

Bioinformatics analysis of microRNA expression between patients with and without latent tuberculosis infections

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Abstract. Tuberculosis (TB) is a globally prevalent infectious disease. The mechanisms of latent TB infection (LTBI) remain to be fully elucidated and may provide novel approaches for diagnosis. As therapeutic targets and molecular diagnostic markers, microRNAs (miRs) have been studied and utilized in various diseases. In the present study, the differentially expressed miRs (DEMs) in LTBI were screened and analyzed to determine the underlying mechanisms and identify potential biomarkers, thereby contributing to the diagnosis of LTBI. The GSE25435 and GSE29190 datasets from Gene Expression Omnibus were selected for analysis. The 2 datasets were analyzed individually using the Bioconductor package to screen the DEMs with specific cut-off criteria [$P < 0.01$ and $\log_2(\text{fold change}) \geq 1$]. Target gene prediction and interaction network construction were performed using Targetscan, the Search Tool for the Retrieval of Interacting Genes and Proteins and Cytoscape individually, and were merged using the latter tool. The hub genes were finally selected based on their degree of connectivity (DC). Gene Ontology and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were performed using the KEGG and GENCLIP. A total of 144 DEMs were identified from the 2 datasets. By exploring the overlapping miRs in the two datasets, *Homo sapiens* (hsa)-miR-29a and hsa-miR-15b were identified to be decreased, while hsa-miR-576-5p, hsa-miR-500 and hsa-miR-155 were identified to be upregulated. hsa-miR-500a-3p and hsa-miR-29a-3p,

as well as 4 genes, namely cell division cycle (CDC)42, actin $\alpha 1$, skeletal muscle (ACTA1), phosphatase and tensin homolog (PTEN) and fos proto-oncogene (FOS), were selected as the key factors in this regulatory network. A total of 9 signaling pathways, including phosphoinositide-3 kinase (PI3K)/AKT and 11 biological processes, were identified to be associated with LTBI. In conclusion, the present analysis identified hsa-miR-500a-3p and hsa-miR-29a-3p, as well as CDC42, ACTA1, PTEN and FOS, as the most promising biomarkers and therapeutic candidates for LTBI. The PI3K/AKT signaling pathway is the key signaling pathway implicated in LTBI, and an in-depth investigation of the efficiency of PI3K/AKT signaling inhibitors may be used to prevent a chronic state of infection in LTBI.

Introduction

Tuberculosis (TB) is a global infectious disease caused by infection with the pathogen *Mycobacterium tuberculosis* (MTB) and poses a serious risk to human health. It is a contagious disease that spreads by inhalation of bacteria contained in the breath of an infected individual. China ranks second out of the countries with a high TB burden. According to the 2017 World Health Organization (WHO) global TB report (1), TB is the ninth most lethal disease in the world and ranks first among infectious diseases, with a mortality rate much higher than that of acquired immune deficiency syndrome. Latent TB infection (LTBI) is defined as a state of persistent immune response to stimulation by MTB antigens with no evidence of any clinical manifestations of active TB (2). Within the population of individuals with LTBI, most cases remain asymptomatic and are not contagious; however, 5-10% of those who are infected progress to active TB disease and become contagious (2,3). LTBI is a continuum between self-healed and asymptomatic (4). At present, there are no unified diagnostic criteria for LTBI. The current method used is the tuberculin skin test (TST) and interferon- γ release assay (IGRA), however both have certain limitations, which include poor specificity and high cost of diagnosis, as well as being complex techniques (5-8). At present, the WHO recommends the use of the IGRA to detect LTBI in middle- and high-income countries with greater resources (9), while the TST

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is recommended in countries with limited resources. Therefore, it is necessary to develop a rapid, low-cost, non-invasive and efficient diagnostic method for the prevention and control of TB.

MicroRNAs (miRs) are a class of non-coding RNAs composed of 21–23 nucleotides, which are not translated into proteins. They are cut from precursor miRs with 6,070 nucleotides and a hairpin structure. The major function of miRs is to regulate gene expression at the post-transcriptional level, primarily by binding to the 3'-untranslated region of the mRNAs of their target genes to degrade the mRNA or inhibit its translation, thus inhibiting the expression of the target genes (10). When disease occurs, the specific miRs of the lesion organs may be released into the blood. In numerous diseases, the miR content in the blood is significantly different from that of healthy individuals (11). To date, miRs as molecular diagnostic markers have been studied and applied in cancer, diabetes, psychiatric disorders, heart disease and various infectious diseases (12–16). More recent evidence suggests the use of miRs as biomarkers for LTBI. However, compared with the well-known role of certain miRs in cancer, the biological functions and diagnostic utility of miRs in LTBI remain largely elusive. Wu *et al.* (17) identified that the expression of *Homo sapiens* (hsa)-miR-142-3p and hsa-miR-21-5p was enhanced in the peripheral blood samples from patients with LTBI compared with patients with TB. Meng *et al.* (18) indicated that miR-93-3p is a potential diagnostic marker for distinguishing LTBI from active TB. Using bioinformatics to further analyze gene expression data, the present study unveiled certain characteristics and mechanisms involved in TB latency, which may provide a theoretical basis for the early diagnosis and treatment of, and experimental research into TB (19), and may serve as a reference for further investigation of molecular events indicative of LTBI.

In previous decades, microarray analysis has been frequently used to identify candidate biomarkers and therapeutic targets by studying changes in non-coding RNA and gene expression profiles at a genome-wide scale (20). A previous study on diagnosing latent TB infection identified dysregulated miRs and associated pathways of LTBI (21). However, only a small number of miRs have been identified to be significantly differentially expressed between patients with and without LTBI. Due to these discordant results, the reliability of these data for further development into useful clinical diagnostic biomarkers and therapeutic targets for TB is limited. It has been well recognized that small sample sizes, different microarray platforms and different statistical methods are among the limiting factors contributing to the discordant results. To overcome this limitation, meta-analysis represents a powerful approach to combine datasets from different studies to improve the reliability and generalizability of the results by increasing the statistical power. Meta-analysis of gene expression data or non-coding RNA profiles has yielded novel biological insight, and has identified more robust and reliable candidate biomarkers and therapeutic targets.

In the present study, to increase the understanding of latent TB infection, the GSE25435 and GSE29190 datasets were downloaded from the GEO database and analyzed using R tools for differentially expressed miRs (DEMs). The regulatory network of these DEMs and their target genes was constructed to explore the potential regulatory interactions

involved in LTBI. Enrichment analysis of these target genes in Gene Ontology (GO) terms in the category biological process (BP), molecular function (MF), cellular component (CC) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways was performed for further identification of LTBI-associated pathways and molecular mechanisms.

Materials and methods

Selection of microarray datasets. To retrieve suitable LTBI-associated miR profiling data for the meta-analysis, a web-based search in the Gene Expression Omnibus database (GEO; <http://www.ncbi.nlm.nih.gov/geo>) and ArrayExpress (<http://www.ebi.ac.uk/arrayexpress>) database was performed using the key words 'latent TB infection' and 'microRNA'. In total, 5 array datasets were identified in the GEO and ArrayExpress databases. The datasets were manually reviewed and only those fulfilling the following criteria were included for further analysis: i) miR expression profiling by array; and ii) paired samples from patients with and without LTBI. Finally, a total of 2 datasets, GSE25435 and GSE29190, were selected for analysis. miR expression data of peripheral blood mononuclear cells (PBMCs) from 6 donors with LTBI and 3 healthy controls are included in GSE29190. GSE25435 contains miR expression data from PBMCs of 3 donors with LTBI and 3 healthy controls.

Screening of DEMs. The 2 datasets were analyzed individually by using the Bioconductor package to screen the DEMs with specific cut-off criteria [$P < 0.01$ and $|\log$ fold change (FC)| ≥ 1] (22). Paired samples t-tests and the FC method were used to obtain DEMs. The DEMs of the 2 datasets were then uploaded to the online VENN tool (<http://bioinformatics.psb.ugent.be/webtools/Venn/>) to identify the overlapping miRs (23).

Target gene prediction and network construction. The prediction of target genes of different miRs was performed by using TargetScan (http://www.targetscan.org/vert_71) (24). A cumulative weighted context++ score online > -0.5 was set as the standard for screening target genes, as previously described (24). The target genes were then uploaded to the online database Search Tool for the Retrieval of Interacting Genes and Proteins (STRING; string-db.org) (25) to obtain the interaction information for these target genes. The miR targeting data and target gene interaction data were imported to Cytoscape, which was used to visualize and merge networks. The key factors were finally selected based on their DC (26,27).

GO and KEGG pathway analysis. Pathway enrichment analysis was performed using KEGG, a collection of databases on genomes, biological pathways, diseases, drugs and chemical substances (28). Target genes identified in the above-mentioned analysis were uploaded to GENCLIP (ci.smu.edu.cn/GenCLIP2/analysis.php) to perform the GO enrichment analysis in the categories BP, CC and MF, and $P < 0.01$ was set as the cut-off criterion (29).

Results

Individual microarray data analysis of DEMs. To identify DEMs in patients with LTBI vs. healthy individuals, $P < 0.01$

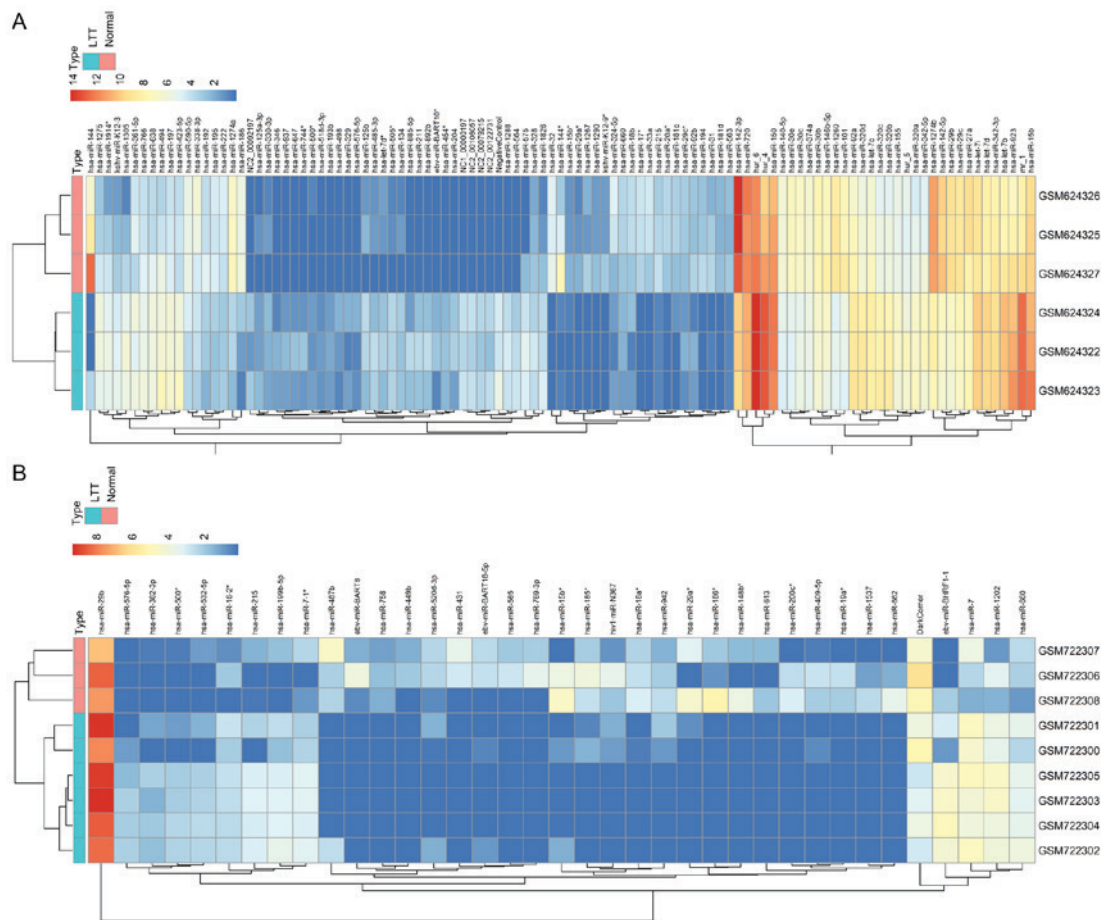


Figure 1. Hierarchical clustering of the DEMs from patients with and without LTBI. The color scale at the top illustrates the relative expression level of an mRNA. Red represents a high relative expression and blue represents a low relative expression. (A) DEMs of GSE25435. (B) DEMs of GSE29190. DEM, differentially expressed miR; LTB, latent tuberculosis; miR, microRNA; hsa, *Homo sapiens*.

and $\log_2FC \geq 1$ were set as cut-off criteria when screening the miR array profiles. A total of 107 miRs, including 44 downregulated and 63 upregulated miRs, were identified to be dysregulated in the GSE25435 dataset (Table I). The GSE29190 dataset was also analyzed, revealing only 37 DEMs, including 24 downregulated and 13 upregulated miRs (Table II). The clustering heatmap analysis of these two datasets indicated a highly significant difference between LTBI and normal patients, meriting further analysis (Fig. 1).

Meta-analysis of the dysregulated miRs provides 5 hub miRs. Due to the limitations of the analyses of the individual datasets, the overlapping miRs between the two datasets were selected for the analysis of hub miRs. By Venn diagram analysis, hsa-miR-29a and hsa-miR-15b were identified as being downregulated in the two datasets (Fig. 2A), while hsa-miR-576-5p, hsa-miR-500 and hsa-miR-155 were identified as being upregulated (Fig. 2B).

Construction of the molecular network provides complex regulatory interactions. The target genes of the abovementioned miRs were assessed using the online tool Targetscan with a pre-defined standard. A total of 216 potential target genes were identified in the present analysis and they are listed in Table III. To illustrate the direct regulatory associations between the hub miRs and their target genes, as well as interactions of these target genes,

the network of these factors was constructed using Cytoscape. A complex regulatory network was obtained, suggesting that dysregulation of miRs during LTBI may cause a widespread dysregulation in gene expression. In addition, dysregulation of miRs during LTBI may lead to functional disorders via signaling pathways or BPs. By exploring the regulatory network, the key miRs and genes may be selected based on the DC in the network. A total of 2 miRs, namely hsa-miR-500a-3p (degree, 54) and hsa-miR-29a-3p (degree, 77), as well as 4 genes, namely cell division cycle (CDC)42 (degree, 21), actin $\alpha 1$, skeletal muscle (ACTA1; degree, 21), phosphatase and tensin homolog (PTEN; degree, 21) and FOS (degree, 21), were selected as the core factors that may have principal roles in LTBI (Fig. 3).

Functional and pathway analysis. To investigate the signaling pathways that are potential associated with LTBI, the target genes were subjected to KEGG pathway enrichment analysis. A total of 9 pathways were enriched by the target genes (Fig. 4), including ‘protein digestion and absorption’, ‘extracellular matrix-receptor interaction’ and ‘phosphoinositide-3 kinase (PI3K)/AKT signaling pathway’. In addition, inclusion of small cell lung cancer pathways in the present analysis indicated that LTBI may be associated with the development of small cell lung cancer. The 185 target genes were subsequently subjected to GO term enrichment analysis which revealed that 90 target genes were enriched in the following pathways:

Table I. Differentially expressed miRs in the GSE25435 dataset.

miR	logFC	AveExpr	t	P-value
hsa-miR-32	-4.83703	2.490441	-32.8327	2.86 x10 ⁻⁶
hsa-let-7d	1.605658	8.870986	25.95471	7.72 x10 ⁻⁶
hsa-miR-1260	-3.67093	6.940923	-21.9504	1.56 x10 ⁻⁵
hsa-miR-181d	-2.24959	1.176625	-21.3805	1.75 x10 ⁻⁵
NC1_00000197	3.79419	1.948923	21.37752	1.75 x10 ⁻⁵
hsa-miR-1288	4.12176	2.112708	20.71065	2.00 x10 ⁻⁵
hur_6	2.153942	13.10695	19.77886	2.43 x10 ⁻⁵
NC2_00079215	3.222861	1.663258	15.16582	7.39 x10 ⁻⁵
hsa-miR-17*	-3.75792	1.930786	-14.9806	7.78 x10 ⁻⁵
NC2_00106057	3.685307	1.894481	14.56989	8.74 x10 ⁻⁵
hsa-miR-142-5p	-3.15876	8.353603	-14.0788	0.000101
hsa-let-7i	1.002891	9.400425	13.92367	0.000106
hsa-miR-30e	-2.12857	6.050006	-13.8563	0.000108
hsa-miR-30b	-1.24457	7.124084	-13.295	0.000128
hsa-miR-454*	2.311672	1.207664	12.96173	0.000142
hsa-miR-1274b	-3.22595	9.539236	-12.8303	0.000148
hsa-miR-20a*	-3.09852	1.688503	-12.5566	0.000162
hsa-miR-564	3.682902	1.893279	11.84041	0.000207
hsa-miR-30c	-1.47533	6.141081	-11.4563	0.000237
hsa-miR-518d-3p	1.098449	0.601052	11.36622	0.000245
hsa-miR-142-3p	-4.08985	11.79739	-11.1118	0.000269
hsa-miR-215	-2.79467	2.085103	-10.6335	0.000323
hsa-miR-320c	2.710049	6.808952	10.40018	0.000353
hsa-miR-29b	-2.6684	7.916884	-10.2334	0.000378
hsa-miR-193b	1.481897	0.792776	10.23095	0.000378
hsa-miR-505*	2.825782	2.002488	10.08595	0.000401
hsa-miR-1274a	-3.96165	5.117785	-10.0041	0.000415
hsa-miR-320d	2.530531	7.386848	9.966743	0.000421
hsa-miR-194	-2.9415	1.573092	-9.87762	0.000437
mr_1	4.009616	10.57346	9.598379	0.000491
hsa-miR-197	1.865861	5.328528	9.377518	0.00054
hsa-miR-33a	-3.71272	1.90819	-8.53538	0.000792
hsa-miR-374a	-1.05367	5.99272	-8.48323	0.000812
hsa-miR-320b	2.595755	6.879281	8.346227	0.000867
hsa-miR-211	2.928911	1.516283	8.276387	0.000897
NC2_00122731	3.230483	1.667069	8.074902	0.000990
hsa-miR-29c	-1.60822	8.001147	-7.87047	0.001098
hsa-miR-195	-1.58473	3.691033	-7.80122	0.001138
hsa-let-7b	2.253171	9.216569	7.57616	0.001279
hsa-miR-563	-1.93468	1.56909	-7.14915	0.001612
hsa-miR-140-5p	-1.35331	5.635543	-7.11292	0.001645
hsa-miR-423-5p	2.546925	5.162408	7.075481	0.001679
hsa-miR-892b	2.908221	1.505938	6.779481	0.001988
hsa-let-7c	1.591322	7.493733	6.714825	0.002065
hsa-miR-181c	-2.61189	1.740467	-6.71423	0.002066
hsa-miR-31	-2.69031	1.538948	-6.55738	0.002267
hur_4	2.724996	11.79659	6.333833	0.002596
hsa-miR-155	1.135612	6.034357	6.210813	0.002802
hsa-miR-720	-1.17258	11.34608	-5.89947	0.003420
hsa-miR-222	-1.33272	3.65274	-5.83189	0.003575
hsa-miR-186	-4.09024	3.67533	-5.76641	0.003734
hsa-miR-101	-2.5019	6.584804	-5.69109	0.003927

Table I. Continued.

miR	logFC	AveExpr	t	P-value
hsa-miR-204	2.505782	1.304719	5.150187	0.005734
hsa-miR-134	2.026807	1.755041	4.931725	0.006741
hsa-miR-1290	-1.70206	0.902855	-4.8857	0.006979
hsa-miR-575	3.799179	2.716106	4.762392	0.007670
hsa-miR-937	1.689168	0.896412	4.735594	0.007830
hsa-miR-320a	1.789151	6.023945	4.670974	0.008235
hsa-miR-590-5p	-1.58344	4.687364	-4.6666	0.008263
hsa-miR-1275	2.152322	4.508394	4.391423	0.010302
NC2_00092197	2.244662	1.174159	4.37517	0.010440
hsa-miR-500*	1.085714	0.806853	4.324164	0.010888
hur_5	1.581846	5.898678	4.300761	0.011102
hsa-miR-766	1.2936	4.933253	4.282655	0.011270
hsa-miR-1305	3.51088	3.923818	4.184017	0.012244
hsa-miR-485-3p	2.264246	1.974846	4.137144	0.012742
hsa-miR-29c*	-1.41527	2.134653	-4.08565	0.013317
hsa-miR-328	1.533516	3.063469	4.035669	0.013904
hsa-miR-1826	2.542979	2.772236	4.009905	0.014219
hsa-miR-923	2.864446	9.516405	3.979143	0.014606
hsa-miR-92a	1.491981	8.018584	3.940985	0.015104
hsa-miR-1267	-2.09638	1.132907	-3.91896	0.015401
hsa-miR-885-5p	2.463596	1.283626	3.881055	0.015927
hsa-miR-144	-7.93309	5.22873	-3.85587	0.016289
hsa-miR-660	-1.32829	2.555425	-3.75816	0.017787
hsa-let-7d*	1.904947	1.863162	3.758015	0.017790
hsa-miR-647	1.663436	0.883546	3.748012	0.017952
hsa-miR-342-3p	1.119628	8.920874	3.645006	0.019731
hsa-miR-15b*	-1.18389	0.868758	-3.5706	0.021146
hsa-miR-144*	-4.64489	2.712225	-3.53154	0.021937
kshv-miR-K12-9*	-1.76769	1.026389	-3.48719	0.022877
hsa-miR-15b	1.65062	10.49454	3.437951	0.023976
hsa-miR-638	1.600023	4.980986	3.434029	0.024066
hsa-miR-744*	1.677503	0.890579	3.371348	0.025564
hsa-miR-346	1.392836	0.748246	3.330718	0.026593
hsa-miR-324-5p	-2.61219	2.446374	-3.28872	0.027707
hsa-miR-498	1.277597	0.690626	3.285391	0.027798
hsa-miR-329	1.335942	0.719799	3.261694	0.028454
hsa-miR-125a-3p	1.424537	1.12041	3.210039	0.029946
hsa-miR-192	-1.44406	3.520292	-3.20944	0.029964
hsa-miR-18b	-1.96872	2.472096	-3.1976	0.030319
hsa-miR-27a	-1.44475	8.246285	-3.19705	0.030335
hsa-miR-92b	-1.58151	2.150902	-3.18198	0.030795
hsa-miR-150	1.084065	11.60013	3.12891	0.032478
hsa-miR-342-5p	1.584302	6.234256	3.120644	0.032750
hsa-miR-29a*	-1.22069	0.662175	-3.08183	0.034061
hsa-miR-576-5p	1.233511	0.668583	3.056434	0.034952
ebv-miR-BART10*	1.833523	0.968589	3.013552	0.036519
hsa-miR-1914*	2.359262	4.149506	2.986318	0.037556
hsa-miR-330-3p	1.331046	1.394026	2.970476	0.038175
hsa-miR-494	2.245492	5.43426	2.961052	0.038548
hsa-miR-361-5p	1.350614	4.881747	2.92993	0.039813
hsa-miR-125b	2.025984	2.170862	2.884837	0.041731
hsa-miR-338-3p	-1.63845	4.620754	-2.78552	0.046342

Table I. Continued.

miR	logFC	AveExpr	t	P-value
hsa-miR-146b-5p	-1.29146	6.920841	-2.75528	0.047860

logFC, log fold change; AveExpr, average expression; miR, microRNA; hsa, *Homo sapiens*, *complementary strand of the microRNA.

Table II. Differentially expressed miRs in the dataset GSE29190.

miR	logFC	AveExpr	t	P-value
hsa-miR-18a*	-2.51535	0.9441	-15.4559	6.85x10 ⁻⁷
hsa-miR-758	-1.24903	0.487935	-10.0342	1.46x10 ⁻⁵
hsa-miR-185*	-2.498	1.185303	-5.30749	0.000938
hsa-miR-199b-5p	2.216376	2.08776	4.343231	0.002984
hsa-miR-16-2*	1.558178	1.935857	4.180525	0.003676
hsa-miR-1202	2.150841	3.293889	4.041271	0.004408
hsa-miR-7	1.917814	3.758687	3.950336	0.004971
hsa-miR-660	1.536002	2.837873	3.529016	0.008814
hsa-miR-7-1*	1.79193	2.285376	3.46859	0.009588
hsa-miR-487b	-2.48633	1.308742	-3.28236	0.012470
hsa-miR-409-5p	-1.5458	0.669419	-3.22662	0.013503
hsa-miR-200c*	-1.80803	0.674268	-3.12735	0.015573
hsa-miR-613	-1.11381	0.442862	-3.11342	0.015889
hsa-miR-431	-2.10028	0.771685	-3.09273	0.016372
hsa-miR-19a*	-1.48092	0.565233	-3.06806	0.016968
hsa-miR-186*	-2.5773	0.930693	-3.04035	0.017664
ebv-miR-BART8	-1.86635	0.693708	-2.98042	0.019276
hsa-miR-585	-1.52776	0.594644	-2.97825	0.019337
ebv-miR-BHRF1-1	2.864032	2.647555	2.977097	0.019370
hsa-miR-576-5p	1.428135	1.023683	2.942374	0.020379
hsa-miR-942	-1.45888	1.013753	-2.92631	0.020864
hsa-miR-449b	-1.03809	0.422371	-2.90996	0.021370
hsa-miR-29a*	-2.36068	0.96128	-2.89317	0.021904
hsa-miR-769-3p	-1.81466	0.676479	-2.87074	0.022639
hsa-miR-362-3p	1.258509	0.984151	2.859353	0.023021
hsa-miR-500*	1.483617	1.126051	2.787552	0.025596
hiv1-miR-N367	-1.63534	0.967286	-2.70054	0.029123
ebv-miR-BART18-5p	-1.43074	0.748889	-2.57279	0.035240
hsa-miR-148b*	-1.72537	0.646717	-2.55501	0.036190
hsa-miR-532-5p	1.417317	1.319251	2.552325	0.036336
hsa-miR-15b*	-2.3785	1.211755	-2.54192	0.036907
hsa-miR-1537	-1.0943	0.436358	-2.5317	0.037477
hsa-miR-215	1.887456	1.812279	2.514436	0.038460
hsa-miR-662	-1.57886	0.59788	-2.4756	0.040769
hsa-miR-29b	1.103322	8.302687	2.406804	0.045214
hsa-miR-520d-3p	-1.23035	0.943423	-2.34466	0.049651

logFC, log fold change; AveExpr, average expression; miR, microRNA; hsa, *Homo sapiens*; ebv, Epstein-Barr.

'Structural molecule activity', 'cardiovascular system development', 'organ morphogenesis', 'negative regulation of nitrogen compound metabolic process' with ≥ 15 target genes enriched

in several of these functional pathways, the most relevant of which were the 'cardiovascular system development' and 'circulatory system development' (Fig. 5).

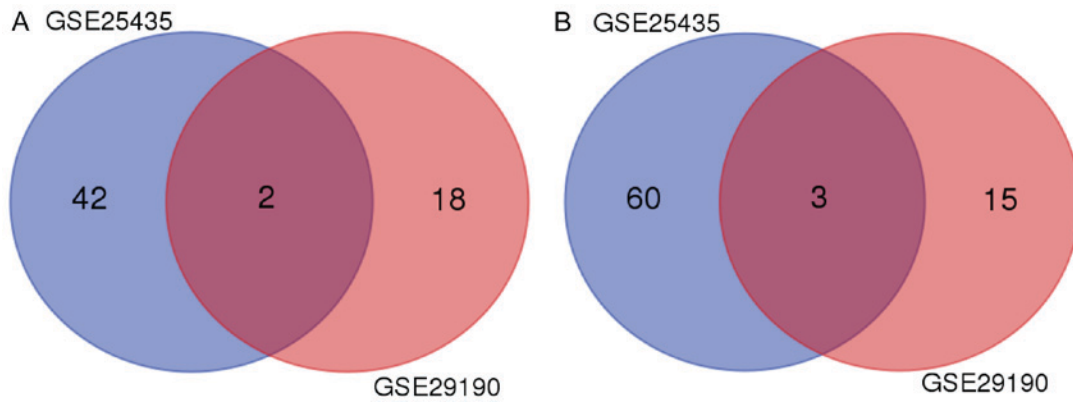


Figure 2. Venn diagram of (A) downregulated microRNAs and (B) upregulated microRNAs from the 2 datasets.

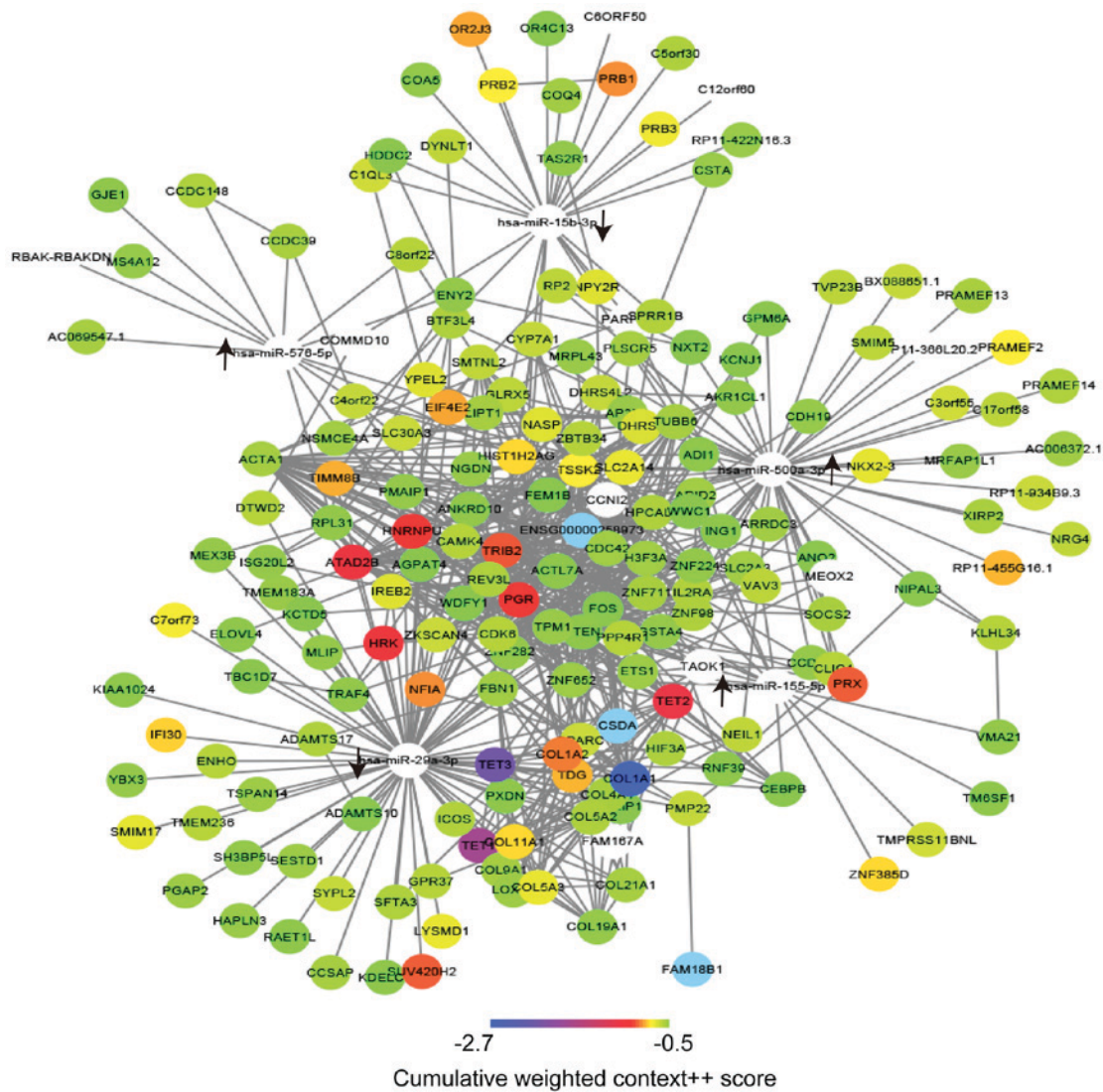


Figure 3. Regulatory and interaction networks of the differentially expressed miRNAs and their target genes. The upward arrow indicates the upregulation of the miRNAs and the downward arrow indicates the downregulation of the miRNAs. The color scale presented at the bottom illustrates the cumulative weighted context++ scores of the target genes. miR, microRNA; hsa, *Homo sapiens*.

Discussion

The incomplete understanding of the underlying mechanisms and deficiencies in the diagnosis and treatment make it difficult

to prevent and cure LTBI. Various host factors are included in this complex process. In the present study, miRNAs, a type of non-coding RNAs, were the focus, as they have been applied in multiple fields of life science and medicine. Microarray

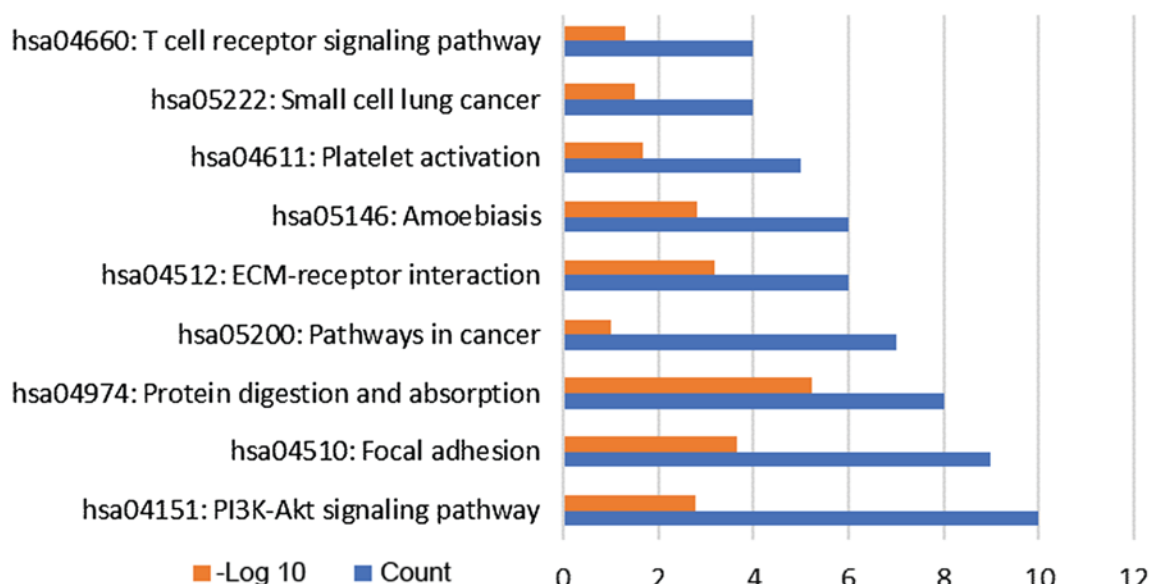


Figure 4. Kyoto Encyclopedia of Genes and Genomes signaling pathway enrichment of target genes. Red indicates the $-\log P$ of the enriched pathway and blue indicates the counts of genes enriched in each pathway. PI3K, phosphoinositide-3 kinase; ECM, extracellular matrix; hsa, *Homo sapiens*.

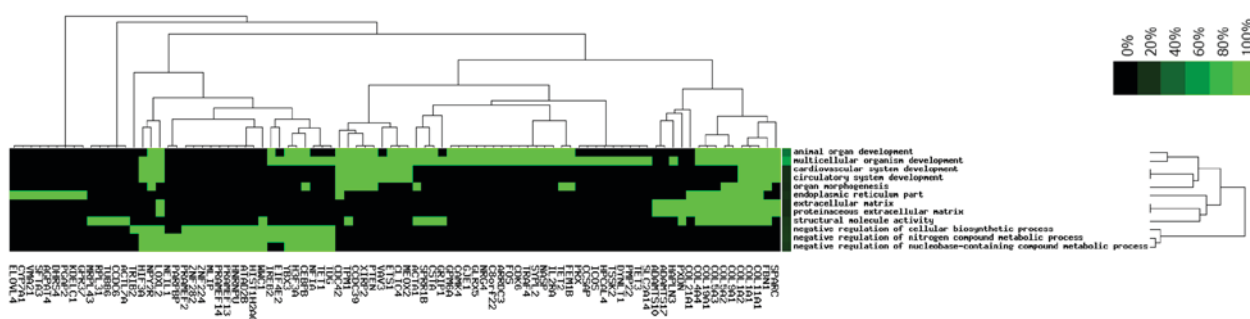


Figure 5. Gene Ontology analysis of the target genes. The color scale illustrates the corresponding gene-term associations. Green indicates the presence of a corresponding gene-term association and black indicates the absence of a corresponding gene-term association in the database.

analysis has been widely used to investigate DEMs, dysregulated genes and pathways associated with LTBI (21,30,31). In previous decades, a large amount of research investigating this matter has emerged (18,32-34); however, identifying novel miRNAs can be used to investigate further the underlying mechanism of LTBI. Thus, in the present study, expression data obtained from patients with and without LTBI were subjected to a bioinformatics analysis to provide more reliable data.

In the analysis of the present study, 2 datasets were included. A total of 144 DEMs were identified from these 2 datasets. By exploring the overlapping miRNAs in the two datasets, hsa-miR-29a and hsa-miR-15b were identified as being decreased, while hsa-miR-576-5p, hsa-miR-500 and hsa-miR-155 were identified to be upregulated. Of note, miR-29a was previously reported to be significantly decreased in patients with LTBI (35,36). miRNAs encoded by the miR-15b/16-2 cluster may act as tumor suppressors. Aberrant regulation of miR-15b in human cancers reportedly has an important role in cancer development, contributing to reduced proliferation, cell death, angiogenesis and metabolic reprogramming and metastasis resistance, as well as tumor-associated inflammation and genomic instability (37). miR-576 is directly targeted by hepatitis B virus-encoded X

protein (38). miR-155 and miR-500 were reported as potential markers of aflatoxin exposure (39), which suggests that miRNAs may be used as potential biomarkers for the diagnosis of LTBI. However, the possible association of hsa-miR-15b, hsa-miR-576-5p, hsa-miR-500 and hsa-miR-155 with LTBI identified in the present analysis remains to be verified.

To gain a broad understanding of the effects of miRNAs in LTBI, the target genes of the 5 abovementioned DEMs were predicted. By merging the miR regulatory network and protein-protein interaction network, a degree of crosstalk among these miRNAs via cancer-associated genes, including FOS, ACTA1, CDC42 and PTEN, was identified (40-43). In the current study, pathways associated with small cell lung cancer also appeared in the KEGG pathway analysis, which suggests that LTBI may participate in the development of small cell lung cancer. By exploring the topological structure of the interaction network, it was revealed that hsa-miR-500a-3p and hsa-miR-29a-3p, as well as the 4 genes CDC42, ACTA1, PTEN and FOS, have essential roles in this regulatory network. These miRNAs and genes may be core factors in the diagnosis of LTBI.

To determine the molecular mechanisms underlying the LTBI process, GO term and KEGG pathway analysis

Table III. Target gene prediction of miRs by TargetScan.

miR/gene symbol	Cumulative weighted context++ score
hsa-miR-29a-3p	
COL1A1	-2.72
TET3	-2.16
TET1	-1.78
TET2	-1.17
ATAD2B	-1.12
HRK	-1.07
TRIB2	-0.96
SUV420H2	-0.95
COL1A2	-0.91
NFIA	-0.89
EIF4E2	-0.86
TIMM8B	-0.85
TDG	-0.84
IFI30	-0.81
COL11A1	-0.80
C7orf73	-0.75
LYSMD1	-0.73
COL5A3	-0.73
SMIM17	-0.72
IREB2	-0.71
YPEL2	-0.71
NASP	-0.71
SPARC	-0.67
ZKSCAN4	-0.67
SLC30A3	-0.67
PMP22	-0.66
SYPL2	-0.66
SMTNL2	-0.65
DTWD2	-0.63
TMEM236	-0.63
TMEM236	-0.63
ZBTB34	-0.63
GPR37	-0.63
ENHO	-0.63
ICOS	-0.62
HIF3A	-0.61
ADAMTS17	-0.61
SFTA3	-0.61
REV3L	-0.61
COL5A2	-0.61
COL4A4	-0.60
COL21A1	-0.59
COL9A1	-0.59
CCSAP	-0.59
ARRDC3	-0.58
FBN1	-0.58
CDC42	-0.58
PGAP2	-0.57
TSPAN14	-0.57
SESTD1	-0.57

Table III. Continued.

miR/gene symbol	Cumulative weighted context++ score
LOXL2	-0.57
TMEM183A	-0.56
TPM1	-0.56
HAPLN3	-0.56
MLIP	-0.55
GSTA4	-0.55
MEX3B	-0.55
ZNF282	-0.55
COL19A1	-0.55
YBX3	-0.55
ISG20L2	-0.55
TRAF4	-0.54
SH3BP5L	-0.54
PTEN	-0.53
RNF39	-0.53
KDELC1	-0.53
KIAA1024	-0.53
ELOVL4	-0.53
RAET1L	-0.53
PXDN	-0.53
GRIP1	-0.52
FEM1B	-0.52
WDFY1	-0.52
TBC1D7	-0.52
AGPAT4	-0.52
KCTD5	-0.52
ADAMTS10	-0.52
FAM167A	-0.51
hsa-miR-15b-3p	
PGR	-1
PRB1	-0.89
OR2J3	-0.86
PRB2	-0.77
PRB3	-0.74
NPY2R	-0.71
C1QL3	-0.68
CYP7A1	-0.66
DYNLT1	-0.65
GLRX5	-0.64
SPRR1B	-0.62
C5orf30	-0.61
TUBB6	-0.59
NSMCE4A	-0.58
COQ4	-0.58
RP11-422N16.3	-0.56
CSTA	-0.56
TAS2R1	-0.54
COA5	-0.54
OR4C13	-0.52
HDDC2	-0.52
C12orf60	-0.51

Table III. Continued.

miR/gene symbol	Cumulative weighted context++ score
C6ORF50	-0.51
PARPBP	-0.51
COMMD10	-0.51
hsa-miR-576-5p	
HNRNPU	-1
HIST1H2AG	-0.80
C4orf22	-0.66
C8orf22	-0.63
CCDC148	-0.61
CCDC39	-0.60
AC069547.1	-0.59
LIPT1	-0.59
MS4A12	-0.55
RPL31	-0.54
ENY2	-0.54
GJE1	-0.53
RBAK-RBAKDN	-0.51
hsa-miR-500a-3p	
PRX	-0.95
RP11-455G16.1	-0.84
PRAMEF2	-0.77
TSSK2	-0.77
SLC2A14	-0.73
NKX2-3	-0.72
DHRS4	-0.70
C3orf55	-0.68
RP11-934B9.3	-0.67
DHRS4L2	-0.66
BX088651.1	-0.66
NRG4	-0.65
NEIL1	-0.65
SMIM5	-0.64
TVP23B	-0.64
RP2	-0.64
C17orf58	-0.64
PPP4R1	-0.63
KLHL34	-0.63
CAMK4	-0.63
CLIC4	-0.63
CDK6	-0.63
IL2RA	-0.62
BTF3L4	-0.61
PRAMEF14	-0.61
HPCAL4	-0.61
ZNF98	-0.61
SOCS2	-0.60
PRAMEF13	-0.60
XIRP2	-0.60
ZNF711	-0.60
PLSCR5	-0.59
SLC2A3	-0.58

Table III. Continued.

miR/gene symbol	Cumulative weighted context++ score
AC006372.1	-0.58
AP3B2	-0.56
MRPL43	-0.56
NGDN	-0.56
MRFAP1L1	-0.56
CCDC6	-0.55
CDH19	-0.55
PMAIP1	-0.55
ZNF224	-0.54
ANKRD10	-0.54
AKR1CL1	-0.54
ADI1	-0.53
ING1	-0.53
NIPAL3	-0.52
KCNJ1	-0.52
GPM6A	-0.52
NXT2	-0.52
ANO2	-0.52
RP11-366L20.2	-0.51
MEOX2	-0.51
CCNI2	-0.51
hsa-miR-155-5p	
ZNF385D	-0.80
TMPRSS11BNL	-0.68
VAV3	-0.64
ACTA1	-0.59
ARID2	-0.58
H3F3A	-0.58
ETS1	-0.57
ZNF652	-0.57
VMA21	-0.54
ACTL7A	-0.53
TM6SF1	-0.53
CEBPB	-0.52
FOS	-0.52
WWC1	-0.52
TAOK1	-0.51

miR, microRNA; hsa, *Homo sapiens*.

were included in the present study to perform a comprehensive analysis of the roles of the key miRs and genes. In the KEGG pathway enrichment analysis, 'PI3K/AKT signaling pathway' appeared to be the most enriched pathway with the largest number of enriched genes. The PI3K/AKT signaling pathway, including its downstream pathway, mammalian target of rapamycin (mTOR), is well known to be involved in a variety of BPs, and it has been reported that hyperactivation of mTOR has a pathogenetic role in human immunodeficiency virus infection (44-46). Due to the important role of the AKT

pathway in cellular metabolism, growth and division, apoptosis suppression and angiogenesis, the regulation of the AKT signaling may represent a valuable therapeutic strategy. For this reason, AKT inhibitors have become the hotspots of research in a number of clinical diseases (47), and dual PI3K/mTOR inhibitors, including PF-04691502 and NVP-BEZ235, may have important therapeutic applications in cancer (48,49). Anti-retroviral protease inhibitors have been re-purposed as inhibitors of the PI3K/AKT/mTOR pathway, and such drugs that are also available as a generic and cheap formulation may be immediately available for testing in patients with LTBI (50). Studies have also indicated on upregulated activation of the PI3K/AKT/mTOR pathway in autoimmune diseases, including multiple sclerosis and liver fibrosis (51,52). It has been demonstrated that the PI3K/AKT signaling pathway is inhibited in patients with active TB (53,54). However, there is no direct evidence indicating PI3K/AKT signaling pathway alterations in patients with LTBI. 'Protein digestion and absorption' was the most significant pathway. These signaling pathways constitute important potential mechanisms underlying the processes of LTBI and merit further validation.

In conclusion, the present analysis identified hsa-miR-500a-3p and hsa-miR-29a-3p, as well as 4 target genes CDC42, ACTA1, PTEN and FOS, which may be used as potential biomarkers of LTBI. The PI3K/AKT signaling pathway is the key genetic event implicated in LTBI, and an in-depth investigation of the efficiency of PI3K/AKT signaling inhibitors in the prevention of LTBI is warranted. The novel key factors and molecular pathways provided in the present study may contribute to the current understanding of LTBI and may facilitate the development of a molecular diagnostic platform for its detection.

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Availability of data and materials

All datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

YL, HD, XiaW, PY, LH, YW, ZZ and WZ analyzed the data. YL, XinW, HD and LZ designed the study and prepared the manuscript.

Ethical approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interest.

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