Original Article

A Bioinformatics Analysis of Exosomal MicroRNAs Released following Mycobacterial Infection

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Abstract

Background: Tuberculosis (TB) still remains a major health threat worldwide. The current TB diagnostics are suboptimal, and there is a high clinical need for identifying novel biomarkers of disease prevalence. Circulating exosomes have been currently attractive as novel biomarkers in a wide range of pathological conditions. **Methods:** In this study, we performed bioinformatics analysis on the downstream targets of a dysregulated microRNA (miRNA) cluster induced by Bacillus Calmette–Guerin infection of human macrophages to provide greater understanding of their potential roles in disease pathogenesis. **Results:** Our analysis demonstrated that these dysregulated miRNAs have central roles in the host metabolic and energy pathways. **Conclusion:** This suggests that the host miRNA network is perturbed by *Mycobacterium* to re-patterning host metabolism machinery to favor its intracellular survival. The dysregulated miRNAs can be delivered to local and distal cells by exosomes and thereby modulate their function.

Keywords: Clusters, exosomal, microRNAs, tuberculosis

Introduction

Tuberculosis (TB) is a potentially serious infection that still remains the most common cause of death from infection, despite the global advances in health and disease.[1,2] According to a recent World Health Organization report, 9.6 million new TB cases and 1.5 million TB deaths occur each year.[3,4] In addition, about one-third of the world's population has latent TB showing no overt symptoms of disease but acting as a source for spreading the infection.[5] Since the early detection of TB is important in controlling and preventing infections from spreading, and because of the challenges with current TB diagnostics, the development of novel biomarkers is essential.[6,7] Exosomes have been proposed as novel diagnostic biomarkers in a wide range of pathological conditions, such as cancers and infectious diseases.[8-11]

Exosomes are 30–100 nm vesicles containing functional molecules, such as nucleic acids (mRNA and non-coding RNAs, DNA), proteins, metabolites, and lipid mediators.[12,13] Exosomes can be derived from nearly all cell types and are isolated from almost all human biofluids.[12,13] Exosomes play a key role in cell–cell communication and transfer biological information by shuttling their cargo to either local or more distant cells and thereby modulate the function of the recipient cells.[13-15] Exosomal contents have been used as disease signatures in various cancers[16-18] as well as in Alzheimer's disease.[19]

MicroRNAs (miRNAs) are small 18–22 ntRNAs that have an important role in modulating gene expression and translation. miRNAs can affect most biological functions, and their dysregulation is associated with several pathologies.[20]

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Functional miRNAs may be encapsulated inside exosomes, delivered to target cells causing effects on the recipient cell function by affecting their transcriptome and/or proteome.[21]

miRNAs are involved in the regulation of inflammatory processes during *Mycobacterium tuberculosis* (Mtb) infection.[22,23] Mtb infection triggers a range of physiological responses in infected cells, leading to host immune dysregulation and metabolic re-patterning.[24,25] These modulations in host cell functions enable bacteria to sequester vital host factors to supply their food and energy requirements to enable intracellular survival.[25] These processes may be controlled by subversion of host miRNA networks that are involved in the regulation of carbon, nitrogen, and lipid metabolism in the infected host cells.[26,27]

We have previously observed that infection of human monocyte-derived macrophages (MDMs) with *Mycobacterium bovis* Bacillus Calmette–Guerin (BCG) induced the secretion of a specific set of exosomal miRNAs, including mir-1224, mir-484, mir-1293, mir-423, and miR-96, which are involved in modulating key host metabolic and energy production pathways as well as regulating immunological and cell signaling events.[22] In this study, we hypothesized that further bioinformatic analysis of the downstream targets of dysregulated miRNA cluster induced by BCG infection may provide further insight into their potential roles.

Methods

MicroRNAs network and enrichment analysis

The isolation and detection of exosomal miRNAs from BCG-exposed human MDMs have been described previously.[22]

The target genes of the interested miRNAs were determined using miRTarBase[28] and microT-CDS algorithms.[29] Protein–protein interaction (PPI) networks were constructed and analyzed by Cytoscape v. 3.4.0.[30] Briefly, miRNA target genes were converted to seed proteins and PPI networks were constructed using GeneMANIA plugin and visualized and analyzed in Cytoscape.

GeneMANIA provides information on both the experimental and the predicted interactions based on their gene neighborhood, gene co-occurrence, gene fusions, gene co-expression, experiments, and literature mining.

The highly interconnected regions (clusters) were extracted using the Molecular COmplex DEtection (MCODE) plugin within Cytoscape. This clustering method isolates the dense proteins based on their local neighborhood density and distance from a local seed protein. Enrichment analysis was performed using EnrichR[31] and KEGG pathway[32] database for nodes within each cluster.

Results

Protein–protein interaction network of the candidate microRNA target genes and enrichment analysis

Differentially regulated miRNAs were called according to a log2-fold difference of greater than 2 and/or an adjusted $P < 0.05$. This gave a total of five miRNAs – miR-1224, miR-484, miR-1293, miR-423, and miR-96. To survey the interactions between the genes targeted by the identified dysregulated miRNAs, a PPI network was constructed. We first constructed an extended network of the seed proteins, their direct PPI neighbors, and their interactions. The extended network consisted of 96 nodes connected via 1022 edges. Extracting the highly connected regions from this network using MCODE identified five clusters [Figure 1]. The number of nodes in clusters 1–5 was 16, 12, 11, 10, and 15 with 90, 45, 27, 20, and 28 edges, respectively. The score of these subnetworks was 1 = 7.86; 2 = 5.81; 3 = 4.4; 4 = 4.22; and 5 = 3.57 [Table 1]. To understand the biological meaning behind the highly connected regions, enrichment analysis was performed on the nodes within each cluster. Using the EnrichR tool, the most significant gene ontology biological process terms identified within the clusters are provided in Table 2. The results showed that cluster 1 with the highest score of 7.8 contains proteins that participate in B-cell activation involved in immune response; cluster 2 was most associated with positive regulation of phosphatidylinositol 3-kinase signaling; cluster 3 with positive regulation of lipid metabolic process; cluster 4 with inositol phosphate biosynthetic process; and cluster 5 with the Fc receptor signaling pathway.

Pathway analysis utilizing KEGG, Reactome, and WikiPathways within EnrichR revealed that the target genes within each cluster were mostly involved in signaling pathways, metabolism, and immunological pathways [Table 3]. In particular, cluster 1 was most associated with endocytosis, bacterial invasion of epithelial cells, and

Table 1: Protein–protein interaction data of intersection network identified by Molecular COmplex DEtection plugin

Cluster	Score	Nodes	Edges
1	7.86	16	90
2	5.81	12	45
3	4.4	11	27
4	4.22	10	20
5	3.57	15	28

Table 2: Gene ontology biological process identified in the Molecular COmplex DEtection clusters using the EnrichR tool

MCODE cluster	Description	*P*
1	B cell activation involved in immune response	7.1×10^{-5}
2	Positive regulation of phosphatidylinositol 3-kinase signaling	3.9×10^{-3}
3	Positive regulation of lipid metabolic process	1.3×10^{-6}
4	Inositol phosphate biosynthetic process	1.4×10^{-6}
5	Fc receptor signaling pathway	5.5×10^{-4}

MCODE: Molecular COmplex DEtection

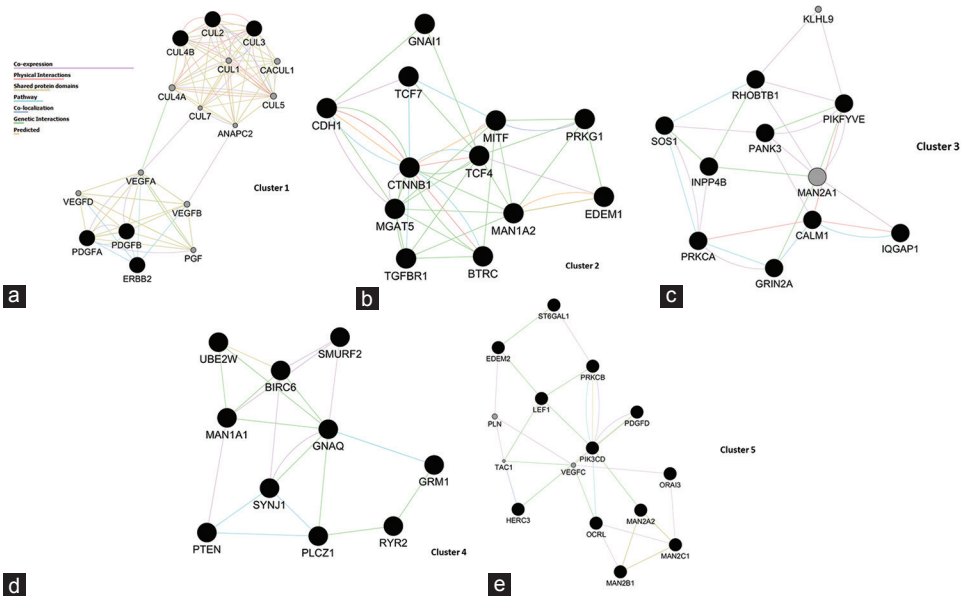

Figure 1: Protein–protein interaction network of the dysregulated microRNA gene targets. Protein–protein interaction networks were constructed and the clusters contained within the highly connected regions in the network identified by Molecular COmplex DEtection plugin. (a) Cluster 1, (b) cluster 2, (c) cluster 3, (d) cluster 4, and (e) cluster 5. Nodes represent proteins and edges represent interactions between two proteins. Node size indicates the generality of each gene ontology term (larger = more specific). Node color indicates the *P* values of each gene ontology term in each intersection (darker = more significant)

Table 3: Molecular COmplex DEtection clusters and their related pathways

Cluster	Nodes ID in cluster	Pathways
1	VEGFD, VEGFB, VEGFA, PGF, PDGFB, PDGFA, ERBB2, CUL7, CUL5, CUL4B, CUL4A, CUL3, CUL2, CUL1, CACUL1, ANAPC2	Endocytosis, Bacterial invasion of epithelial cells, EGF/EGFR signaling pathway, Golgi-associated vesicle biogenesis; signal transduction; clathrin-derived vesicle budding; trans-Golgi network vesicle budding, focal adhesion
2	TGFBR1, TCF7, TCF4, PRKG1, MITF, MGAT5, MAN1A2, GNAI1, EDEM1, CTNNB1, CDH1, BTRC	Adherents junction; focal adhesion; regulation of lipid metabolism; fatty acid, triacylglycerol and ketone body metabolism
3	KLHL9, CALM1, GRIN2A, SOS1, PANK3, IQGAP1, RHOBTB1, PRKCA, INPP4B, PIKFYVE, MAN2A1	N-Glycan biosynthesis, proteasome degradation, metabolism pathways, asparagine N-linked glycosylation; T-cell receptor and co-stimulatory signaling; ErbB signaling pathway; downstream signaling of activated FGFRs; VEGFA-VEGFR2 pathway
4	UBE2W, SYNJ1, SMURF2, RYR2, PTEN, PLCZ1, MAN1A1, GRM1, GNAQ, BIRC6	Adaptive immune system; antigen processing; ubiquitination and proteasome degradation; calcium signaling pathway; Class I MHC-mediated antigen processing and presentation; estrogen signaling pathway; gap junction; inositol phosphate metabolism; insulin secretion; metabolism; phosphatidylinositol signaling system; phospholipid metabolism; ubiquitin mediated proteolysis
5	VEGFC, TAC1, ST6GAL1, PRKCB, PLN, PIK3CD, PDGFD, ORAI3, OCRL, MAN2C1, MAN2B1, MAN2A2, LEF1, HERC3, EDEM2	Calcium signaling pathway; focal adhesion; asparagine N-linked glycosylation; beta-catenin independent WNT signaling; Downstream signaling events of (BCR); IL-3 signaling pathway; N-glycan antennae elongation in the medial/trans-Golgi; phosphatidylinositol signaling system; PI metabolism; Rap1 signaling pathway; Ras signaling pathway; signaling by receptor tyrosine kinases

BCR: B-Cell Receptor

EGF/EGFR signaling; cluster 2 with adherents junction, focal adhesion, and regulation of lipid metabolism; cluster 3 with N-glycan biosynthesis, proteasome degradation, and metabolic pathways; cluster 4 with the adaptive immune system, antigen processing, and ubiquitination and proteasome degradation; and cluster 5 with calcium signaling, focal adhesion, and B-cell receptor and interleukin-3 signaling.

Discussion

In the current study, we assessed the potential regulatory effect of a cluster of dysregulated exosomal miRNAs released by human MDMs upon infection with *Mycobacterium*. Bioinformatic analysis demonstrated that these miRNAs regulate key metabolic and energy pathways including central carbon metabolism, fatty acids and sugar metabolism, bacterial

invasion, and immunological-related pathways as well as cell signaling pathways.

This finding suggests that *Mycobacterium* may recruit host miRNAs to modulate host metabolic reprograming or re-patterning to favor its intracellular survival. These dysregulated miRNAs can be enclosed inside exosomes and released into the biological fluids to modulate the function of local and distal cells. Exosomes have been reported as novel biomarkers in several diseases, including acute myeloid leukemia, asthma, and sarcoidosis.[18,21] In TB, the exosomal miRNA profile has recently been proposed as a diagnostic biomarker.

miRNAs generally exert their effects in the context of complex regulatory networks.[33] Understanding the role of miRNAs in the modulation of pathological conditions depends on our knowledge of their functions in the context of these networks.[34] Network analysis of miRNA incorporates known and predicted protein–protein interaction data, based on their gene neighborhood, gene fusions, gene co-occurrence, gene co-expression interactions determined from both laboratory and text mining analysis.[35,36] In a given network, nodes are genes and the interactions between the nodes are defined as edges. Node degree shows the number of edges connected to a node, and nodes with high degree may indicate the key proteins with important biological functions.[36]

Our miRNA network gave a total of 1022 interaction pairs over 96 nodes. Cluster 1 was the most connected with the highest score and as with two or the five other clusters contained nodes enriched for metabolism and infection-related pathways. This suggests that these miRNAs and their gene targets have extensive interactions that control the host response to *Mycobacterium* infection. Specific proteins targeted by this cluster include mitochondrial fission protein 1 [Figure 1] that is regulated by miR-484[37] and a number of lipid metabolizing proteins targeted by miR-1224.[38] Furthermore, miR-425 has also been implicated in the control of various metabolic pathways and is associated with several metabolic disorders.[39]

In addition, both miR-425 and miR-96 mediate insulin resistance with miR-96 regulating the expression of multiple genes that fine-tune insulin release.[40,41] For example, miR-96 suppresses insulin secretion by increasing the level of granuphilin, an inhibitor of insulin exocytosis.[35] miR-96 is strongly induced by fatty acids and downregulates the expression of the insulin receptor (INSR) and the INSR substrate 1.[41] On the other hand, Mtb-infected macrophages show a disturbed sugar flux and a significant change in the intracellular levels of glucose, glycogen, NAD/NADP, and lactate.[25,42] Mtb infection also causes increased aerobic glycolysis as a result of the pentose-phosphate shunt and elevated glucose uptake.[25,43]

In this small pilot study, we have obtained a comprehensive network analysis of the pathways affected by the dysregulated miRNAs found in exosomes from Mtb-infected human macrophages. We demonstrated that this cluster of miRNAs modulate not only key host immune functions but also re-pattern critical metabolic and energy production pathways. Since the nutrients required for intracellular survival of Mtb are restricted inside the host cell, it is likely that the bacteria sequester the host's cellular metabolism to optimize the chances of survival and growth.[44] Our data suggest that this is accomplished, at least in part, through dysregulation of host miRNA networks that control the host's carbon and nitrogen metabolism. These Mtb-induced dysregulated miRNAs also impact on amino acid, pyrimidine, and purine nucleotide biosynthesis which are increased in Mtb-infected lung tissue.[45]

Finally, the target genes of the altered miRNAs also were involved in cell membrane and communication pathways, such as cell junction, glycosaminoglycan biosynthesis, and heparan sulfate metabolism. This further emphasizes the ability of Mtb to alter the host cell membrane structure to support its intracellular growth and proliferation.

Conclusion

These findings emphasize the multifactorial effects of miRNAs on cellular metabolism and shed light on the host–pathogen interaction and modulation in cellular function following Mtb infection. The data highlight key PPI networks that have a potential pivotal role in host metabolic reprogramming to favor Mtb survival. Functional analysis of these effects is required to confirm their roles. It also may be possible to use exosomal dysregulated miRNAs as potential biomarkers for TB therapy monitoring and diagnosis of active TB.

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Conflicts of interest

There are no conflicts of interest

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