Distinct RNA Transcriptome Patterns Are Potentially Associated with Angiogenesis in Tie2-Expressing Monocytes

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Abstract

Tie2-expressing Monocytes (TEMs) were previously identified as a novel subset of monocytes and were believed to have prominent pro-angiogenesis activities in human tumors. While the molecular mechanism of the angiogenesis promoting capacity of TEMs remains unclear. RNA transcriptome pattern, including non-coding RNAs as microRNA (miRNA) and long non-coding RNA (lncRNA), plays important role in cell differentiation and functions. However, little is known about the transcriptome patterns of TEMs, including those non-coding RNAs. We explore the transcriptome of TEMs and the matched monocytes that do not express Tie2 (Tie2⁻monocytes) isolated from peripheral blood of healthy adults employing the Agilent Human miRNA (8*60K, Design ID: 046064) microarray and the Agilent lncRNA Gene Expression (4*180K, Design ID: 042818) microarray. A total of 141 mRNAs, 142 lncRNAs and 75 miRNAs were found dysregulated in TEMs compared to Tie2⁻monocytes. TEMs have the distinct RNA transcriptome patterns according to the Hierarchical clustering and then the gene expression patterns were confirmed by quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR). Functional annotation by Gene Ontology (GO) analyses showed that the up-regulated mRNAs in TEMs were associated to blood vessel remodeling and positive regulation of epithelial cell proliferation, and the up-regulated insulin like growth factor 1(IGF1) mRNA was involved in both pathways. For functional analysis of those dysregulated non-coding RNAs, target genes of the miRNAs were predicted and cis/trans-regulation analysis of the lncRNAs were performed.

Keywords: microarray, miRNA, lncRNA, cis/trans-regulation

1.1 Introductions

In the last decade, a novel subset of monocytes that express the Tie2 protein (Dumont et al., 1994) was classified as TEMs (Tie2-expressing monocytes/macrophages). Tie2, the receptor for angiopoietin2 (Ang2), has been shown to play important roles in angiogenesis and was once believed to be expressed only in endothelial cells (Partanen et al., 1992). TEMs with specific surface marker profiles were later described in several studies of human tumors as well as mouse models (De Palma et al., 2003; De Palma et al., 2005; Venneri et al., 2007) as circulating TEMs (Tie2⁺CD45⁺CD11b⁺Gr-1low/neg-mice; Tie2⁺CD14⁺CD16⁺CD11c⁺-humans) (Pucci et al., 2009). The surface marker profiles indicate that circulating TEMs are distinct from classic inflammatory monocytes, which are the precursors of tissue macrophages. Moreover, in tissues, particularly tumors, where TEMs are found much more frequently than in normal tissue, TEMs also present a specific surface marker profile (Tie2⁺Sca-1⁺CD11b⁺) (Pucci et al., 2009) that distinguished them from the majority of macrophages. The lack of expression of CCR2 (Augustin et al., 2009), a receptor for the chemokine MCP1, which plays an important role in the recruitment of monocytes to inflammatory tissues and tumors, indicates that TEMs may be attracted to tissues in a manner unrelated to the classical MCP1-CCR2 axis. Moreover, the specific pro-angiogenic activity of TEMs in many human tumors and other non-neoplastic diseases such as the ischemic limb makes these cells distinct from other Tie2⁺

73 monocytes/macrophages (Patel et al., 2013). In the local tumor environment, TEMs
74 are considered a more potent source of angiogenesis-promoting signals than other
75 Tie2⁺ tumor associated macrophages (TAMs) (Ribatti, 2009; Coffelt et al., 2010;
76 Huang et al., 2011), although they are recruited to tumors in lower numbers.

77 Although the association of TEMs with angiogenesis is well studied, the RNA
78 expression profile that underlies the distinct pro-angiogenic phenotype of TEMs has
79 not been investigated to date. With the rapid development of high-throughput
80 transcriptome analysis, it is possible to obtain a comprehensive view of the distinct
81 genome-wide transcription profiles of TEMs and Tie2⁺ monocytes.

82 With respect to transcriptome analyses, one of the most surprising recent discoveries
83 was that while the approximately 20,000 protein-coding genes of the human genome
84 comprise only 2% of the total genome, at least 90% of the genome is actively
85 transcribed, according to whole genome and transcriptome sequencing technologies.

86 Additional studies have demonstrated that the human genome is transcribed into many
87 different complex RNA families, almost half of which are non-coding RNAs
88 (ncRNAs). These ncRNAs are grouped into the following two major classes
89 depending on transcript size: small non-coding RNAs (sncRNAs) represented by
90 miRNAs and lncRNAs. The function of miRNAs as specific regulators of
91 protein-coding RNAs, primarily through post-transcriptional silencing, has been
92 widely characterized. In contrast, lncRNAs, defined as RNAs longer than 200
93 nucleotides that do not encode proteins (Birney et al., 2007; Clark et al., 2012), were
94 initially described as “transcriptional noise” but are now considered to have major

biological functions in cellular development and metabolism(Lee, 2012). Indeed, these newly discovered lncRNAs were found to have tissue-specific expression patterns, and their aberrant regulation has been related to a variety of biological processes(Nagano and Fraser, 2011; Rinn and Chang, 2012) and diseases, such as cancers(Gupta et al., 2010; Tsai et al., 2011; Brunner et al., 2012). A cis-acting mechanism was described in previous studies through which lncRNAs can regulate transcription of neighboring genes on the same chromosome, and for most lncRNA transcripts, a trans-acting mechanism has also been proposed to regulate gene expression. In the trans-acting manner, the transcription of the neighboring genes of the lncRNAs was not affected(Bertani et al., 2011).

To explore the expression patterns of protein-coding RNAs as well as non-coding RNAs in TEMs, we analyzed three pairs of TEMs in comparison to Tie2⁺ monocytes samples by microarray analysis. Some of the dysregulated RNAs were validated by qRT-PCR in ten paired samples. Then we analyzed the functions of the differentially expressed mRNAs through their annotations in the GO database, while the non-coding-RNAs-mRNAs co-expression profiles were conducted. The cis-/trans-acting analyses of the dysregulated lncRNAs were performed based on the co-expression profiles, and the functions of the dysregulated non-coding-RNAs were predicted by the analysis of their target protein-coding genes.

2.1 Materials and Methods

2.1.1 Cell sorting

Our study was approved by the Ethics Committee of the Shanghai First Maternity and Infant Hospital, School of Medicine, Tongji University and the venous blood was obtained from healthy donors with written informed consent. Human peripheral blood mononuclear cells (PBMCs) were isolated using Lymphoprep (Axis-Shield, Norway). The monocytes were then enriched by positive immunomagnetic selection using anti-CD14 MicroBeads (Miltenyi, Germany). The TEMs (TIE2⁺CD14⁺) and Tie2⁻monocytes (Tie2⁻CD14⁺) were then sorted by flow cytometry using FITC-conjugated anti-CD14 (555397, BD Biosciences, USA) and APC-conjugated anti-Tie2 (FAB3131A, R&D Systems, USA) antibodies. For further western-blot analysis of the sorted TEMs and Tie2⁻monocytes, anti-Tie2 (ab137786, Abcam, USA), anti-CD11c (ab52632, Abcam, USA) and anti-CCR2 (ab202391, Abcam, USA) were used as primary antibodies followed by HRP-conjugated Goat anti-Rabbit antibody (31461, Invitrogen, USA) as the secondary antibody. Moreover, anti-CD31 (ab83959, Abcam, USA) and anti-VEGFR2 (ab39638, Abcam, USA) were used to rule out the sorted TEMs with circulating endothelial cells.

2.1.2 RNA preparation

The total RNA was extracted from the TEMs and Tie2⁻monocytes using the miRNA isolation kit (AM1560, Life Technologies, USA). The RNA was then quantified using the NanoDrop ND-2000 system (Life Technologies, USA), and the RNA integrity was assessed using an Agilent Bioanalyzer 2100 system (Agilent Technologies, USA).

Microarray processing and statistical analysis

The RNAs expression profiling in three paired TEMs and Tie2⁺ monocytes samples was performed using the Agilent lncRNA Gene Expression 4×180K Microarray (Design ID:042818, Agilent Technologies, USA,) and the Agilent Human miRNA microarray (8×60K, Design ID: 046064 Agilent Technologies, USA)following the manufacturer's standard protocols. For microarray analysis, the Feature Extraction software (version10.7.1.1, Agilent Technologies) was used to analyze array images to get raw data. Next, Genespring software (version 12.5; Agilent Technologies) was employed to finish the basic analysis with the raw data. To begin with, the raw data was normalized with the quantile algorithm. The probes that at least 100.0 percent of samples in any 1 out of 2 conditions have flags in Detected were chosen for further data analysis. Differentially expressed RNAs were then identified through fold change as well as P value calculated using t-test. The threshold set for up- and down-regulated genes was a fold change $\geq$ 2.0 and a P value $\leq$ 0.05. Hierarchical Clustering was performed to show the distinguishable miRNAs expression pattern among samples. All microarray data has been submitted to GEO (GSE57783 and GSE68322).

2.1.3 Quantitative real-time PCR (qRT-PCR)

For mRNAs or lncRNAs qRT-PCR analysis, 500ng of total RNA was reversed transcribed using the RT kit (RR037A, TaKaRa, Japan) and cDNA was amplified using the SYBR kit (RR420A, TaKaRa, Japan). For miRNAs qPCR analysis, the miRNA qRT-PCR SYBR Kit (RR716, TaKaRa, Japan) was used. The qRT-PCR

cycling conditions were 5s at 95 °C, 20s at 60°C for 40 amplification cycles. Differences in gene expression were determined using the $2^{\Delta\Delta CT}$ method (β -actin and U6 were used as calibrators). Primers were listed in supplemental data.

2.1.4 Co-expression of non-coding-RNAs with mRNAs and functional predictions

For these differentially expressed mRNAs, GO analyses were applied. However, functional predictions for the dysregulated non-coding-RNAs were based on the functional annotations of their target regulated mRNAs. For miRNAs, we predicted their target mRNAs using three different databases. For lncRNAs, we first identified co-expressed mRNAs for each dysregulated lncRNA by calculating Pearson's correlations with P values<0.05. Based on this co-expression analysis, we further explored the mechanisms through which these dysregulated lncRNAs may exert their functions through cis- and/or trans-regulated mRNAs. We then conducted a functional enrichment analysis of the cis-regulated mRNAs. The enriched GO and KEGG annotations were then assigned to the lncRNA as predicted functions. To further determine the lncRNAs potentially trans-regulating mechanism, the lncRNAs co-expressed mRNAs were compared with mRNAs which are regulatory targets of certain transcription factors (TFs). Then the co-expressed lncRNAs were predicted to possibly participate in pathways regulated by these TFs. In our study, the latest ENCODE data of TFs and their targets were used. The lncRNA-TF and lncRNA-TF-target networks were constructed using MATLAB 2012b, and networks were illustrated using Cytoscape 3.01.

3.1 Results

3.1.1 Specific surface markers of the sorted TEMs

TEMs (Tie2⁺CD14⁺) were sorted from PBMCs and the Tie2 expression in TEMs was confirmed by post-sorting (**Supplementary Figure 1A**), PCR and western blot analysis (**Supplementary Figure 1B-C**). Moreover, the higher expression level of CD11c and the lack of CCR2 expression in TEMs compared to Tie2-monocytes were also confirmed by western blot (**Supplementary Figure 1C**). The endothelial-specific genes such as CD31 and VEGFR2 were found not expressed in TEMs using human umbilical vein endothelial cells (HUVECs) as the positive control (**Supplementary Figure 1D**) though both of them expressed Tie2.

3.1.2 Differentially expressed mRNA profiles in TEMs and their function annotations

According to the microarray, we found 141 dysregulated mRNA transcripts in TEMs compared to Tie2⁻monocytes, with 37 up-regulated and 104 down-regulated. Heat map of the differentially expressed mRNAs (**Figure 1A**) was generated by unsupervised hierarchical clustering analysis. As expected, the dysregulated mRNAs clearly self-segregated into TEMs and Tie2⁻monocytes clusters.

The most up-regulated mRNA transcript was ZNF770 (probe A_33_P3423425), with an FC of 17.89. The two most down-regulated mRNA transcripts (represented by probe A_23_P324885 and A_23_P212354) were two transcript variants of a single protein-coding gene, CCR2, with FCs of 7.60 and 7.08, respectively. The full set of

dysregulated RNA transcripts is listed in supplemental data. We chose a total of 10 differentially expressed mRNAs (six up-regulated and four down-regulated) for qRT-PCR verification (**Figure 1B**).

The functions of the up- and down-regulated mRNAs were annotated separately by GO analysis at the following three levels: cellular component (GOCC, **Figure 1C**), biological process (GOBP, **Figure 1D**) and molecular function (GOMF, **Figure 1E**). The dysregulated mRNAs were clustered into hundreds of GO annotations, and the top 12 annotations that included the most mRNAs are shown. The most frequently predicted annotations of the up-regulated mRNAs included signal transduction (GOBP), extracellular region (GOCC) and actin-binding (GOMF). For the down-regulated mRNAs, the most frequently predicted annotations involved the regulation of small GTPase-mediated signal transduction (GOBP), cytosol (GOCC) and ATP-binding (GOMF). As expected, several up-regulated mRNAs participate in more than one GO pathway involved in angiogenesis and tumor pathogenesis. For example, the mRNA IGF1 (A_23_P13907) is predicted to function in signal transduction, blood vessel remodeling, the positive regulation of epithelial cell proliferation and prostate cancer. Another mRNA, ECM1 (A_23_P160559), is annotated in the positive regulation of angiogenesis and the positive regulation of endothelial cell proliferation.

3.1.3 Differentially expressed lncRNA profiles in TEMs and the cis/trans-regulation analysis

In our microarray analysis, 142 lncRNA transcripts were differentially expressed in

227 TEMs, with 45 up-regulated and 97 down-regulated. Among these dysregulated
 228 lncRNA transcripts, TCONS_00000768 (probe A_21_P0001246) was the most
 229 up-regulated, with an FC of 6.43, whereas Hs.689354 (probe A_21_P0014756) was
 230 the most down-regulated, with an FC of 7.28. Heat map of those dysregulated
 231 lncRNAs (**Figure 2A**) was generated by unsupervised hierarchical clustering analysis.
 232 Six lncRNA transcripts (three up-regulated and three down-regulated) were chosen for
 233 qRT-PCR verification (**Figure 2B**).
 234 We calculated the co-expressed mRNAs for each dysregulated lncRNA and found that
 235 the lncRNAs were co-expressed with hundreds of mRNAs. For example, the
 236 up-regulated lncRNA TCONS_00009753 (A_21_P0004167) was co-expressed with
 237 100 mRNA transcripts, and the down-regulated lncRNA TCONS_00004782
 238 (A_21_P0002594) was co-expressed with 94 mRNA transcripts.
 239 Base on the lncRNA- mRNA co-expression profiles, cis- and trans-regulation between
 240 the lncRNAs and the mRNAs were conducted. For the analyses of trans-regulation,
 241 378 lncRNA–TF pairs were found using $P < .01$ and $FDR < 0.01$ as the threshold,
 242 corresponding to 25 TFs. Then a core network (**Figure 2C**) of the top 100
 243 lncRNA–TF pairs with the lowest P values and FDRs (as the most credibility) was
 244 generated. In the network we found that most of these lncRNAs with potential
 245 trans-regulatory functions participate in pathways regulated by four TFs: PBX3
 246 (participates in 35 of the 100 pairs), NFYA (in 12 pairs), NFYB (in 11 pairs), and
 247 SPI1 (in 12 pairs). We also found a total of 26 lncRNAs with their predicted
 248 cis-regulated mRNAs through accurate genomic mapping. For example, the lncRNA

TCONS_00004782 (A_21_P0002594) is predicted to cis-regulate 4 mRNA transcripts: EPAS1 (A_23_P210210), CRIPT (A_33_P3251989) and two RHOQ transcripts (A_33_P3271323, A_33_P3327921).

The core network is used to illustrate the cis- regulation between these lncRNA and mRNA transcripts (**Figure 2D**). The functions of the differentially expressed lncRNAs were also predicted by GO annotations of their cis-regulated mRNAs. The most frequently predicted annotations included the synaptosome (GOCC, **Figure 2E**), interspecies interactions between organisms (GOBP, **Figure 2F**) and protein binding (GOMF, **Figure 2G**). We found that many of the annotations such as tuberculosis, phagosome, osteoclast differentiation, antigen processing and presentation and differentiation of Fc gamma R-mediated phagocytosis are related to the phagocytic functions of monocytes/macrophages.

3.1.4 Differentially expressed miRNA profiles in TEMs and target genes prediction

A total of 75 miRNAs (34 up-regulated, 41 down-regulated) were found dysregulated in TEMs. The most up- /down-regulated miRNAs with the greatest were found to be miR-361-3p (down-regulated, fold change=17.06) and miR-6722-3p (up-regulated, fold change=23.41). We also found that the four dysregulated miRNAs (hsa-let-7a-5p, hsa-let-7d-5p, hsa-let-7f-5p, and hsa-let-7 g-5p) in the hsa-let-7 family were all down-regulated in TEMs. Unsupervised hierarchical clustering analysis was used to generate the heat map of the differentially expressed miRNAs (**Figure 3A**), and the dysregulated altered miRNAs self-segregated into TEMs and Tie2⁺ monocytes to as

clearly -separated clusters, as expected. Nine miRNA transcripts (three up-regulated and six down-regulated) were chosen for qRT-PCR verification (**Figure 2B**). The target mRNAs regulated by these miRNAs were predicted by three databases (microRNAorg, PITA, and Targetscan), and 909 mRNAs were predicted in by all of three databases at the same time independently (**Figure 3C**). The function of these 909 target mRNAs were annotated by GO analysis (**Figure 2D-F**). The most frequently predicted annotations included nucleus (GOCC), the protein binding (GOMF) and the DNA-dependent regulation of transcription (GOBP).

The mRNA microarray analysis has found that, an interesting candidate gene, IGF1, which might induce the angiogenesis activities of TEMs in a secretion manner, was up-regulated in TEMs. In view of this, we further search light of this knowledge. Each database was queried for the dysregulated miRNAs with altered expression patterns that could potentially regulate the IGF1 and its related genes (IGFBPs, IGF2, and IGF1R) as target genes. As results, this search discovered that 20 dysregulated miRNAs for IGF1, 10 for IGF2, 4 for IGFBP6 and 14 for IGF1R had altered expression in TEMs (listed in supplementary data). Surprisingly, the results show that for these four target genes, most of the miRNAs were found down -regulated (18 in 20 for IGF1, 8 in 10 for IGF2, 3 in 4 for IGFBP6, 8 in 14 for IGF1R) according to the microarray analysis. As the most down-regulated miRNA, hsa-miR-361-3p was predicted to regulate IGF1 and IGFBP6. Another miRNA, hsa-miR-423-5p, as the forth down-regulated miRNA in TEMs with a fold change of 11.83, was predicted to regulate IGF1, IGF2, and IGFBP6. And the four down-regulated miRNA members in

hsa-let-7 miRNA family were predicted to regulate IGF1, IGF2, and IGF1R. We then examined the expression of IGFBP6, IGF2 and the IGF1R by qPCR and these IGF1 related genes were found significantly increased in TEMs (**Supplementary Figure 2A-C**).

4.1 Discussion

In our study, transcriptome patterns of sorted TEMs and Tie2⁺ monocytes were explored by microarray for the first time. Not only mRNA but also non-coding RNAs expression levels were analyzed. We found hundreds of RNAs including 141 mRNAs, 142 lncRNAs and 75 miRNAs were dysregulated in TEMs. These results indicate that TEMs has the distinct transcriptome pattern compared to Tie2⁺ monocytes and these dysregulated RNAs might contribute to TEMs pro-angiogenic capacity in various manners.

IGF1, an mRNA transcript encoding a secreted protein similar to insulin in function and structure and a member of a protein family involved in growth and development, was up-regulated in TEMs. According to the gene ontology annotation, IGF1 was involved in both blood vessel remodeling and the positive regulation of epithelial cell proliferation. Therefore, we can infer that TEMs might promote angiogenesis and even tumor development by secreting IGF1 into tumor microenvironment. Interestingly, target genes prediction of the dysregulated miRNAs in TEMs shows that IGF1, together with several related genes such as IGFBPs and IGF1R, were predicted to be target genes of the dysregulated miRNAs, especially down-regulated miRNAs in TEMs. It is well-accepted that miRNAs can regulate their target protein-coding

mRNAs in an interfering manner and these data indicated that angiogenesis induced via IGF1 might also be regulated by the distinct miRNA expression profiles in TEMs. Moreover, CCR2, which plays an important role in the recruitment of monocytes to tissues as the receptor of the chemokine MCP1, was the most down-regulated mRNA in TEMs, with a fold change more than seven. The result indicates that TEMs are likely attracted to tissues through a mechanism independent of CCR2 and more study should be performed.

In contrast to mRNAs, lncRNAs are thought to function through regulating their co-expressed protein-coding genes. In our study, the lncRNA-mRNA co-expression profiles were conducted firstly, and then the cis/trans-regulation between the lncRNAs and the mRNAs were analyzed based on the co-expression profiles. The cis-regulation analysis confirmed the function of lncRNAs as transcriptional regulators. In addition, the functions of the trans-regulated lncRNAs were predicted through the TFs that possibly regulate their expression. In the core network of top100 lncRNA-TF pairs, the lncRNAs were mostly regulated by four TFs: PBX3, NFYA, NFYB, and SPI1. The PBX protein families, directly binding to HOX proteins, were known to increase DNA-binding/transcriptional activity of the HOX proteins. PBX3, but not PBX1 or PBX2, was found co-expressed with HOXA9 in different subtypes of cytogenetically abnormal acute myeloid leukemia (CA-AML) and appeared to have potential pathologic functions as cofactor of HOXA9 in CA-AML (Li et al., 2013). NFYA and NFYB are two genes that encode the alpha and beta subunits of the nuclear transcription factor Y (NFY). And the NFY was reported to have critical roles for

hematopoietic stem cell proliferation and survival (Bungartz et al., 2012). And the gene SPI1 encodes an ETS-domain transcription factor that has functions during myeloid and B-lymphoid cell development by activating gene expression, and the SPI1/PU.1 transcription factor was reported (Jego et al., 2014) to be essential for macrophage differentiation regulated by the heat shock factor 1 (HSF1). Thus, the trans-regulation analyses indicated that the lncRNAs may affect many biological processes such as proliferation, survival, and differentiation in TEMs.

5.1 Conclusion

In conclusion, our study has revealed a distinct RNA expression profile of TEMs, including mRNA and non-coding-RNAs, compared to Tie2⁺ monocytes. According to the microarray and the qRT-PCR analysis, the absence of an important chemokine receptor, CCR2, was confirmed in TEMs. And the up-regulation of IGF1 indicates a possible molecular basis of the prominent angiogenesis-promoting activity of this novel subset of monocytes. And miRNAs functional analysis showed that the angiogenesis promoted by TEMs might be regulated by a complex RNA interfering mechanism. Moreover, the distinct lncRNA expression profile and associated cis/trans-regulation of key target genes represent an important component of this phenotype and the general immune function of TEMs. However, the molecular mechanisms by which TEMs are attracted from the circulatory system to tissues, the activities that depend on IGF1 and the exact lncRNAs that perform important functions in TEMs remain unclear. Considering that TEMs may represent an important target of anti-angiogenesis therapy in human diseases like tumors,

additional studies of their characteristics should be conducted in the future.

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8.1 Figure legends

8.1.1 Figure 1. Differentially expressed mRNA profiles and the GO annotation

A. Heatmap of the differentially expressed mRNAs in TEMs and Tie2⁺ Monocytes

B.qRT-PCR of ten dysregulated mRNAs shows the same regulation of mRNAs with microarray analysis

C. GOCC annotations of the mRNAs

D. GOBP annotations of the mRNAs

E. GOMF annotations of the mRNAs

8.1.2 Figure 2. Differentially expressed lncRNA profiles and the cis/trans-regulation analysis

A. Heatmap of the differentially expressed lncRNAs in TEMs and Tie2⁺ Monocytes

B.qRT-PCR of six dysregulated lncRNAs

C.Network of the lncRNAs and their trans-regulated mRNAs

D.Network of the lncRNAs and their cis-regulated mRNAs

E.GOCC annotations of the cis-regulated mRNAs

F.GOBP annotations of the cis-regulated mRNAs

G.GOMF annotations of the cis-regulated mRNAs

8.1.3 Figure 3. Differentially expressed miRNA profiles and the target genes analysis

A. Heatmap of the differentially expressed miRNAs in TEMs and Tie2 Monocytes

B.qRT-PCR of nine dysregulated miRNAs

C.Target mRNA prediction of the dysregulated miRNAs

D.GOCC annotations of the target mRNAs

E.GOBP annotations of the target mRNAs

F.GOMF annotations of the target mRNAs

9.1 Figures

9.1.1 Figure 1

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