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Short Communication

Sequence comparison of six human microRNAs genes between tuberculosis patients and healthy individuals

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ABSTRACT

Objective/Background: MicroRNAs (miRNAs) play an important role in diseases development. Therefore, human miRNAs may be able to inhibit the survival of *Mycobacterium tuberculosis* (Mtb) in the human host by targeting critical genes of the pathogen. Mutations within miRNAs can alter their target selection, thereby preventing them from inhibiting Mtb genes, thus increasing host susceptibility to the disease.

Methods: This study was undertaken to investigate the genetic association of pulmonary tuberculosis (TB) with six human miRNAs genes, namely, *hsa-miR-370*, *hsa-miR-520d*, *hsa-miR-154*, *hsa-miR-497*, *hsa-miR-758*, and *hsa-miR-593*, which have been predicted to interact with Mtb genes. The objective of the study was to determine the possible sequence variation of selected miRNA genes that are potentially associated with the inhibition of critical Mtb genes in TB patients.

Results: The study did not show differences in the sequences compared with healthy individuals without antecedents of TB.

Conclusion: This result could have been influenced by the sample size and the selection of miRNA genes, which need to be addressed in future studies.

Introduction

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* (Mtb) is commonly manifested as a pulmonary disease [1,2]. Estimates indicate that 8.8 million new cases of active TB and 1.4 million deaths are caused by this disease every year. One third of the human population is latently infected with Mtb [1,2]. Only approximately 10% of infected individuals develop the disease, and the remaining 90% do not progress to clinical TB, although the bacteria remains

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in the dormant state and can be reactivated at any time [1,2].

Pathogenic and environmental factors influence host's susceptibility to TB and host genetic factors also contribute to resistance/susceptibility to this disease [3–6].

One of the elements of great importance in gene expression and regulation is the microRNAs (miRNAs) [7]. Mature miRNAs (20–22 nt) are formed from long hairpins containing miRNA precursors (pre-miRNAs; 70–100 nt) as small single-stranded noncoding RNAs controlling the expression levels of their target genes by messenger RNA (mRNA) degradation and protein translation inhibition [7].

There are currently about 1000 human miRNA sequences available in the miRNA registry representing approximately 60% of all mammalian genes [8]. This indicates that miRNAs are involved in the fundamental and global functions in human biology. The miRNAs are also known to be potential regulators of immune reactivity [9].

Interindividual differences of miRNAs expression are likely to influence the expression of miRNA target genes, contributing to some changes in human phenotype, conditioning the evolution, and influencing the susceptibility/resistance to multiple diseases [9]. The association of miRNAs with multiple diseases, including infectious diseases, has been extensively studied [9–12].

The potential role of miRNAs in the susceptibility and evolution of TB has been studied extensively, with reports indicating changes in several miRNAs, highlighting the potential role of these molecules in the disease [13–15].

In general, the main alterations related with TB have been found associated with miRNAs involved in the regulation of the immune responses and inflammation [13–15].

The potential impact resulting from interactions of human miRNAs with the expression of Mtb genes has not yet been explored. Human miRNAs directed at critical genes of Mtb associated with virulence and survival of the bacteria could have an important role in the control of the infection. Mutations in such host miRNA genes could abolish the inhibition of the expression of critical Mtb genes and increase the host's susceptibility to the disease. Guo et al. [16] predicted 26 candidate Mtb genes that are expressed in macrophages and in the lungs of humans and mice that may be targeted by 31 human miRNAs [16]. These 31 human miRNAs were selected as they are expressed in the lungs and macrophages based on miRNAs expression atlas [17].

In this study, we selected six of the miRNAs predicted by Guo et al. [16], which target Mtb genes that are responsible for the virulence and survival of Mtb. These genes have been reported to be expressed *in vivo* and/or in macrophages in culture [18–22]. The objective of this study was to explore possible polymorphisms within these miRNAs using DNA sequencing.

Materials and methods

Study population

Newly diagnosed adult pulmonary TB patients ($n = 33$) from the Hospital Universiti Sains Malaysia (Kubang Kerian, Kelantan, Malaysia) and healthy individuals without TB antecedents ($n = 38$) were included in the study. A peripheral blood sample from each participant was collected into a Vacutainer tube containing the anticoagulant EDTA after obtaining informed consent from each participant. This study was approved by the Human Ethics Committee of Universiti Sains Malaysia.

Selection of miRNAs

Thirty-one human miRNAs expressed in the lungs and macrophages and their 26 potential target Mtb genes were previously predicted by Guo et al. [16]. From this previous study, we chose to study six human miRNAs, which are predicted to target five important Mtb genes, using their overexpression after infection in mice (lung and artificial granuloma) [18,19] and/or humans (lung, sputum, and macrophage cell lines) as the main selection criterion [20,22], because they may be important in virulence/survival of Mtb *in vivo*.

Primer design

Primers were designed to flank the sites of miRNAs using NCBI Primer-Basic Local Alignment Search Tool. Hairpin formation, primer dimerization, and self-primer dimerization were carefully inspected using an online software from integrated DNA Technologies. Each primer set was designed to amplify the amplicon containing the sequence of specific miRNA to give a bigger product than the pre-miRNA to ensure that the full pre-miRNA sequence is obtained. The gene sequence of pre-miRNAs of each miRNA was obtained from the NCBI reference sequence [23].

Polymerase chain reaction

Genomic DNA was extracted using the QIAamp DNA blood mini kit (QIAGEN, Germantown, Maryland, USA). Polymerase chain reaction (PCR) was performed using specific primers for each miRNA. The PCR was performed in a 20-μL reaction mix comprising 10 pmol of each primer (Sigma–Aldrich, Singapore), 2 μL of 10 × *Taq* reaction buffer, and 3 μL of deoxynucleotide triphosphate mix (2.5 mM each; Geneall Biotechnology, Seoul, Korea).

The mixture was initially heated at 95 °C for 3 min to activate the polymerase. The DNA amplification was performed for 30 cycles as follows: at 95 °C for 30 s, annealing at 58 °C for 30 s, and extension at 72 °C for 50 s. The final elongation step was performed at 72 °C for 10 min and the reaction was held at 4 °C. PCR products were purified and sequenced using the ABI 3130xl genetic analyzer.

DNA sequencing

Termination cycle

The termination cycle of the purified PCR product was carried out in a final volume of 10 μL in a 0.5-mL tube containing 3 μL of the purified PCR product, 3.75 μL of distilled water, 1 μL of primer (each tube contained only 1 type of primer F1 or R1), 1.75 μL of 5× buffer, and 0.5 μL of big dye (Applied Biosystems, Carlsbad, California, USA). The cycle sequence amplification

condition was as follows: 96 °C for 1 min, 35 cycles at 96 °C for 10 s, 50 °C for 5 s, and 60 °C for 4 min and a final hold at 4 °C.

Ethanol precipitation

To purify the amplification product, ethanol precipitation was performed after the termination cycle was completed. As much as 10 μL of the cycle sequence product was transferred into a 1.5-mL microfuge tube to which 1.5 μL of 2 M sodium acetate (pH 5.2) and 20 μL of 95% ethanol were added. The mixture was vortexed and left at room temperature for 30 min. The mixture was then centrifuged at 6000 rpm (4 °C for 40 min) and the supernatant was discarded. Then, 150 μL of cold 70% ethanol was added into the tube, and the tube was vortexed and centrifuged at 6000 rpm (4 °C for 10 min) and the step was repeated two more times. The supernatant was discarded and the tube was dried at 65 °C for 10 min in a thermal cycler. To this sample, 10 μL of Hi-Di (Applied Biosystems, Carlsbad, California, USA) was added and the tube was placed into the thermal cycler for 3 min at 96 °C. Then, the sample was loaded into 96-well plates and sequenced using the ABI 3130xl genetic analyzer. The sequencing runs up to 16 samples and yielded read length greater than 500 bp.

Sequencing analysis

Sequence analysis was carried out with the ABI 3130xl genetic analyzer using the Ultra "Sep36_POP7" sequencing run module. The sequence results were analyzed using the BioEdit software by comparing them with the sequence of miRNAs from the miRNA database [8].

Results and discussion

All the Mtb genes predicted by Guo et al. [16] were reported to be overexpressed *in vivo* in mice during infection [18,19] (Table 1). In addition, Mtb genes, *Rv0208c/trmB*, *Rv0682/rpsL*, *Rv0291/mycP3*, *Rv0440/groEL2*, and *Rv0685/tuf*, were reported to be overexpressed during infection in mice [18,19] and humans [20–22] (Table 1), suggesting their roles in the virulence/survival of Mtb *in vivo*, and thus, miRNAs targeting them were selected for this study.

Table 2 presents the sequences of the pre-miRNAs of each miRNA selected. Sequences of all the primer sets designed are showed in Table 3. The designed primers were able to amplify the target human miRNAs genes with the expected size as shown in Fig. 1.

The sequence of the six miRNAs in TB patients and healthy individuals did not show any variations among them as well as with the reference miRNA sequences retrieved [23].

Genetic association with susceptibility and resistance to TB has been described previously [3–6]. The presence of different polymorphisms in different Toll-like receptors (TLRs) has been reported to be associated with TB. In the case of TB in children, the progression of the disease was found to be influenced by the TLR2 variant R753Q [24]. Tumor necrosis factor

Table 1 – Mtb gene expression during infection.

	Mtb gene[a]	Human miRNA[a]	Mice[b]	Human[c]
1	*Rv0208c/trmB*	hsa-miR-154[d]	+	+
2	*Rv0291/mycP3*	hsa-miR-497, hsa-miR-593, hsa-miR-758[d]	+	+
3	*Rv0337c/aspC*	hsa-miR-557	+	–
4	*Rv0351/grpE*	hsa-miR-328, hsa-miR-453, hsa-miR-483	+	–
5	*Rv0352/dnaJ1*	hsa-miR-425-3p	+	–
6	*Rv0353/hspR*	hsa-miR-210, hsa-miR-375, hsa-miR-423, hsa-miR-636	+	–
7	*Rv0415/thiO*	hsa-miR-128a, hsa-miR-128b, hsa-miR-324-5p, hsa-miR-339	+	–
8	*Rv0416/thiS*	hsa-miR-324-5p, hsa-miR-423	+	–
9	*Rv0432/sodC*	hsa-miR-453, hsa-miR-638, hsa-miR-654	+	–
10	*Rv0440/groEL*	hsa-miR-370, hsa-miR-486, hsa-miR-520d*[d]	+	+
11	*Rv0462/Lpd*	hsa-miR-324-3p	+	–
12	*Rv0500/proC*	hsa-miR-518a	+	–
13	*Rv0509/hemA*	hsa-miR-331, hsa-miR-433, hsa-miR-324-3p	+	–
14	*Rv0510/HemC*	hsa-miR-453, hsa-miR-593	+	–
15	*Rv0528*	hsa-miR-593	+	–
16	*Rv0555/mend*	hsa-miR-593	+	–
17	*Rv0558/ubiE*	hsa-miR-134, hsa-miR-486	+	–
18	*Rv0651/rplJ*	hsa-miR-370, hsa-miR-433, hsa-miR-432	+	–
19	*Rv0682/rpsL*	hsa-miR-370[d]	+	+
20	*Rv0685/tuf*	hsa-miR-593[d]	+	+
21	*Rv0707/rpsC*	hsa-miR-453	+	–
22	*Rv0709/rpmC*	hsa-miR-187	+	–
23	*Rv0714/rplN*	hsa-miR-614	+	–
24	*Rv0720/rplR*	hsa-miR-636, hsa-miR-328, hsa-miR-639	+	–
25	*Rv0721/rpsE*	hsa-miR-525	+	–
26	*Rv0722/rpmD*	hsa-miR-671	+	–

Note: miRNA = microRNA.

[a] Mtb genes–miRNA pairs reported by Guo et al [16].

[b] Mtb gene expressed *in vivo* in the lungs [18] and/or artificial granuloma [19] in mice.

[c] Mtb gene expressed in the lungs [20], sputum [21], and/or macrophage cell line [22] in humans.

[d] The miRNA–Mtb genes combinations selected for this study.

Table 2 – Pre-miRNA gene sequences.

miRNA	Pre-miRNA gene sequence including the mature miRNA (marked in bold)	Chromosome coordinates of the gene sequence including the selected miRNA sequences (GRCh37.p5)
hsa-miR-154	[GenBank:NR_029704] 5′-GTGGTACTTGAAGA**TAGGTTATCCGTGTTG**CCTTCGCTTTATTTGT GACGAATCATACACGGTTGACCTATTTTTCAGTACCAA-3′	Chr14: 101524492-101526775
hsa-miR-370	[GenBank:NR_029863] 5′-AGACAGAGAAGCCAGGTCACGTCTCTGCAGTTACACA **GCTCACGAGTGCCTGCTGGGGTGGAACCTGGT**CTGTCT-3′	Chr 14: 101376876-10138150
hsa-miR-497	[GenBank:NR_030178] 5′-CCACCCCGGTCCTGCTCCCGCCC**CAGCAGCACACTGTGGT** **TTGT**ACGGCACTGTGGCCACGTCCAAACCACACTGTGGTGTT AGAGCGAGGGTGGGGGAGGCACCGCCGAGG-3′	Chr17: 6920630-6921941
hsa-miR-520d	[GenBank:NR_030204] 5′-TCTCAAGCTGTGAGTCTACAAAGGGAAGCCCTTTCTGTTGT CTAAAAGAAAAG**AAAG** **TGCTTCTCTTTGGTGGGT**TACGGTTTGAGA-3′	Chr19: 54222750-54224036
hsa-miR-593	[GenBank:NR_030324] 5′-CCCCCAGAAT**CTGTCAGGCACAGCCAGGCATTG** **CTCAGC**CCGTTTCCCTCTGGGGGAGCAAGGAGTG GT GCTGGGTTTG TCTCTGCTGGGGTTTCTCCT-3′	Chr17: 127721313-127722610
hsa-miR-758	[GenBank:NR_030406] 5′-CCTGGATACATGAGATGGTTGACCAGAGAGCACACG CTTTAT**TTGTGCCGTTTGTG** **ACCTGGTCCACTAACC**CTCAGTATCTAATGC3′	Chr14: 101491757-101493044

Note: miRNA = microRNA.

Table 3 – Primer Used for Polymerase Chain Reaction and Sequencing of Selected miRNAs.

Primer	miRNA	Label	Primer Sequence (5′–3′)	Size (mer)	GC (%)	T_m (°C)	Product size (bp)
Pair 1	hsa-miR-154	F1	GCA TTG AGG TCT GGA TGG CTG	21	57	69	424
		R1	GCA CCA TCT CTA GAA ACC TCC C	22	54	65	
Pair 2	hsa-miR-370	F2	GCT ACT TGA GGG ATG GGC GA	20	60	68	374
		R2	GTC TCT GTG CCT GTT TCC CC	20	60	66	
Pair 3	hsa-miR-497	F3	GTC CAA TCT TAC ACT GTG AGC	21	48	59	545
		R3	GCC AAT ATT TCT GTG CTG CTA G	22	45	63	
Pair 4	hsa-miR-520d	F4	CTG GAG ATG GTC TTT GTA TCG G	22	50	64	542
		R4	CCT GAC CTG AGA TGA TCT ACC	21	52	60	
Pair 5	hsa-miR-593	F5	CTT GGA GTT ACT TCA GGA GC	20	50	58	510
		R5	GTA AGC GGT TGG AGG TGT AG	20	55	61	
Pair 6	hsa-miR-758	F6	GGA GCT AAC ATC AAC TGC GG	20	55	65	338
		R6	CTT CAG AGT CTA ACA GAG TGC	21	48	56	

Note: GC (%) = guanosine–cytosine percentage; T_m (°C) = melting temperature.

receptor 1 gene was found to be associated with susceptibility to TB in a study conducted among Ugandan population [25].

The function and mechanism of miRNAs in the regulation of innate and acquired immune responses have been described [26]. Single-nucleotide polymorphisms (SNPs) may alter the function of miRNAs, and therefore, may influence the outcome of a particular disease. Most studies on miRNAs have been carried out in cancer. The importance of miRNAs in cancer is highlighted by the observation that approximately 50% of miRNA genes located in cancer-associated genomic regions or fragile sites [27] are frequently amplified or deleted in tumorigenesis.

A study performed by Wu et al. [28], found that a G → A mutation in nucleotide 19 downstream of the miRNA let-7e reduced its expression, and suggested that the mutation could contribute to tumorigenesis, thus identifying that genetic polymorphisms in miRNA genes may have therapeutic and diagnostic values [28]. Calin et al. [27] found that the mutation in two miRNA genes, *mir-15* and *mir-16*, resulted in nonexpression of these genes and downregulation in chronic lymphocytic leukemia patients in comparison to normal tissues and lymphocytes, and suggested that these miRNA genes may be involved in the pathogenesis of the disease. Previous studies have also confirmed that SNPs in either

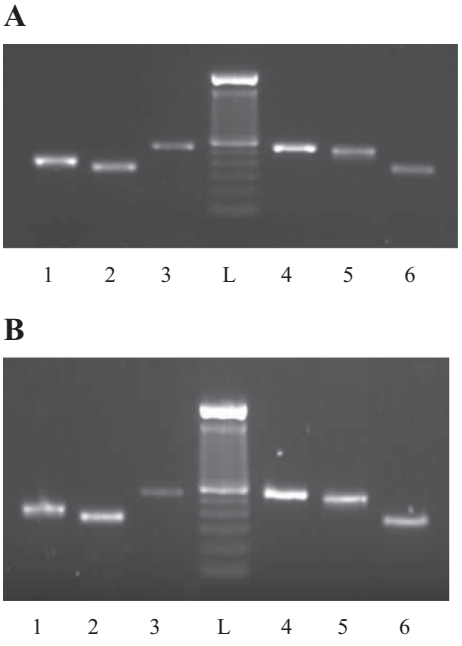

Fig. 1 – (A) Polymerase chain reaction (PCR) products of all six primer sets from a tuberculosis patient. (B) PCR product of all six primer sets from a healthy individual. *Note:* 1 = hsa-miR-154; 2 = hsa-miR-370; 3 = hsa-miR-497; L = 100-bp ladder as reference; 4 = hsa-miR-520d; 5 = hsa-miR-593; 6 = hsa-miR-758.

pre-miRNAs or miRNAs could increase the risk of diseases and caused phenotypic changes [29,30].

It has been reported that infection of macrophages with live mycobacteria (Mtb and Bacillus Calmette–Guérin vaccine) induced an miRNA signature including miR-155, miR-146a, miR-145, miR-222*, miR-27a, and miR-27b, which potentially targets genes associated with the immune response [31].

Only two reports have been related to the study of SNPs in the miRNAs machinery in association with susceptibility and resistance to TB [32,33]. In one of these studies, a genetic variant of the DICER protein was associated with resistance to TB in the Tibetan population [32]. Genetic variants in miRNAs regulating the TLR pathway were associated with resistance/-susceptibility in the other study in Tibetan and Han populations [33].

Our study did not demonstrate any sequence variation in all the six human miRNAs between newly diagnosed TB patients and healthy individuals without antecedents of TB. This may indicate that they may not be involved directly in susceptibility or resistance to TB. Mutation in miRNAs is not the only reason for loss of function of miRNAs. There are also other factors affecting the function of miRNAs. The transcription of miRNA gene, *mir-124a*, was shown to be deactivated by hypermethylation of its promoter in various human tumors [34]. The functions of miRNA could also be regulated by the loss of its binding sites in the target gene. It has been reported that when chromosomal translocations occur in oncogenes, the high mobility group A2 (Hmga2) will cause the loss of let-7 miRNA-binding site in mRNA, thereby promoting oncogenic transformation and growth in mammalian cells. These studies have shown that disrupting the interaction between miRNA and its target binding sites can alter the normal phenotype in mammalian cells [35,36]. Thomson et al. [37] found that the downregulation of miRNAs in cancer was not influenced by the reduction in the level of pre-miRNA transcript but is due to the failure at the Drosha processing step [37].

Some of the previous mechanisms, beyond the sequence of the miRNAs genes, could be operative too in the induction of resistance or susceptibility to TB.

Conclusion

The negative results of our study could be associated with the small amount of individuals included or with the selection of the miRNAs. In addition, the presence of latent infection in the healthy individuals of the study was not determined, which could have influenced the results. It might be worthwhile to study the potential genetic variability in the predicted target sites of the human miRNAs in the genome of Mtb strains. In future studies with bigger samples, including infected and noninfected healthy individuals and including other miRNAs, the potential role of the interaction of miRNAs and Mtb in the susceptibility/resistance to TB could be clarified.

Conflicts of interest

All contributing authors declare no conflicts of interest.

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REFERENCES

[1] D. Falzon, M. Raviglione, TB control: achievements, obstacles and the role of vaccines, in: M.N. Norazmi, A. Acosta, M.E. Sarmiento (Eds.), The Art and Science of Tuberculosis Vaccine Development, second ed., Oxford University Press, Selangor, Malaysia, 2014, pp. 13–29.
[2] A. Zumla, M. Raviglione, R. Hafner, et al, Tuberculosis, N. Engl. J. Med. 368 (2013) 745–755.
[3] C.G. Meyer, T. Thye, Host genetic studies in adult pulmonary tuberculosis, Semin. Immunol. 26 (2014) 445–453.
[4] C. Deffert, J. Cachat, K.H. Krause, Phagocyte NADPH oxidase, chronic granulomatous disease and mycobacterial infections, Cell. Microbiol. 18 (2014) 1168–1178.
[5] C. Wang, Z.L. Chen, Z.F. Pan, et al, NOD2 polymorphisms and pulmonary tuberculosis susceptibility: a systematic review and meta-analysis, Int. J. Biol. Sci. 10 (2013) 103–108.
[6] C. Chen, Q. Liu, L. Zhu, et al, Vitamin D receptor gene polymorphisms on the risk of tuberculosis, a meta-analysis of 29 case-control studies, PLoS One 8 (2013) e83843.
[7] K. Ranganathan, V. Sivasankar, MicroRNAs—biology and clinical applications, J. Oral Maxillofac. Pathol. 18 (2014) 229–234.

[8] miRBase. Available from: http://www.mirbase.org/, (last accessed: May 2, 2015).
[9] L.L. Coutinho, L.K. Matukumalli, T.S. Sonstegard, et al, Discovery and profiling of bovine microRNAs from immune-related and embryonic tissues, Physiol. Genomics 29 (2007) 35–43.
[10] C. Maudet, M. Mano, A. Eulalio, MicroRNAs in the interaction between host and bacterial pathogens, FEBS Lett. 588 (2014) 4140–4147.
[11] H.X. Fan, H. Tang, Complex interactions between microRNAs and hepatitis B/C viruses, World J. Gastroenterol. 20 (2014) 13477–13492.
[12] K.L. Xie, Y.G. Zhang, J. Liu, et al, MicroRNAs associated with HBV infection and HBV-related HCC, Theranostics 4 (2014) 1176–1192.
[13] M.D. Mehta, P.T. Liu, MicroRNAs in mycobacterial disease: friend or foe?, Front Genet. 5 (2014) 231.
[14] H. Harapan, F. Fitra, I. Ichsan, et al, The roles of microRNAs on tuberculosis infection: meaning or myth?, Tuberculosis (Edinb) 93 (2013) 596–605
[15] P.K. Singh, A.V. Singh, D.S. Chauhan, Current understanding on micro RNAs and its regulation in response to mycobacterial infections, J. Biomed. Sci. 20 (2013) 14.
[16] W. Guo, J.-T. Li, X. Pan, et al, Candidate *Mycobacterium tuberculosis* genes targeted by human microRNAs, Protein Cell. 1 (2010) 419–421.
[17] P. Landgraf, M. Rusu, R. Sheridan, et al, A mammalian microRNA expression atlas based on small RNA library sequencing, Cell 129 (2007) 1401–1414.
[18] A.M. Talaat, R. Lyons, S.T. Howard, et al, The temporal expression profile of *Mycobacterium tuberculosis* infection in mice, Proc. Natl. Acad. Sci. USA 101 (2004) 4602–4607.
[19] P.C. Karakousis, T. Yoshimatsu, G. Lamichhane, et al, Dormancy phenotype displayed by extracellular *Mycobacterium tuberculosis* within artificial granulomas in mice, J. Exp. Med. 200 (2004) 647–657.
[20] H. Rachman, M. Strong, T. Ulrichs, et al, Unique transcriptome signature of *Mycobacterium tuberculosis* in pulmonary tuberculosis, Infect. Immun. 74 (2006) 1233–1242.
[21] N.J. Garton, S.J. Waddell, A.L. Sherratt, et al, Cytological and transcript analyses reveal fat and lazy persister-like bacilli in tuberculous sputum, PLoS Med. 5 (2008) e75.
[22] I.M. Monahan, J. Betts, D.K. Banerjee, et al, Differential expression of mycobacterial proteins following phagocytosis by macrophages, Microbiology 147 (2001) 459–471.
[23] NCBI reference sequence. Available from: <http://www.ncbi.nlm.nih.gov> (last accessed: May 2, 2015).
[24] N. Dalgic, D. Tekin, Z. Kayaalti, et al, Arg753Gln polymorphism of the human Toll-like receptor 2 gene from infection to disease in pediatric tuberculosis, Hum. Immunol. 72 (2011) 440–445.
[25] C.M. Stein, S. Zalwango, A.B. Chiunda, et al, Linkage and association analysis of candidate genes for TB and TNFalpha cytokine expression: evidence for association with IFNGR1, IL-10, and TNF receptor 1 genes, Hum. Genet. 121 (2007) 663–673.
[26] N. Iwai, H. Naraba, Polymorphisms in human pre-miRNAs, Biochem. Biophys. Res. Commun. 331 (2005) 1439–1444.
[27] G.A. Calin, C. Sevignani, C.D. Dumitru, et al, Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers, Proc. Natl. Acad. Sci. USA 101 (2004) 2999–3004.
[28] M. Wu, N. Jolicoeur, Z. Li, et al, Genetic variations of microRNAs in human cancer and their effects on the expression of miRNAs, Carcinogenesis 29 (2008) 1710–1716.
[29] K. Jazdzewski, S. Liyanarachchi, M. Swierniak, et al, Polymorphic mature microRNAs from passenger strand of pre-miR-146a contribute to thyroid cancer, Proc. Natl. Acad. Sci. USA 106 (2009) 1502–1505.
[30] K.D. Taganov, M.P. Boldin, K.J. Chang, et al, NF-kappaB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses, Proc. Natl. Acad. Sci. USA 103 (2006) 12481–12486.
[31] L. Furci, E. Schena, P. Miotto, et al, Alteration of human macrophages microRNA expression profile upon infection with *Mycobacterium tuberculosis*, Int. J. Mycobacteriol. 2 (2013) 128–134.
[32] X. Song, S. Li, M. QuCuo, et al, Association between SNPs in microRNA-machinery genes and tuberculosis susceptibility in Chinese Tibetan population, Mol. Biol. Rep. 40 (2013) 6027–6033.
[33] D. Li, T. Wang, X. Song, et al, Genetic study of two single nucleotide polymorphisms within corresponding microRNAs and susceptibility to tuberculosis in a Chinese Tibetan and Han population, Hum. Immunol. 72 (2011) 598–602.
[34] A. Lujambio, S. Ropero, E. Ballestar, et al, Genetic unmasking of an epigenetically silenced microRNA in human cancer cells, Cancer Res. 67 (2007) 1424–1429.
[35] C. Mayr, M.T. Hemann, D.P. Bartel, Disrupting the pairing between let-7 and Hmga2 enhances oncogenic transformation, Science 315 (2007) 1576–1579.
[36] Y.S. Lee, A. Dutta, The tumor suppressor microRNA let-7 represses the HMGA2 oncogene, Genes Dev. 21 (2007) 1025–1030.
[37] J.M. Thomson, M. Newman, J.S. Parker, et al, Extensive post-transcriptional regulation of microRNAs and its implications for cancer, Genes Dev. 20 (2006) 2202–2207.