    |
| hsa-let-7b-5p | 7.081014395 | 0.000227207 | 0.004955503 | up    |
| hsa-miR-1298-5p | 6.74576804 | 2.58948E-05  | 0.001820878 | up    |
| hsa-miR-193a-5p | 6.602101886 | 7.49325E-05  | 0.002851128 | up    |
| hsa-miR-855-5p | 6.418510241 | 0.000272081 | 0.005628786 | up    |
| hsa-miR-let-7c-5p | 6.297028039 | 2.95983E-09  | 1.52628E-06  | up    |
| hsa-miR-363-3p | 6.269409954 | 1.54789E-13  | 1.19729E-10  | up    |
| hsa-miR-618 | 6.231316715 | 0.000276527 | 0.005628786 | up    |
| hsa-miR-29b-3p | 6.164685475 | 0.00733793  | 0.011519171 | up    |
| hsa-miR-99b-3p | 6.147758159 | 4.93893E-14  | 7.64053E-11  | up    |
| hsa-miR-485-3p | 6.083130973 | 6.25082E-07  | 0.000123803 | up    |
| hsa-miR-1243 | 6.080205186 | 6.40223E-07  | 0.000123803 | up    |
| hsa-miR-193a-5p | 6.069448799 | 1.32848E-07  | 3.42525E-05  | up    |
| hsa-miR-137-5p | 6.047009206 | 0.000763775 | 0.011815601 | up    |
| hsa-miR-590-5p | 6.037288962 | 0.000383296 | 0.007144091 | up    |
| hsa-miR-511-5p | 5.905742669 | 1.7233E-06   | 0.000242359 | up    |
| hsa-miR-1267 | 5.896199662 | 3.28785E-05  | 0.002088438 | up    |
| hsa-miR-148a-3p | 5.865441423 | 0.001261164 | 0.016395129 | up    |
| hsa-miR-30d-5p | 5.831116516 | 0.002248497 | 0.024495949 | up    |
| hsa-miR-486-5p | 5.564447631 | 1.41289E-05  | 0.001264144 | up    |
| hsa-miR-548a-3p | 5.552914827 | 7.19604E-05  | 0.002851128 | up    |
| hsa-miR-99a-5p | 5.39603918  | 5.92159E-06  | 0.000654335 | up    |
| hsa-miR-520d-5p | 5.3697579  | 0.000148742 | 0.003900067 | up    |
| hsa-miR-518b-3p | 5.51551199 | 0.001133096 | 0.015360113 | up    |
| hsa-miR-140-3p | 5.481357457 | 0.000181949 | 0.004264777 | up    |
| miRNA         | Value       | P-value   | FDR         | Status |
|--------------|-------------|-----------|-------------|--------|
| hsa-miR-508-5p | 5.458111243 | 0.006282607 | 0.048114816 | up     |
| hsa-miR-592   | 5.447297769 | 0.000167126 | 0.004147149 | up     |
| hsa-miR-324-3p| 5.445343033 | 0.004338065 | 0.03791518  | up     |
| hsa-miR-296-5p| 5.440772306 | 0.000518532 | 0.008680444 | up     |
| hsa-miR-1285-3p| 5.426482304 | 0.001343155 | 0.017066652 | up     |
| hsa-miR-598-3p| 5.413461255 | 0.00110185  | 0.003626728 | up     |
| hsa-miR-766-3p| 5.384247124 | 0.002518532 | 0.027271096 | up     |
| hsa-miR-614   | 5.346661476 | 0.002538845 | 0.036066845 | up     |
| hsa-miR-1183  | 5.313071929 | 0.000110185 | 0.003626728 | up     |
| hsa-miR-339-3p| 5.306714553 | 0.00124834  | 0.003696458 | up     |
| hsa-miR-1233-3p| 5.264430569 | 0.000274062 | 0.005628786 | up     |
| hsa-miR-188-3p| 5.226803605 | 0.001100185 | 0.003626728 | up     |
| hsa-miR-625-3p| 5.192911687 | 0.000778011 | 0.011916657 | up     |
| hsa-miR-875-3p| 5.174336191 | 0.00086226  | 0.012950644 | up     |
| hsa-miR-758-3p| 5.167747909 | 2.39966E-06 | 0.000309356 | up     |
| hsa-miR-54a-5p| 5.152096449 | 1.20951E-07 | 3.42525E-05 | up     |
| hsa-miR-27a   | 5.141395196 | 0.002041626 | 0.023395526 | up     |
| hsa-miR-34a   | 5.138801488 | 0.00125247  | 0.016395129 | up     |
| hsa-miR-125b-5p| 5.122676194 | 1.33884E-05 | 0.001264144 | up     |
| hsa-miR-15a   | 5.110036409 | 0.000359735 | 0.006786705 | up     |
| hsa-miR-558   | 5.051441121 | 0.005306746 | 0.043667749 | up     |
| hsa-miR-483-5p| 4.985278141 | 6.82155E-05 | 0.002851128 | up     |
| hsa-miR-1277-3p| 4.96612105  | 0.001033352 | 0.014666017 | up     |
| hsa-miR-1260a | 4.931265335 | 0.000137917 | 0.003743124 | up     |
| hsa-miR-122-3p| 4.882978843 | 6.46948E-05 | 0.002851128 | up     |
| hsa-miR-16-5p | 4.870571508 | 4.4788E-05  | 0.002474535 | up     |
| hsa-miR-378c  | 4.865473522 | 3.50998E-05 | 0.002088438 | up     |
| hsa-miR-192-5p| 4.819906165 | 0.00130442  | 0.003696458 | up     |
| miRNA          | Fold Change | Log2 Fold Change | P-Value  | Adj. P-Value | Status |
|---------------|-------------|------------------|----------|--------------|--------|
| hsa-miR-122b-5p | 4.813043234 | 7.55632E-05      | 0.002851128 | up          |
| hsa-miR-548b-3p | 4.787050472 | 0.005772356      | 0.046320826 | up          |
| hsa-miR-194-5p  | 4.781402495 | 0.0015155        | 0.003907475 | up          |
| hsa-miR-658     | 4.766242612 | 0.004135298      | 0.036983224 | up          |
| hsa-miR-195-5p  | 4.723631785 | 0.003657478      | 0.035361395 | up          |
| hsa-miR-19a-3p  | 4.701557418 | 0.003604788      | 0.035294977 | up          |
| hsa-miR-885-5p  | 4.670729991 | 0.000147017      | 0.003900067 | up          |
| hsa-miR-25-3p   | 4.668210424 | 0.000147017      | 0.003900067 | up          |
| hsa-miR-676-3p  | 4.635212949 | 0.000147017      | 0.003900067 | up          |
| hsa-miR-30a-5p  | 4.633464441 | 0.000147017      | 0.003900067 | up          |
| hsa-miR-378e    | 4.573833884 | 6.24059E-05      | 0.002851128 | up          |
| hsa-miR-320a    | 4.545202766 | 7.5698E-07       | 0.000130116 | up          |
| hsa-miR-518d-3p | 4.523946854 | 1.83062E-05      | 0.001415983 | up          |
| hsa-miR-532-3p  | 4.438273684 | 0.005937185      | 0.046435358 | up          |
| hsa-miR-152-3p  | 4.434831855 | 1.47089E-05      | 0.001264144 | up          |
| hsa-miR-885-3p  | 4.343597305 | 0.000104981      | 0.003530551 | up          |
| hsa-miR-548c-3p | 4.298413102 | 0.001422001      | 0.017598688 | up          |
| hsa-miR-597-5p  | 4.291099833 | 0.00423662       | 0.037238931 | up          |
| hsa-miR-636     | 4.286856265 | 0.00041197       | 0.00741067 | up          |
| hsa-miR-645     | 4.261328603 | 0.000119754      | 0.003696458 | up          |
| hsa-miR-720     | 4.245115    | 0.004098041      | 0.036983224 | up          |
| hsa-miR-378g    | 4.242244907 | 7.1321E-05       | 0.002851128 | up          |
| hsa-miR-449b-3p | 4.190458798 | 0.005779387      | 0.046320826 | up          |
| hsa-miR-886-5p  | 4.181509179 | 0.000737167      | 0.011519171 | up          |
| hsa-miR-133a-5p | 4.163298014 | 5.89909E-05      | 0.002851128 | up          |
| hsa-miR-92a-3p  | 4.117957927 | 0.001603042      | 0.019526823 | up          |
| hsa-miR-549a    | 4.093083013 | 0.02382692       | 0.025776395 | up          |
| hsa-miR-455-3p  | 4.085035465 | 0.000201408      | 0.004650421 | up          |
| hsa-miR-875-5p  | 4.084769606 | 0.004764889      | 0.04028024  | up          |
| miRNA          | log2FoldChange | p-value 1 | p-value 2 | Status |
|----------------|----------------|-----------|-----------|--------|
| hsa-miR-378j   | 4.067935221    | 0.000172496 | 0.004147149 | up     |
| hsa-miR-100-5p | 4.064359746    | 0.000528185 | 0.004880873 | up     |
| hsa-miR-378f   | 4.058976779    | 0.000214544 | 0.004880873 | up     |
| hsa-miR-323b-5p| 4.049295395    | 0.002076178 | 0.023569945 | up     |
| hsa-miR-378d   | 3.967150304    | 0.000303754 | 0.006024464 | up     |
| hsa-miR-378a-3p| 3.887692985    | 0.000172964 | 0.004147149 | up     |
| hsa-miR-99b-5p | 3.87989185     | 0.000860374 | 0.012950644 | up     |
| hsa-miR-675-3p | 3.863440724    | 0.000984691 | 0.014370908 | up     |
| hsa-miR-125b-1-3p| 3.858649521  | 0.000355423 | 0.006786705 | up     |
| hsa-miR-133a-3p| 3.856130758    | 0.000397748 | 0.007316155 | up     |
| hsa-miR-156-3p | 3.828665539    | 0.001070782 | 0.014909039 | up     |
| hsa-miR-422a   | 3.821697972    | 0.002089088 | 0.023569945 | up     |
| hsa-miR-508-5p | 3.773055535    | 0.004143019 | 0.036983224 | up     |
| hsa-miR-455-5p | 3.74855908     | 3.54643E-06 | 0.000422025 | up     |
| hsa-miR-30a-5p | 3.722860052    | 1.74445E-05 | 0.001415983 | up     |
| hsa-miR-362-3p | 3.708667691    | 0.002089088 | 0.023569945 | up     |
| hsa-miR-210-3p | 3.706738617    | 0.001018893 | 0.014594692 | up     |
| hsa-miR-80     | 3.690830266    | 0.004398526 | 0.038227641 | up     |
| hsa-miR-428    | 3.690701315    | 0.00017425  | 0.004147149 | up     |
| hsa-miR-686-5p | 3.563674461    | 0.004159716 | 0.036983224 | up     |
| hsa-miR-557-3p | 3.54859907     | 0.005170255 | 0.042854747 | up     |
| hsa-miR-127-5p | 3.534359038    | 0.005180244 | 0.042854747 | up     |
| hsa-miR-574-5p | 3.460709408    | 0.001686106 | 0.020064661 | up     |
| hsa-miR-95-5p  | 3.450983644    | 0.004151843 | 0.036983224 | up     |
| hsa-miR-672-5p | 3.426905205    | 0.000131419 | 0.003696458 | up     |
| hsa-miR-378h   | 3.399669168    | 0.001305573 | 0.016831014 | up     |
| hsa-miR-499b-3p| 3.367588817    | 0.000512354 | 0.008680444 | up     |
| hsa-miR-499a-5p| 3.321524527    | 0.000401987 | 0.007316155 | up     |
| hsa-miR-873-5p | 3.303504919    | 0.002108672 | 0.023569945 | up     |
| miRNA      | Fold Change | P-value   | q-value  | Status |
|------------|-------------|-----------|----------|--------|
| hsa-miR-468 | 3.242848143 | 0.000313963 | 0.006071253 | up     |
| hsa-miR-188-5p | 3.202653388 | 0.001920073 | 0.022166816 | up     |
| hsa-miR-501-5p | 3.201586587 | 0.001662516 | 0.019937302 | up     |
| hsa-miR-375-3p | 3.198625055 | 0.004051092 | 0.036983224 | up     |
| hsa-miR-601 | 3.183032738 | 0.001142479 | 0.015360113 | up     |
| hsa-miR-313-3p | 3.181596542 | 0.003044582 | 0.031191848 | up     |
| hsa-miR-125b-5p | 3.177573994 | 0.001729595 | 0.020425067 | up     |
| hsa-miR-480-3p | 3.139771854 | 0.000225445 | 0.004955503 | up     |
| hsa-miR-141-5p | 3.135945816 | 0.004088257 | 0.036983224 | up     |
| hsa-miR-475-5p | 3.123371628 | 0.004584576 | 0.039401888 | up     |
| hsa-miR-660-3p | 3.107484568 | 0.001847427 | 0.021651279 | up     |
| hsa-miR-208b-3p | 3.076327192 | 0.003236933 | 0.032306675 | up     |
| hsa-miR-619-5p | 3.068163387 | 0.000131118 | 0.003696458 | up     |
| hsa-miR-36a-5p | 3.0546317   | 0.000115998 | 0.003666773 | up     |
| hsa-miR-650-3p | 3.050923963 | 0.003925388 | 0.036983224 | up     |
| hsa-miR-497-5p | 3.01762823  | 2.81112E-05  | 0.001890783 | up     |
| hsa-miR-480-5p | 3.014405265 | 0.001079387 | 0.014909039 | up     |
| hsa-miR-148a-3p | 2.988162684 | 0.005949996 | 0.046435358 | up     |
| hsa-miR-124-5p | 2.951062151 | 0.00395291  | 0.036983224 | up     |
| hsa-miR-21-3p  | 2.934507665 | 0.004686581 | 0.039835941 | up     |
| hsa-miR-391   | 2.929568628 | 0.000559096 | 0.009009607 | up     |
| hsa-miR-193b-3p | 2.918888063 | 0.005446315 | 0.044344472 | up     |
| hsa-miR-95-3p | 2.908344044 | 0.000872945 | 0.01298506 | up     |
| hsa-miR-365b-3p | 2.888195604 | 0.001576713 | 0.019358531 | up     |
| hsa-miR-675-5p | 2.883829012 | 0.001414023 | 0.017598688 | up     |
| hsa-miR-500a-3p | 2.828996605 | 0.005840891 | 0.046320826 | up     |
| hsa-miR-463   | 2.820851921 | 8.29863E-05  | 0.002989247 | up     |
| hsa-miR-770   | 2.80885692  | 0.000460923 | 0.008011765 | up     |
| hsa-miR-133b  | 2.733475223 | 0.002157145 | 0.023667401 | up     |
| miRNA          | Log2 Fold Change | p-value  | FDR          | Status |
|---------------|-----------------|----------|--------------|--------|
| hsa-miR-467   | 2.727764422     | 0.006033295 | 0.046435358 | up     |
| hsa-miR-130-5p| 2.583022076     | 0.004642189 | 0.039676606 | up     |
| hsa-miR-125a-5p| 2.581138745   | 0.003159096 | 0.031941968 | up     |
| hsa-miR-99    | 2.579135083     | 0.003921596 | 0.031941968 | up     |
| hsa-miR-365a-3p| 2.507688657   | 0.005501005 | 0.044555261 | up     |
| hsa-miR-448   | 2.39972093      | 0.004084038 | 0.036983224 | up     |
| hsa-miR-394-5p| 2.283892989     | 0.006021612 | 0.046435358 | up     |
| hsa-miR-29a-3p| 2.268261556     | 0.00115176  | 0.015360113 | up     |
| hsa-miR-512-3p| 2.126801502     | 0.005430677 | 0.044344472 | up     |
| hsa-miR-475-3p| 1.990553632     | 0.005843288 | 0.046320826 | up     |
| hsa-miR-30e-3p| -1.835550942    | 0.004945721 | 0.0415597   | down   |
| hsa-miR-222-3p| -1.856156913    | 0.00164635  | 0.019897684 | down   |
| hsa-miR-584-5p| -2.009084984    | 0.002266844 | 0.037238931 | down   |
| hsa-miR-671-3p| -2.014744985    | 0.002636901 | 0.02813301  | down   |
| hsa-miR-340-5p| -2.030456926    | 0.003124684 | 0.031803918 | down   |
| hsa-miR-654-5p| -2.110003521    | 0.003565424 | 0.035131917 | down   |
| hsa-miR-30b-3p| -2.192120837    | 0.004969971 | 0.0415597   | down   |
| hsa-miR-766-5p| -2.212703259    | 0.000521837 | 0.008680444 | down   |
| hsa-miR-30c-1-3p| -2.31408293   | 0.003994987 | 0.036983224 | down   |
| hsa-miR-374b-5p| -2.343572476   | 0.003917189 | 0.036983224 | down   |
| hsa-miR-432-5p| -2.358199166    | 0.002717244 | 0.028791614 | down   |
| hsa-miR-374c-3p| -2.358739972   | 0.002871652 | 0.029815068 | down   |
| hsa-miR-331-3p| -2.435047906    | 0.001030414 | 0.014507302 | down   |
| hsa-miR-130b-5p| -2.469418509   | 0.002127476 | 0.023569945 | down   |
| hsa-miR-224-3p| -2.477360429    | 0.006010819 | 0.04635358  | down   |
| hsa-miR-135a-5p| -2.506163407   | 0.001895531 | 0.022045503 | down   |
| hsa-miR-199a-5p| -2.513372321   | 0.003633528 | 0.035326256 | down   |
| hsa-miR-98-5p | -2.557471672    | 0.004966068 | 0.007904952 | down   |
| hsa-miR-186-5p| -2.72167449     | 0.001115982 | 0.015278093 | down   |
| miRNA               | Log2 Fold Change | P-value | FDR   | Status |
|---------------------|-----------------|---------|-------|--------|
| hsa-miR-487b-3p     | -2.729215601    | 0.000241089 | 0.005180058 | down   |
| hsa-miR-454-3p      | -2.748583439    | 0.0002930923 | 0.030227591 | down   |
| hsa-miR-495-3p      | -2.758027548    | 0.000420334 | 0.00747421  | down   |
| hsa-miR-26a-5p      | -2.763384301    | 0.003216004 | 0.03230622  | down   |
| hsa-miR-409-3p      | -2.820796229    | 0.001345916 | 0.017066652 | down   |
| hsa-miR-543         | -2.850172237    | 0.000680847 | 0.010858462 | down   |
| hsa-miR-296-5p      | -2.931642939    | 8.30883E-05  | 0.002989247 | down   |
| hsa-miR-1185-1-3p   | -2.947501718    | 0.000538761 | 0.008773296 | down   |
| hsa-miR-329-3p      | -3.010672012    | 0.000102978 | 0.003530551 | down   |
| hsa-miR-340-3p      | -3.09443468     | 0.000302301 | 0.006024464 | down   |
| hsa-miR-200a-5p     | -3.107804542    | 6.20481E-05  | 0.002851128 | down   |
| hsa-miR-221-3p      | -3.134305694    | 0.001390292 | 0.017486025 | down   |
| hsa-miR-26a-5p      | -3.171505823    | 4.01985E-05  | 0.002303226 | down   |
| hsa-miR-758-3p      | -3.216623778    | 0.000256334 | 0.005432171 | down   |
| hsa-miR-323b-3p     | -3.255988987    | 0.000508585 | 0.008680444 | down   |
| hsa-miR-224-5p      | -3.363071212    | 0.002133027 | 0.023569945 | down   |
| hsa-miR-485-3p      | -3.422140644    | 0.004446777 | 0.038431083 | down   |
| hsa-miR-487a-5p     | -3.426603802    | 0.000123266 | 0.003696458 | down   |
| hsa-miR-194-5p      | -3.902033656    | 0.000116142 | 0.003666773 | down   |
| hsa-miR-411-3p      | -3.936102911    | 5.22449E-05  | 0.002694095 | down   |
| hsa-miR-196a-5p     | -3.960486658    | 7.3528E-05   | 0.002851128 | down   |
| hsa-miR-379-3p      | -4.028025819    | 3.48353E-05  | 0.002088438 | down   |
| hsa-miR-16-5p       | -4.037789955    | 0.000311387 | 0.006071253 | down   |
| hsa-miR-151a-3p     | -4.170054878    | 1.18001E-06  | 0.000182547 | down   |
| hsa-miR-146a-5p     | -4.181358731    | 0.000158479 | 0.004019119 | down   |
| hsa-miR-150-3p      | -4.335973238    | 0.000134263 | 0.003709015 | down   |
Table 4 Differentially expressed targeted mRNAs associated with inflammatory response
| Gene symbol | Protein Name |
|-------------|--------------|
| NFAM1       | NFAT activating protein with ITAM motif 1 |
| LYN         | LYN proto-oncogene, Src family tyrosine kinase |
| NRROS       | negative regulator of reactive oxygen species |
| DAB2IP      | DAB2 interacting protein |
| IGFBP4      | insulin-like growth factor binding protein 4 |
| PRKD1       | protein kinase D1 |
| SP100       | SP100 nuclear antigen |
| IL25        | interleukin 25 |
| CRLF2       | cytokine receptor-like factor 2 |
| PSTPIP1     | proline-serine-threonine phosphatase interacting protein 1 |
| NLRP2       | NLR family, pyrin domain containing 2 |
| NFKBIZ      | nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, zeta |
| AOAH        | acyloxyacyl hydrolase (neutrophil) |
| PIK3C2A     | phosphatidylinositol-4-phosphate 3-kinase, catalytic subunit type 2 alpha |
| CXCR1       | chemokine (C-X-C motif) receptor 1 |
| THEMIS2     | thymocyte selection associated family member 2 |
| RELA        | v-rel avian reticuloendotheliosis viral oncogene homolog A |
| IL17C       | interleukin 17C |
| CCR4        | chemokine (C-C motif) receptor 4 |
| NDST1       | N-deacetylase/N-sulfotransferase (heparan glucosaminyl) 1 |
| FOLR2       | folate receptor 2 (fetal) |
| CHST4       | carbohydrate (N-acetylglucosamine 6-O) sulfotransferase 4 |
| TBXA2R      | thromboxane A2 receptor |
| CCL5        | chemokine (C-C motif) ligand 5 |
| APOL3       | apolipoprotein L, 3 |
| IL13        | interleukin 13 |
| TNIP2       | TNFAIP3 interacting protein 2 |
| TGFB1       | transforming growth factor, beta 1 |
| Gene Symbol | Description                        |
|-------------|------------------------------------|
| HRH1        | histamine receptor H1              |
| CCL21       | chemokine (C-C motif) ligand 21     |
| PROK2       | prokineticin 2                     |
| CXCR6       | chemokine (C-X-C motif) receptor 6 |
| BMPR1B      | bone morphogenetic protein receptor, type IB |
| PTGDR       | prostaglandin D2 receptor (DP)     |
| PRDX5       | peroxiredoxin 5                    |
| CXCL6       | chemokine (C-X-C motif) ligand 6   |
| CXCL10      | chemokine (C-X-C motif) ligand 10  |
| ADORA2A     | adenosine A2a receptor             |
| NLRP4       | NLR family, pyrin domain containing 4 |
| CCL2        | chemokine (C-C motif) ligand 2     |
| CYBA        | cytochrome b-245, alpha polypeptide |
| JAK3        | Janus kinase 3                     |
| PIK3CD      | phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit delta |
| CXCR4       | chemokine (C-X-C motif) receptor 4 |
| CCR5        | chemokine (C-C motif) receptor 5 (gene/pseudogene) |
| TNFAIP6     | tumor necrosis factor, alpha-induced protein 6 |
| APOC3       | apolipoprotein C-III               |
| IKBKG       | inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase gamma |
| CD40LG      | CD40 ligand                        |
| CHST2       | carbohydrate (N-acetylglucosamine-6-O) sulfotransferase 2 |
| AIMP1       | aminoacyl tRNA synthetase complex-interacting multifunctional protein 1 |
| ADORA1      | adenosine A1 receptor              |
| TSPAN2      | tetraspanin 2                      |
| TLR7        | toll-like receptor 7               |
| CXCL11      | chemokine (C-X-C motif) ligand 11  |
| KIT         | v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog |
| KDM6B       | lysine (K)-specific demethylase 6B  |
| Gene Symbol | Description |
|-------------|-------------|
| LGALS9      | lectin, galactoside-binding, soluble, 9 |
| AFAP1L2     | actin filament associated protein 1-like 2 |
| VNN1        | vanin 1 |
| S100A9      | S100 calcium binding protein A9 |
| PIK3CB      | phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit beta |
| NOD1        | nucleotide-binding oligomerization domain containing 1 |
| TNIP3       | TNFAIP3 interacting protein 3 |
| CCL26       | chemokine (C-C motif) ligand 26 |
| NFKB2       | nuclear factor of kappa light polypeptide gene enhancer in B-cells 2 (p49/p100) |
| NFATC3      | nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 3 |
| FFAR2       | free fatty acid receptor 2 |
| IL1RAP      | interleukin 1 receptor accessory protein |
| IL2RA       | interleukin 2 receptor, alpha |
| CCL22       | chemokine (C-C motif) ligand 22 |
| PIK3C2G     | phosphatidylinositol-4-phosphate 3-kinase, catalytic subunit type 2 gamma |
| TLR5        | toll-like receptor 5 |
| TPST1       | tyrosylprotein sulfotransferase 1 |
| BMP6        | bone morphogenetic protein 6 |
| CCR3        | chemokine (C-C motif) receptor 3 |
| HDAC9       | histone deacetylase 9 |
| CCL7        | chemokine (C-C motif) ligand 7 |
| OLR1        | oxidized low density lipoprotein (lectin-like) receptor 1 |
| IDO1        | indoleamine 2,3-dioxygenase 1 |
| IL23R       | interleukin 23 receptor |
| TRIL        | TLR4 interactor with leucine-rich repeats |
| TUSC2       | tumor suppressor candidate 2 |
| C5AR1       | complement component 5a receptor 1 |
| S1PR3       | sphingosine-1-phosphate receptor 3 |
| IL10RB      | interleukin 10 receptor, beta |
| Gene Symbol | Gene Name |
|-------------|-----------|
| ELF3 | E74-like factor 3 (ets domain transcription factor, epithelial-specific) |
| JMJD7-PLA2G4B | JMJD7-PLA2G4B readthrough |
| CD40 | CD40 molecule, TNF receptor superfamily member 5 |
| SCN9A | sodium channel, voltage-gated, type IX, alpha subunit |
| ITGAL | integrin, alpha L (antigen CD11A (p180), lymphocyte function-associated antigen 1; alpha polypeptide) |
| CAMK1D | calcium/calmodulin-dependent protein kinase ID |
| LY86 | lymphocyte antigen 86 |
| PLA2G4C | phospholipase A2, group IVC (cytosolic, calcium-independent) |
| TNFRSF1A | tumor necrosis factor receptor superfamily, member 1A |
| PIK3CA | phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha |
| RPS6KA5 | ribosomal protein S6 kinase, 90kDa, polypeptide 5 |
| FPR2 | formyl peptide receptor 2 |
| TNFRSF1B | tumor necrosis factor receptor superfamily, member 1B |
| GPR68 | G protein-coupled receptor 68 |
| ATRN | attractin |
| SYK | spleen tyrosine kinase |
| XCL2 | chemokine (C motif) ligand 2 |
| MS4A2 | membrane-spanning 4-domains, subfamily A, member 2 |
| F2RL1 | coagulation factor II (thrombin) receptor-like 1 |
| IL36A | interleukin 36, alpha |
| PXK | PX domain containing serine/threonine kinase |
| HDAC5 | histone deacetylase 5 |
| PRKCQ | protein kinase C, theta |
| SPHK1 | sphingosine kinase 1 |
| ADAM8 | ADAM metallopeptidase domain 8 |
| CXCL9 | chemokine (C-X-C motif) ligand 9 |
| TICAM2 | toll-like receptor adaptor molecule 2 |
| PLA2G2D | phospholipase A2, group IID |
| Gene Symbol | Description |
|-------------|-------------|
| CCL25       | chemokine (C-C motif) ligand 25 |
| IL22        | interleukin 22 |
| MIF         | macrophage migration inhibitory factor (glycosylation-inhibiting factor) |
| LYZ         | lysozyme |
| NGF         | nerve growth factor (beta polypeptide) |
| PDPN        | podoplanin |
| PRKCZ       | protein kinase C, zeta |
| PIK3C2B     | phosphatidylinositol-4-phosphate 3-kinase, catalytic subunit type 2 beta |
| KNG1        | kininogen 1 |
| CCL3L3      | chemokine (C-C motif) ligand 3-like 3 |
| NFATC4      | nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 4 |
| AZU1        | azurocidin 1 |
| IL5         | interleukin 5 |
| ADGRE2      | egf-like module containing, mucin-like, hormone receptor-like 2 |
| CEBPB       | CCAAT/enhancer binding protein (C/EBP), beta |
| HRH4        | histamine receptor H4 |
| CCR1        | chemokine (C-C motif) receptor 1 |
| CSF1        | colony stimulating factor 1 (macrophage) |
| IL10        | interleukin 10 |
| THBS1       | thrombospondin 1 |
| NAIP        | NLR family, apoptosis inhibitory protein |
| HYAL3       | hyaluronoglucosaminidase 3 |
| CALCA       | calcitonin-related polypeptide alpha |
| MMP17       | matrix metalloproteinase 17 (membrane-inserted) |
| ALOX15      | arachidonate 15-lipoxygenase |
| IL24        | interleukin 24 |
| CNR2        | cannabinoid receptor 2 (macrophage) |
| CYP26B1     | cytochrome P450, family 26, subfamily B, polypeptide 1 |
| CCL24       | chemokine (C-C motif) ligand 24 |
| Gene Symbol | Protein Name |
|-------------|--------------|
| IL18        | interleukin 18 |
| TNFRSF4     | tumor necrosis factor receptor superfamily, member 4 |
| BCL6        | B-cell CLL/lymphoma 6 |
| CCL4L2      | chemokine (C-C motif) ligand 4-like 2 |
| ITGB2       | integrin, beta 2 (complement component 3 receptor 3 and 4 subunit) |
| IL20        | interleukin 20 |
| HCK         | HCK proto-oncogene, Src family tyrosine kinase |
| IL23A       | interleukin 23, alpha subunit p19 |
| GGT5        | gamma-glutamyltransferase 5 |
| BDKRB2      | bradykinin receptor B2 |
| TLR9        | toll-like receptor 9 |
| GPER1       | G protein-coupled estrogen receptor 1 |
| CCL8        | chemokine (C-C motif) ligand 8 |
| TLR8        | toll-like receptor 8 |
| TLR1        | toll-like receptor 1 |
| NFKBID      | nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, delta |
| MEFV        | Mediterranean fever |
| HDAC4       | histone deacetylase 4 |
| IL18RAP     | interleukin 18 receptor accessory protein |
| CSF1R       | colony stimulating factor 1 receptor |
| KLRG1       | killer cell lectin-like receptor subfamily G, member 1 |
| NOX4        | NADPH oxidase 4 |
| C3AR1       | complement component 3a receptor 1 |
| ADGRE5      | CD97 molecule |
| TLR10       | toll-like receptor 10 |
| KCNJ10      | potassium inwardly-rectifying channel, subfamily J, member 10 |
| ITGB6       | integrin, beta 6 |
| C4A         | complement component 4A (Rodgers blood group) |
| CXCR2       | chemokine (C-X-C motif) receptor 2 |
| Gene Symbol | Description |
|-------------|-------------|
| P2RX7       | purinergic receptor P2X, ligand-gated ion channel, 7 |
| MMP25       | matrix metallopeptidase 25 |
| CXCL5       | chemokine (C-X-C motif) ligand 5 |
| SELE        | selectin E |
| NCF1        | neutrophil cytosolic factor 1 |
| SDC1        | syndecan 1 |
| ALOX5       | arachidonate 5-lipoxygenase |
| BLNK        | B-cell linker |
| IL17D       | interleukin 17D |
| RPS6KA4     | ribosomal protein S6 kinase, 90kDa, polypeptide 4 |
| IL1RL2      | interleukin 1 receptor-like 2 |
| F2R         | coagulation factor II (thrombin) receptor |
| CXCR3       | chemokine (C-X-C motif) receptor 3 |
| FFAR3       | free fatty acid receptor 3 |
| SCG2        | secretogranin II |
| PTAFR       | platelet-activating factor receptor |
| SCUBE1      | signal peptide, CUB domain, EGF-like 1 |
| TICAM1      | toll-like receptor adaptor molecule 1 |
| IL17A       | interleukin 17A |
| NLRP3       | NLR family, pyrin domain containing 3 |
| AKT1        | v-akt murine thymoma viral oncogene homolog 1 |
| NFE2L1      | nuclear factor, erythroid 2-like 1 |
| TLR6        | toll-like receptor 6 |
| SERPINA3    | serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 3 |
| TNFAIP3     | tumor necrosis factor, alpha-induced protein 3 |
| PLA2G4B     | phospholipase A2, group IVB (cytosolic) |
| PTGS1       | prostaglandin-endoperoxide synthase 1 (prostaglandin G/H synthase and cyclooxygenase) |
| TLR2        | toll-like receptor 2 |
| MECOM       | MDS1 and EVI1 complex locus |
| Gene Symbol | Description |
|-------------|-------------|
| REL | v-rel avian reticuloendotheliosis viral oncogene homolog |
| CCL16 | chemokine (C-C motif) ligand 16 |
| MGLL | monoglyceride lipase |
| CYBB | cytochrome b-245, beta polypeptide |
| XCR1 | chemokine (C motif) receptor 1 |
| CAMK4 | calcium/calmodulin-dependent protein kinase IV |
| CHST1 | carbohydrate (keratan sulfate Gal-6) sulfotransferase 1 |
| IL6 | interleukin 6 |
| IFI16 | interferon, gamma-inducible protein 16 |
| NCR3 | natural cytotoxicity triggering receptor 3 |
| TP73 | tumor protein p73 |
| TACR1 | tachykinin receptor 1 |
| CCL13 | chemokine (C-C motif) ligand 13 |
| C4B | complement component 4B (Chido blood group) |
| NFRKB | nuclear factor related to kappaB binding protein |
| CHIA | chitinase, acidic |
| LAT | linker for activation of T cells |
| CCR2 | chemokine (C-C motif) receptor 2 |
| F11R | F11 receptor |
| LTB4R | leukotriene B4 receptor |
| MEP1B | meprin A, beta |
| CCL19 | chemokine (C-C motif) ligand 19 |
| TNFSF4 | tumor necrosis factor (ligand) superfamily, member 4 |
| CRP | C-reactive protein, pentraxin-related |
| RARRES2 | retinoic acid receptor responder (tazarotene induced) 2 |
| PIK3CG | phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit gamma |
| LY75 | lymphocyte antigen 75 |
| SEMA7A | semaphorin 7A, GPI membrane anchor (John Milton Hagen blood group) |
| HNRNPA0 | heterogeneous nuclear ribonucleoprotein A0 |
| Gene Symbol | Description |
|-------------|-------------|
| RAC1        | ras-related C3 botulinum toxin substrate 1 (rho family, small GTP binding protein Rac1) |
| SIGLEC1     | sialic acid binding Ig-like lectin 1, sialoadhesin |
| TNIP1       | TNFAIP3 interacting protein 1 |
| NLRP1       | NLR family, pyrin domain containing 1 |
| SELP        | selectin P (granule membrane protein 140kDa, antigen CD62) |
| TLR3        | toll-like receptor 3 |
| CCR7        | chemokine (C-C motif) receptor 7 |
| SERPINA1    | serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1 |
| PTGS2       | prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase) |
| ACKR2       | atypical chemokine receptor 2 |
| ITCH        | itchy E3 ubiquitin protein ligase |
| AOX1        | aldehyde oxidase 1 |
| IL1B        | interleukin 1, beta |
| RXRA        | retinoid X receptor, alpha |
| MYD88       | myeloid differentiation primary response 88 |
| CCL17       | chemokine (C-C motif) ligand 17 |
| SLC11A1     | solute carrier family 11 (proton-coupled divalent metal ion transporter), member 1 |
| NTRK2       | neurotrophic tyrosine kinase, receptor, type 2 |
| LIPA        | lipase A, lysosomal acid, cholesterol esterase |
| CYP4F11     | cytochrome P450, family 4, subfamily F, polypeptide 11 |
| CD180       | CD180 molecule |
| HYAL1       | hyaluronoglucosaminidase 1 |
| IL34        | interleukin 34 |
| KLKB1       | kallikrein B, plasma (Fletcher factor) 1 |
| ADORA3      | adenosine A3 receptor |
| IL27        | interleukin 27 |
| IL1A        | interleukin 1, alpha |
| PLP1        | proteolipid protein 1 |
| Protein   | Description                                                                 |
|-----------|------------------------------------------------------------------------------|
| REG3A     | regenerating islet-derived 3 alpha                                           |
| RELB      | v-rel avian reticuloendotheliosis viral oncogene homolog B                   |
| MAP2K3    | mitogen-activated protein kinase kinase 3                                    |
| CHUK      | conserved helix-loop-helix ubiquitous kinase                                 |
| CRH       | corticotropin releasing hormone                                              |
| HMGB1     | high mobility group box 1                                                    |
| BMP2      | bone morphogenetic protein 2                                                |
| IL19      | interleukin 19                                                               |
| IRAK2     | interleukin-1 receptor-associated kinase 2                                   |
| CCL18     | chemokine (C-C motif) ligand 18 (pulmonary and activation-regulated)         |
| NOS2      | nitric oxide synthase 2, inducible                                           |
| CHI3L1    | chitinase 3-like 1 (cartilage glycoprotein-39)                               |
| LY75-CD302| LY75-CD302 readthrough                                                       |
| AXL       | AXL receptor tyrosine kinase                                                 |
| MAPKAPK2  | mitogen-activated protein kinase-activated protein kinase 2                  |

**Table 5** Differentially expressed targeted mRNAs associated with platelet activation
| Gene Symbol | Description |
|-------------|-------------|
| LYN         | LYN proto-oncogene, Src family tyrosine kinase |
| MMRN1       | multimerin 1 |
| VWF         | von Willebrand factor |
| RAF1        | Raf-1 proto-oncogene, serine/threonine kinase |
| ENTPD1      | ectonucleoside triphosphate diphosphohydrolase 1 |
| RASGRP1     | RAS guanyl releasing protein 1 (calcium and DAG-regulated) |
| PFN1        | profilin 1 |
| EGF         | epidermal growth factor |
| PIK3C2A     | phosphatidylinositol-4-phosphate 3-kinase, catalytic subunit type 2 alpha |
| DGKH        | diacylglycerol kinase, eta |
| MAPK14      | mitogen-activated protein kinase 14 |
| GNAI3       | guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 3 |
| SERPING1    | serpin peptidase inhibitor, clade G (C1 inhibitor), member 1 |
| TBXA2R      | thromboxane A2 receptor |
| IGF2        | insulin-like growth factor 2 |
| PRKCD       | protein kinase C, delta |
| RAPGEF4     | Rap guanine nucleotide exchange factor (GEF) 4 |
| TGFB1       | transforming growth factor, beta 1 |
| GP1BB       | glycoprotein Ib (platelet), beta polypeptide |
| SPARC       | secreted protein, acidic, cysteine-rich (osteonectin) |
| F5          | coagulation factor V (proaccelerin, labile factor) |
| P2RY12      | purinergic receptor P2Y, G-protein coupled, 12 |
| MAPK1       | mitogen-activated protein kinase 1 |
| RAP2B       | RAP2B, member of RAS oncogene family |
| APBB1IP     | amyloid beta (A4) precursor protein-binding, family B, member 1 interacting protein |
| PIK3CD      | phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit delta |
| PPIA        | peptidylprolyl isomerase A (cyclophilin A) |
| YWHAZ       | tyrosine 3-monoxygenase/tryptophan 5-monoxygenase activation protein, zeta |
| Gene Symbol | Description |
|-------------|-------------|
| ENTPD2      | ectonucleoside triphosphate diphosphohydrolase 2 |
| CD9         | CD9 molecule |
| GNA11       | guanine nucleotide binding protein (G protein), alpha 11 (Gq class) |
| CSK         | c-src tyrosine kinase |
| CD40LG      | CD40 ligand |
| CFL1        | cofilin 1 (non-muscle) |
| ARRB2       | arrestin, beta 2 |
| PTK2        | protein tyrosine kinase 2 |
| BCAR1       | breast cancer anti-estrogen resistance 1 |
| RASGRP2     | RAS guanyl releasing protein 2 (calcium and DAG-regulated) |
| PIK3R5      | phosphoinositide-3-kinase, regulatory subunit 5 |
| PIK3CB      | phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit beta |
| LAMP2       | lysosomal-associated membrane protein 2 |
| DAGLA       | diacylglycerol lipase, alpha |
| HGF         | hepatocyte growth factor (hepapoietin A; scatter factor) |
| ACTN2       | actinin, alpha 2 |
| CRK         | v-crk avian sarcoma virus CT10 oncogene homolog |
| PIK3C2G     | phosphatidylinositol-4-phosphate 3-kinase, catalytic subunit type 2 gamma |
| DGKI        | diacylglycerol kinase, iota |
| TLN1        | talin 1 |
| F2RL2       | coagulation factor II (thrombin) receptor-like 2 |
| PLSCR1      | phospholipid scramblase 1 |
| TGFB3       | transforming growth factor, beta 3 |
| RHOG        | ras homolog family member G |
| TGFB2       | transforming growth factor, beta 2 |
| PIK3R6      | phosphoinositide-3-kinase, regulatory subunit 6 |
| TRPC3       | transient receptor potential cation channel, subfamily C, member 3 |
| DGKA        | diacylglycerol kinase, alpha 80kDa |
| GNG2        | guanine nucleotide binding protein (G protein), gamma 2 |
| Gene   | Description                                                                 |
|--------|-----------------------------------------------------------------------------|
| CD40   | CD40 molecule, TNF receptor superfamily member 5                             |
| ACTN1  | actinin, alpha 1                                                            |
| DGKK   | diacylglycerol kinase, kappa                                                 |
| PIK3CA | phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha     |
| ITPR3  | inositol 1,4,5-trisphosphate receptor, type 3                               |
| SYK    | spleen tyrosine kinase                                                      |
| PTPN1  | protein tyrosine phosphatase, non-receptor type 1                           |
| SOS1   | son of sevenless homolog 1 (Drosophila)                                     |
| PDPK1  | 3-phosphoinositide dependent protein kinase 1                               |
| CALM2  | calmodulin 2 (phosphorylase kinase, delta)                                  |
| PRKCQ  | protein kinase C, theta                                                     |
| RHOB   | ras homolog family member B                                                 |
| SERPINF2 | serpin peptidase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor), member 2 |
| COL1A1 | collagen, type I, alpha 1                                                   |
| PRKCZ  | protein kinase C, zeta                                                      |
| PIK3C2B| phosphatidylinositol-4-phosphate 3-kinase, catalytic subunit type 2 beta   |
| LEFTY2 | left-right determination factor 2                                           |
| VAV3   | vav 3 guanine nucleotide exchange factor                                    |
| KNG1   | kininogen 1                                                                 |
| DGKG   | diacylglycerol kinase, gamma 90kDa                                          |
| RHOA   | ras homolog family member A                                                 |
| FCER1G | Fc fragment of IgE, high affinity I, receptor for; gamma polypeptide        |
| ADRA2A | adrenoceptor alpha 2A                                                        |
| ITPR1  | inositol 1,4,5-trisphosphate receptor, type 1                               |
| THBS1  | thrombospondin 1                                                            |
| GNAQ   | guanine nucleotide binding protein (G protein), q polypeptide               |
| TIMP1  | TIMP metallopeptidase inhibitor 1                                           |
| VAV1   | vav 1 guanine nucleotide exchange factor                                    |
| GNA14  | guanine nucleotide binding protein (G protein), alpha 14                    |
| Gene   | Description                                                                 |
|--------|-----------------------------------------------------------------------------|
| GNAT3  | guanine nucleotide binding protein, alpha transducing 3                      |
| SRC    | SRC proto-oncogene, non-receptor tyrosine kinase                             |
| GRB2   | growth factor receptor-bound protein 2                                      |
| P2RY1  | purinergic receptor P2Y, G-protein coupled, 1                                |
| GNA13  | guanine nucleotide binding protein (G protein), alpha 13                     |
| FN1    | fibronectin 1                                                               |
| PLEK   | pleckstrin                                                                  |
| PRKCA  | protein kinase C, alpha                                                     |
| GP9    | glycoprotein IX (platelet)                                                   |
| PLA2G4A| phospholipase A2, group IVA (cytosolic, calcium-dependent)                   |
| TREML1 | triggering receptor expressed on myeloid cells-like 1                       |
| CAP1   | CAP, adenylyl cyclase-associated protein 1 (yeast)                           |
| COL3A1 | collagen, type III, alpha 1                                                 |
| F8A1   | coagulation factor VIII-associated 1                                        |
| PRKCH  | protein kinase C, eta                                                        |
| SHC1   | SHC (Src homology 2 domain containing) transforming protein 1               |
| GNB1   | guanine nucleotide binding protein (G protein), beta polypeptide 1           |
| FLNA   | filamin A, alpha                                                            |
| LCK    | LCK proto-oncogene, Src family tyrosine kinase                              |
| CLU    | clusterin                                                                   |
| APOA1  | apolipoprotein A-I                                                          |
| TMSB4X | thymosin beta 4, X-linked                                                    |
| FYN    | FYN proto-oncogene, Src family tyrosine kinase                              |
| TYRO3  | TYRO3 protein tyrosine kinase                                                |
| ABCC4  | ATP-binding cassette, sub-family C (CFTR/MRP), member 4                      |
| VEGFA  | vascular endothelial growth factor A                                        |
| LCP2   | lymphocyte cytosolic protein 2 (SH2 domain containing leukocyte protein of 76kDa) |
| VCL    | vinculin                                                                    |
| F2R    | coagulation factor II (thrombin) receptor                                   |
| Gene Symbol | Description |
|-------------|-------------|
| DGKB | diacylglycerol kinase, beta 90kDa |
| ADRA2B | adrenoceptor alpha 2B |
| PSAP | prosaposin |
| AKT1 | v-akt murine thymoma viral oncogene homolog 1 |
| BRPF3 | bromodomain and PHD finger containing, 3 |
| MPL | MPL proto-oncogene, thrombopoietin receptor |
| ITPR2 | inositol 1,4,5-trisphosphate receptor, type 2 |
| PDGFB | platelet-derived growth factor beta polypeptide |
| ITGA2B | integrin, alpha 2b (platelet glycoprotein IIb of IIb/IIIa complex, antigen CD41) |
| ARRB1 | arrestin, beta 1 |
| ACTN4 | actinin, alpha 4 |
| POTEM | POTE ankyrin domain family, member M |
| GNAI2 | guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 2 |
| CALM1 | calmodulin 1 (phosphorylase kinase, delta) |
| PRKCG | protein kinase C, gamma |
| DAGLB | diacylglycerol lipase, beta |
| MGLL | monoglyceride lipase |
| F2RL3 | coagulation factor II (thrombin) receptor-like 3 |
| VEGFB | vascular endothelial growth factor B |
| IGF1 | insulin-like growth factor 1 (somatomedin C) |
| IL6 | interleukin 6 |
| GAS6 | growth arrest-specific 6 |
| CALM3 | calmodulin 3 (phosphorylase kinase, delta) |
| VAV2 | vav 2 guanine nucleotide exchange factor |
| ADRA2C | adrenoceptor alpha 2C |
| CD63 | CD63 molecule |
| LAT | linker for activation of T cells |
| F8 | coagulation factor VIII, procoagulant component |
| ADAMTS13 | ADAM metallopeptidase with thrombospondin type 1 motif, 13 |
| Gene Symbol | Description |
|-------------|-------------|
| SCG3        | secretogranin III |
| SRF         | serum response factor (c-fos serum response element-binding transcription factor) |
| WNT3A       | wingless-type MMTV integration site family, member 3A |
| PIK3CG      | phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit gamma |
| PLCG2       | phospholipase C, gamma 2 (phosphatidylinositol-specific) |
| RAC2        | ras-related C3 botulinum toxin substrate 2 (rho family, small GTP binding protein Rac2) |
| GNA12       | guanine nucleotide binding protein (G protein) alpha 12 |
| RAC1        | ras-related C3 botulinum toxin substrate 1 (rho family, small GTP binding protein Rac1) |
| CALU        | calumenin |
| GNA15       | guanine nucleotide binding protein (G protein), alpha 15 (Gq class) |
| WDR1        | WD repeat domain 1 |
| DGKQ        | diacylglycerol kinase, theta 110kDa |
| SELP        | selectin P (granule membrane protein 140kDa, antigen CD62) |
| VEGFC       | vascular endothelial growth factor C |
| SERPINA1    | serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1 |
| THPO        | thrombopoietin |
| HSPA5       | heat shock 70kDa protein 5 (glucose-regulated protein, 78kDa) |
| TRPC7       | transient receptor potential cation channel, subfamily C, member 7 |
| PIK3R1      | phosphoinositide-3-kinase, regulatory subunit 1 (alpha) |
| FGB         | fibrinogen beta chain |
| ITGB3       | integrin, beta 3 (platelet glycoprotein IIIa, antigen CD61) |
| BLOC1S3     | biogenesis of lysosomal organelles complex-1, subunit 3 |
| RAPGEF3     | Rap guanine nucleotide exchange factor (GEF) 3 |
| RAP1A       | RAP1A, member of RAS oncogene family |
| GP5         | glycoprotein V (platelet) |
| CFD         | complement factor D (adipsin) |
| PRKCB       | protein kinase C, beta |
| PECAM1      | platelet/endothelial cell adhesion molecule 1 |
| Protein | Description |
|---------|-------------|
| PLG     | plasminogen |
| AXL     | AXL receptor tyrosine kinase |
| SERPINE1| serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1 |
| CX3CL1  | chemokine (C-X3-C motif) ligand 1 |
| TUBA4A  | tubulin, alpha 4a |

**Table 6** Differentially expressed targeted mRNAs associated with blood coagulation
| Gene symbol | Protein Name |
|-------------|--------------|
| LYN         | LYN proto-oncogene, Src family tyrosine kinase |
| GGCX        | gamma-glutamyl carboxylase |
| PDE9A       | phosphodiesterase 9A |
| SIRPA       | signal-regulatory protein alpha |
| MMRN1       | multimerin 1 |
| EFEMP2      | EGF containing fibulin-like extracellular matrix protein 2 |
| HPS1        | Hermansky-Pudlak syndrome 1 |
| VWF         | von Willebrand factor |
| RAF1        | Raf-1 proto-oncogene, serine/threonine kinase |
| KIF9        | kinesin family member 9 |
| KDM1A       | lysine (K)-specific demethylase 1A |
| ENTPD1      | ectonucleoside triphosphate diphosphohydrolase 1 |
| LRRC16A     | leucine rich repeat containing 16A |
| ANO6        | anoctamin 6 |
| ITGAV       | integrin, alpha V |
| CDC42       | cell division cycle 42 |
| RASGRP1     | RAS guanyl releasing protein 1 (calcium and DAG-regulated) |
| PFN1        | profilin 1 |
| SELPLG      | selectin P ligand |
| FGR         | FGR proto-oncogene, Src family tyrosine kinase |
| HDAC1       | histone deacetylase 1 |
| EGF         | epidermal growth factor |
| KIF23       | kinesin family member 23 |
| DGKH        | diacylglycerol kinase, eta |
| AKAP1       | A kinase (PRKA) anchor protein 1 |
| ITGA1       | integrin, alpha 1 |
| EHD2        | EH-domain containing 2 |
| MAPK14      | mitogen-activated protein kinase 14 |
| Gene Symbol | Description |
|-------------|-------------|
| ITPK1       | inositol-tetrakisphosphate 1-kinase |
| CD59        | CD59 molecule, complement regulatory protein |
| GNAI3       | guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 3 |
| KRAS        | Kirsten rat sarcoma viral oncogene homolog |
| EHD1        | EH-domain containing 1 |
| SERPING1    | serpin peptidase inhibitor, clade C1 inhibitor, member 1 |
| TBXA2R      | thromboxane A2 receptor |
| IRF2        | interferon regulatory factor 2 |
| CDK2        | cyclin-dependent kinase 2 |
| IGF2        | insulin-like growth factor 2 |
| F13B        | coagulation factor XIII, B polypeptide |
| CAV1        | caveolin 1, caveolae protein, 22kDa |
| MAG         | myelin associated glycoprotein |
| PRKCD       | protein kinase C, delta |
| RAPGEF4     | Rap guanine nucleotide exchange factor (GEF) 4 |
| TGFB1       | transforming growth factor, beta 1 |
| PRKAR1B     | protein kinase, cAMP-dependent, regulatory, type I, beta |
| INPP5D      | inositol polyphosphate-5-phosphatase, 145kDa |
| GP1BB       | glycoprotein Ib (platelet), beta polypeptide |
| AKAP10      | A kinase (PRKA) anchor protein 10 |
| IFNB1       | interferon, beta 1, fibroblast |
| SPARC       | secreted protein, acidic, cysteine-rich (osteonectin) |
| F5          | coagulation factor V (proaccelerin, labile factor) |
| P2RY12      | purinergic receptor P2Y, G-protein coupled, 12 |
| MAPK1       | mitogen-activated protein kinase 1 |
| DOK2        | docking protein 2, 56kDa |
| HMCN1       | hemicentin 1 |
| NFE2        | nuclear factor, erythroid 2 |
| H3F3B       | H3 histone, family 3B (H3.3B) |
| Gene       | Description                                                                 |
|------------|-----------------------------------------------------------------------------|
| PRKACB     | protein kinase, cAMP-dependent, catalytic, beta                             |
| ADORA2A    | adenosine A2a receptor                                                      |
| PRKG1      | protein kinase, cGMP-dependent, type I                                      |
| CAPZB      | capping protein (actin filament) muscle Z-line, beta                         |
| JAM2       | junctional adhesion molecule 2                                              |
| LMAN1      | lectin, mannose-binding, 1                                                 |
| PDE3A      | phosphodiesterase 3A, cGMP-inhibited                                        |
| APBB1IP    | amyloid beta (A4) precursor protein-binding, family B, member 1 interacting protein |
| PPIA       | peptidylprolyl isomerase A (cyclophilin A)                                  |
| YWHAZ      | tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta |
| KIF3A      | kinesin family member 3A                                                   |
| CD9        | CD9 molecule                                                                |
| STIM1      | stromal interaction molecule 1                                              |
| SERPINA10  | serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 10 |
| PRTN3      | proteinase 3                                                                |
| GNA11      | guanine nucleotide binding protein (G protein), alpha 11 (Gq class)         |
| KIF11      | kinesin family member 11                                                   |
| TEK        | TEK tyrosine kinase, endothelial                                            |
| CSK        | c-src tyrosine kinase                                                       |
| ITGAM      | integrin, alpha M (complement component 3 receptor 3 subunit)              |
| SHH        | sonic hedgehog                                                             |
| LYST       | lysosomal trafficking regulator                                             |
| ACTB       | actin, beta                                                                |
| CFL1       | cofilin 1 (non-muscle)                                                     |
| CD2        | CD2 molecule                                                                |
| IRF1       | interferon regulatory factor 1                                              |
| ARRB2      | arrestin, beta 2                                                            |
| NOS3       | nitric oxide synthase 3 (endothelial cell)                                  |
| ATP2B2     | ATPase, Ca++ transporting, plasma membrane 2                               |
| Gene   | Description                                                                 |
|--------|-----------------------------------------------------------------------------|
| PRKG2  | protein kinase, cGMP-dependent, type II                                     |
| PTK2   | protein tyrosine kinase 2                                                   |
| BCAR1  | breast cancer anti-estrogen resistance 1                                   |
| SLC16A8| solute carrier family 16 (monocarboxylate transporter), member 8           |
| GUCY1A2| guanylate cyclase 1, soluble, alpha 2                                       |
| EHD3   | EH-domain containing 3                                                     |
| RASGRP2| RAS guanyl releasing protein 2 (calcium and DAG-regulated)                  |
| PIK3R5 | phosphoinositol-3-kinase, regulatory subunit 5                             |
| F3     | coagulation factor III (thromboplastin, tissue factor)                      |
| RCOR1  | REST corepressor 1                                                          |
| SLC8A1 | solute carrier family 8 (sodium/calcium exchanger), member 1               |
| PDE2A  | phosphodiesterase 2A, cGMP-stimulated                                       |
| RAB27A | RAB27A, member RAS oncogene family                                          |
| ITGA10 | integrin, alpha 10                                                          |
| PIK3CB | phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit beta     |
| LRP8   | low density lipoprotein receptor-related protein 8, apolipoprotein e receptor|
| ESAM   | endothelial cell adhesion molecule                                          |
| LAMP2  | lysosomal-associated membrane protein 2                                     |
| DAGLA  | diacylglycerol lipase, alpha                                                |
| GATA2  | GATA binding protein 2                                                      |
| HGF    | hepatocyte growth factor (hepapoitin A; scatter factor)                     |
| ACTN2  | actinin, alpha 2                                                            |
| CRK    | v-crk avian sarcoma virus CT10 oncogene homolog                            |
| DGKI   | diacylglycerol kinase, iota                                                 |
| TLN1   | talin 1                                                                      |
| F2RL2  | coagulation factor II (thrombin) receptor-like 2                            |
| OLR1   | oxidized low density lipoprotein (lectin-like) receptor 1                   |
| CBX5   | chromobox homolog 5                                                         |
| TGFB3  | transforming growth factor, beta 3                                           |
| Abbreviation | Description |
|--------------|-------------|
| PLAT         | plasminogen activator, tissue |
| CD47         | CD47 molecule |
| APOB         | apolipoprotein B |
| SIRPG        | signal-regulatory protein gamma |
| SLC7A5       | solute carrier family 7 (amino acid transporter light chain, L system), member 5 |
| GRB7         | growth factor receptor-bound protein 7 |
| RHOG         | ras homolog family member G |
| TGFB2        | transforming growth factor, beta 2 |
| PIK3R6       | phosphoinositide-3-kinase, regulatory subunit 6 |
| TRPC3        | transient receptor potential cation channel, subfamily C, member 3 |
| GATA6        | GATA binding protein 6 |
| DGKA         | diacylglycerol kinase, alpha 80kDa |
| CD44         | CD44 molecule (Indian blood group) |
| HNF4A        | hepatocyte nuclear factor 4, alpha |
| GNG2         | guanine nucleotide binding protein (G protein), gamma 2 |
| MFN2         | mitofusin 2 |
| ITGAL        | integrin, alpha L (antigen CD11A (p180), lymphocyte function-associated antigen 1; alpha polypeptide) |
| TREM1        | triggering receptor expressed on myeloid cells 1 |
| GATA5        | GATA binding protein 5 |
| KCNMB2       | potassium large conductance calcium-activated channel, subfamily M, beta member 2 |
| ACTN1        | actinin, alpha 1 |
| ATP1B1       | ATPase, Na+/K+ transporting, beta 1 polypeptide |
| KLC1         | kinesin light chain 1 |
| DGKK         | diacylglycerol kinase, kappa |
| PIK3CA       | phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha |
| PAPSS2       | 3'-phosphoadenosine 5'-phosphosulfate synthase 2 |
| AP3B1        | adaptor-related protein complex 3, beta 1 subunit |
| ITPR3        | inositol 1,4,5-trisphosphate receptor, type 3 |
| GATA3        | GATA binding protein 3 |
| Gene      | Description                                                                 |
|-----------|-----------------------------------------------------------------------------|
| CD84      | CD84 molecule                                                               |
| KLC2      | kinesin light chain 2                                                       |
| PDE1B     | phosphodiesterase 1B, calmodulin-dependent                                  |
| SYK       | spleen tyrosine kinase                                                      |
| ITGA5     | integrin, alpha 5 (fibronectin receptor, alpha polypeptide)                 |
| PTPN1     | protein tyrosine phosphatase, non-receptor type 1                           |
| SOS1      | son of sevenless homolog 1 (Drosophila)                                    |
| PDPK1     | 3-phosphoinositide dependent protein kinase 1                               |
| F2RL1     | coagulation factor II (thrombin) receptor-like 1                            |
| CALM2     | calmodulin 2 (phosphorylase kinase, delta)                                  |
| CDK5      | cyclin-dependent kinase 5                                                   |
| PRKCQ     | protein kinase C, theta                                                     |
| CYP4F2    | cytochrome P450, family 4, subfamily F, polypeptide 2                        |
| ATP2B4    | ATPase, Ca++ transporting, plasma membrane 4                                |
| RHOB      | ras homolog family member B                                                |
| HMG20B    | high mobility group 20B                                                     |
| SERPINF2  | serpin peptidase inhibitor, clade F (alpha-2 antilasmin, pigment epithelium derived factor), member 2 |
| SLC7A6    | solute carrier family 7 (amino acid transporter light chain, y+L system), member 6 |
| VKORC1    | vitamin K epoxide reductase complex, subunit 1                              |
| JAM3      | junctional adhesion molecule 3                                              |
| KCNMB4    | potassium large conductance calcium-activated channel, subfamily M, beta member 4 |
| ANXA8     | annexin A8                                                                  |
| COL1A1    | collagen, type I, alpha 1                                                   |
| MAFK      | v-maf avian musculoaponeurotic fibrosarcoma oncogene homolog K              |
| PRKCZ     | protein kinase C, zeta                                                      |
| DOCK9     | dedicator of cytokinesis 9                                                 |
| SLC8A3    | solute carrier family 8 (sodium/calcium exchanger), member 3                |
| SERPINA5  | serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 5 |
| C6orf25   | chromosome 6 open reading frame 25                                          |
| Gene Symbol | Description |
|-------------|-------------|
| KIF2C       | kinesin family member 2C |
| LEFTY2      | left-right determination factor 2 |
| VAV3        | vav 3 guanine nucleotide exchange factor |
| PLAUR       | plasminogen activator, urokinase receptor |
| GATA4       | GATA binding protein 4 |
| KNG1        | kininogen 1 |
| PRKAR1A     | protein kinase, cAMP-dependent, regulatory, type I, alpha |
| RAD51B      | RAD51 paralog B |
| KIAA1715    | KIAA1715 |
| DGKG        | diacylglycerol kinase, gamma 90kDa |
| RHOA        | ras homolog family member A |
| FCER1G      | Fc fragment of IgE, high affinity I, receptor for; gamma polypeptide |
| ADRA2A      | adrenoceptor alpha 2A |
| ITPR1       | inositol 1,4,5-trisphosphate receptor, type 1 |
| THBS1       | thrombospondin 1 |
| KCNMA1      | potassium large conductance calcium-activated channel, subfamily M, alpha member 1 |
| DOCK1       | dedicator of cytokinesis 1 |
| GNAQ        | guanine nucleotide binding protein (G protein), q polypeptide |
| TIMP1       | TIMP metallopeptidase inhibitor 1 |
| VAV1        | vav 1 guanine nucleotide exchange factor |
| PDE5A       | phosphodiesterase 5A, cGMP-specific |
| PLAU        | plasminogen activator, urokinase |
| GNA14       | guanine nucleotide binding protein (G protein), alpha 14 |
| GNAT3       | guanine nucleotide binding protein, alpha transducing 3 |
| ATP2A3      | ATPase, Ca++ transporting, ubiquitous |
| SRC         | SRC proto-oncogene, non-receptor tyrosine kinase |
| GRB2        | growth factor receptor-bound protein 2 |
| P2RY1       | purinergic receptor P2Y, G-protein coupled, 1 |
| CXADR       | coxsackie virus and adenovirus receptor |
| Gene   | Description                                                                 |
|--------|-----------------------------------------------------------------------------|
| PDE1A  | phosphodiesterase 1A, calmodulin-dependent                                  |
| GNA13  | guanine nucleotide binding protein (G protein), alpha 13                    |
| FN1    | fibronectin 1                                                               |
| PLEK   | pleckstrin                                                                  |
| PRKCA  | protein kinase C, alpha                                                     |
| ITGB2  | integrin, beta 2 (complement component 3 receptor 3 and 4 subunit)          |
| GP9    | glycoprotein IX (platelet)                                                  |
| PLA2G4A| phospholipase A2, group IVA (cytosolic, calcium-dependent)                  |
| ABL1   | ABL proto-oncogene 1, non-receptor tyrosine kinase                          |
| CAP1   | CAP, adenylate cyclase-associated protein 1 (yeast)                         |
| PRKAR2B| protein kinase, cAMP-dependent, regulatory, type II, beta                   |
| CAPZA1 | capping protein (actin filament) muscle Z-line, alpha 1                     |
| AK3    | adenylate kinase 3                                                          |
| PRKCH  | protein kinase C, eta                                                       |
| TSPAN32| tetraspanin 32                                                              |
| SHC1   | SHC (Src homology 2 domain containing) transforming protein 1              |
| GNB1   | guanine nucleotide binding protein (G protein), beta polypeptide 1          |
| ANGPT2 | angiopoietin 2                                                              |
| FLNA   | filamin A, alpha                                                            |
| LCK    | LCK proto-oncogene, Src family tyrosine kinase                             |
| SLC7A8 | solute carrier family 7 (amino acid transporter light chain, L system), member 8 |
| CLU    | clusterin                                                                   |
| KCNMB1 | potassium large conductance calcium-activated channel, subfamily M, beta member 1 |
| PTPN11 | protein tyrosine phosphatase, non-receptor type 11                          |
| APOA1  | apolipoprotein A-I                                                          |
| KIF5A  | kinesin family member 5A                                                   |
| DOCK6  | dedicator of cytokinesis 6                                                  |
| TMSB4X | thymosin beta 4, X-linked                                                   |
| C4BPB  | complement component 4 binding protein, beta                               |
| Gene Symbol | Description |
|-------------|-------------|
| YES1        | YES proto-oncogene 1, Src family tyrosine kinase |
| ZFPM1       | zinc finger protein, FOG family member 1 |
| CD177       | CD177 molecule |
| FYN         | FYN proto-oncogene, Src family tyrosine kinase |
| PLCG1       | phospholipase C, gamma 1 |
| SELE        | selectin E |
| ITGA3       | integrin, alpha 3 (antigen CD49C, alpha 3 subunit of VLA-3 receptor) |
| WAS         | Wiskott-Aldrich syndrome |
| KIF15       | kinesin family member 15 |
| SLC16A1     | solute carrier family 16 (monocarboxylate transporter), member 1 |
| CABLES1     | Cdk5 and Abl enzyme substrate 1 |
| THBD        | thrombomodulin |
| ABCC4       | ATP-binding cassette, sub-family C (CFTR/MRP), member 4 |
| KIF2A       | kinesin heavy chain member 2A |
| MMP1        | matrix metallopeptidase 1 (interstitial collagenase) |
| VEGFA       | vascular endothelial growth factor A |
| LCP2        | lymphocyte cytosolic protein 2 (SH2 domain containing leukocyte protein of 76kDa) |
| ATP1B3      | ATPase, Na+/K+ transporting, beta 3 polypeptide |
| MAFG        | v-maf avian musculoaponeurotic fibrosarcoma oncogene homolog G |
| VCL         | vinculin |
| RACGAP1     | Rac GTPase activating protein 1 |
| ATP1B2      | ATPase, Na+/K+ transporting, beta 2 polypeptide |
| F2R         | coagulation factor II (thrombin) receptor |
| ATP2A2      | ATPase, Ca++ transporting, cardiac muscle, slow twitch 2 |
| DGKB        | diacylglycerol kinase, beta 90kDa |
| ADRA2B      | adrenoceptor alpha 2B |
| SCUBE1      | signal peptide, CUB domain, EGF-like 1 |
| KIF3C       | kinesin family member 3C |
| PTPRJ       | protein tyrosine phosphatase, receptor type, J |
| Gene   | Description                                                                 |
|--------|-----------------------------------------------------------------------------|
| PSAP   | prosaposin                                                                  |
| AKT1   | v-akt murine thymoma viral oncogene homolog 1                               |
| BRPF3  | bromodomain and PHD finger containing, 3                                    |
| RBSN   | zinc finger, FYVE domain containing 20                                      |
| MPL    | MPL proto-oncogene, thrombopoietin receptor                                 |
| HPS5   | Hermansky-Pudlak syndrome 5                                                 |
| ITPR2  | inositol 1,4,5-trisphosphate receptor, type 2                               |
| CD48   | CD48 molecule                                                                |
| ITGA4  | integrin, alpha 4 (antigen CD49D, alpha 4 subunit of VLA-4 receptor)        |
| PDGFB  | platelet-derived growth factor beta polypeptide                             |
| ORAI1  | ORAI calcium release-activated calcium modulator 1                          |
| GATA1  | GATA binding protein 1 (globin transcription factor 1)                      |
| PRKAR2A| protein kinase, cAMP-dependent, regulatory, type II, alpha                  |
| ITGA2B | integrin, alpha 2b (platelet glycoprotein IIb of IIb/IIIa complex, antigen CD41) |
| ARRB1  | arrestin, beta 1                                                            |
| ACTN4  | actinin, alpha 4                                                            |
| GUCY1A3| guanylate cyclase 1, soluble, alpha 3                                       |
| SELL   | selectin L                                                                  |
| VPS45  | vacuolar protein sorting 45 homolog (S. cerevisiae)                         |
| POTEM  | POTE ankyrin domain family, member M                                        |
| GNAI2  | guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 2 |
| CALM1  | calmodulin 1 (phosphorylase kinase, delta)                                  |
| PRKCG  | protein kinase C, gamma                                                     |
| KIF4B  | kinesin family member 4B                                                    |
| F11    | coagulation factor XI                                                       |
| DAGLB  | diacylglycerol lipase, beta                                                 |
| MGLL   | monoglyceride lipase                                                        |
| ITGAX  | integrin, alpha X (complement component 3 receptor 4 subunit)               |
| SH2B1  | SH2B adaptor protein 1                                                      |
| Gene Symbol | Description |
|-------------|-------------|
| ITGA6 | integrin, alpha 6 |
| F2RL3 | coagulation factor II (thrombin) receptor-like 3 |
| PRCP | prolylcarboxypeptidase (angiotensinase C) |
| VEGFB | vascular endothelial growth factor B |
| IGF1 | insulin-like growth factor 1 (somatomedin C) |
| GAS6 | growth arrest-specific 6 |
| CALM3 | calmodulin 3 (phosphorylase kinase, delta) |
| VAV2 | vav 2 guanine nucleotide exchange factor |
| CEACAM8 | carcinoembryonic antigen-related cell adhesion molecule 8 |
| PTGIR | prostaglandin I2 (prostacyclin) receptor (IP) |
| ADRA2C | adrenoceptor alpha 2C |
| CD63 | CD63 molecule |
| LAT | linker for activation of T cells |
| SLC7A10 | solute carrier family 7 (neutral amino acid transporter light chain, asc system), member 10 |
| ATP2B1 | ATPase, Ca++ transporting, plasma membrane 1 |
| F11R | F11 receptor |
| PTPN6 | protein tyrosine phosphatase, non-receptor type 6 |
| NRAS | neuroblastoma RAS viral (v-ras) oncogene homolog |
| F8 | coagulation factor VIII, procoagulant component |
| ADAMTS13 | ADAM metalloproteinase with thrombospondin type 1 motif, 13 |
| SLC8A2 | solute carrier family 8 (sodium/calcium exchanger), member 2 |
| NOS1 | nitric oxide synthase 1 (neuronal) |
| SCG3 | secretogranin III |
| PPIL2 | peptidylprolyl isomerase (cyclophilin)-like 2 |
| MRVI1 | murine retrovirus integration site 1 homolog |
| SH2B2 | SH2B adaptor protein 2 |
| NBEAL2 | neurobeachin-like 2 |
| ATP2A1 | ATPase, Ca++ transporting, cardiac muscle, fast twitch 1 |
| HRAS | Harvey rat sarcoma viral oncogene homolog |
| Gene Symbol | Description |
|-------------|-------------|
| PDE11A      | phosphodiesterase 11A |
| PIK3CG      | phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit gamma |
| SIN3A       | SIN3 transcription regulator family member A |
| PRKACA      | protein kinase, cAMP-dependent, catalytic, alpha |
| PLCG2       | phospholipase C, gamma 2 (phosphatidylinositol-specific) |
| ENPP4       | ectonucleotide pyrophosphatase/phosphodiesterase 4 (putative) |
| RAC2        | ras-related C3 botulinum toxin substrate 2 (rho family, small GTP binding protein Rac2) |
| GNA12       | guanine nucleotide binding protein (G protein) alpha 12 |
| MAFF        | v-maf avian musculoaponeurotic fibrosarcoma oncogene homolog F |
| RAC1        | ras-related C3 botulinum toxin substrate 1 (rho family, small GTP binding protein Rac1) |
| CALU        | calumenin |
| GNA15       | guanine nucleotide binding protein (G protein), alpha 15 (Gq class) |
| WDR1        | WD repeat domain 1 |
| CEACAM1     | carcinoembryonic antigen-related cell adhesion molecule 1 (biliary glycoprotein) |
| SLC7A11     | solute carrier family 7 (anionic amino acid transporter light chain, xc- system), member 11 |
| DGKQ        | diacylglycerol kinase, theta 110kDa |
| SELP        | selectin P (granule membrane protein 140kDa, antigen CD62) |
| KIF26A      | kinesin family member 26A |
| VEGFC       | vascular endothelial growth factor C |
| MYB         | v-myb avian myeloblastosis viral oncogene homolog |
| ANGPT4      | angiopoietin 4 |
| SERPINA1    | serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1 |
| CEACAM6     | carcinoembryonic antigen-related cell adhesion molecule 6 (non-specific cross reacting antigen) |
| THPO        | thrombopoietin |
| HSPA5       | heat shock 70kDa protein 5 (glucose-regulated protein, 78kDa) |
| TRPC7       | transient receptor potential cation channel, subfamily C, member 7 |
| GUCY1B3     | guanylate cyclase 1, soluble, beta 3 |
| Gene Symbol | Description |
|-------------|-------------|
| PIK3R1      | phosphoinositide-3-kinase, regulatory subunit 1 (alpha) |
| IFNA8       | interferon, alpha 8 |
| TP53        | tumor protein p53 |
| KIFAP3      | kinesin-associated protein 3 |
| FBLN5       | fibulin 5 |
| L1CAM       | L1 cell adhesion molecule |
| CYP4F11     | cytochrome P450, family 4, subfamily F, polypeptide 11 |
| BSG         | basigin (Ok blood group) |
| FGB         | fibrinogen beta chain |
| KLKB1       | kallikrein B, plasma (Fletcher factor) 1 |
| SLC16A3     | solute carrier family 16 (monocarboxylate transporter), member 3 |
| ITGB3       | integrin, beta 3 (platelet glycoprotein IIIa, antigen CD61) |
| BLOC1S3     | biogenesis of lysosomal organelles complex-1, subunit 3 |
| RAPGEF3     | Rap guanine nucleotide exchange factor (GEF) 3 |
| RAP1A       | RAP1A, member of RAS oncogene family |
| TFPI        | tissue factor pathway inhibitor (lipoprotein-associated coagulation inhibitor) |
| GP5         | glycoprotein V (platelet) |
| CFD         | complement factor D (adipsin) |
| PRKCB       | protein kinase C, beta |
| PECAM1      | platelet/endothelial cell adhesion molecule 1 |
| F7          | coagulation factor VII (serum prothrombin conversion accelerator) |
| NOS2        | nitric oxide synthase 2, inducible |
| PLG         | plasminogen |
| CD58        | CD58 molecule |
| KIF4A       | kinesin family member 4A |
| SERPINE1    | serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1 |
| HPS6        | Hermansky-Pudlak syndrome 6 |
| SPN         | sialophorin |
| SH2B3       | SH2B adaptor protein 3 |
TUBA4A  tubulin, alpha 4a