Biochemical and Biophysical Research Communications 441 (2013) 438–446

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Network signatures of cellular immortalization in human lymphoblastoid cell lines

Sung-Mi Shim [a,1], So-Young Jung [a,1], Hye-Young Nam [a], Hye-Ryun Kim [a], Mee-Hee Lee [a], Jun-Woo Kim [a], Bok-Ghee Han [a], Jae-Pil Jeon [b,*]

[a] National Biobank of Korea, Center for Genome Science, Korea National Institute of Health, Osong 363-951, Republic of Korea
[b] Division of Brain Diseases, Center for Biomedical Science, Korea National Institute of Health, Osong 363-951, Republic of Korea

ARTICLE INFO

Article history:
Received 10 October 2013
Available online 24 October 2013

Keywords:
LCL
miRNA
mRNA
Integrated transcriptomic analysis

ABSTRACT

Human lymphoblastoid cell line (LCL) has been used as an *in vitro* cell model in genetic and pharmacogenomic studies, as well as a good model for studying gene expression regulatory machinery using integrated genomic analyses. In this study, we aimed to identify biological networks of LCL immortalization from transcriptomic profiles of microRNAs and their target genes in LCLs. We first selected differentially expressed genes (DEGs) and microRNAs (DEmiRs) between early passage LCLs (eLCLs) and terminally differentiated late passage LCLs (tLCLs). The *in silico* and correlation analysis of these DEGs and DEmiRs revealed that 1098 DEG–DEmiR pairs were found to be positively ($n = 591$ pairs) or negatively ($n = 507$ pairs) correlated with each other. More than 41% of DEGs are possibly regulated by miRNAs in LCL immortalizations. The target DEGs of DEmiRs were enriched for cellular functions associated with apoptosis, immune response, cell death, JAK–STAT cascade and lymphocyte activation while non-miRNA target DEGs were over-represented for basic cell metabolisms. The target DEGs correlated negatively with miR-548a-3p and miR-219-5p were significantly associated with protein kinase cascade, and the lymphocyte proliferation and apoptosis, respectively. In addition, the miR-106a and miR-424 clusters located in the X chromosome were enriched in DEmiR–mRNA pairs for LCL immortalization. In this study, the integrated transcriptomic analysis of LCLs could identify functional networks of biologically active microRNAs and their target genes involved in LCL immortalization.

1. Introduction

Human lymphoblastoid cell lines (LCLs) have been widely used as biological resources in various research fields. For example, LCLs have provided not only unlimited genetic materials for human genetic studies but also an *in vitro* cell model for pharmacogenomic studies exploring genetic variation by drug dosage or cytotoxicity [1–3]. LCL panels were also applied as novel tools *in vitro* for evaluating drug targets and pathways [4]. In addition, gene expression profiles of LCLs from patients with autism spectrum disorder and control subjects have been used for identification of disease-associated genes [5]. Transcriptomic signatures of LCLs were exploited to identify genes or miRNA-targeted genes related with diseases or complex traits [6–8]. Recent reports highlighted LCLs to be the valuable tool for integrated genomic analyses to study microRNA-mediated regulation of gene expression [9–11]. In addition to applications of LCLs, it is important to understand biological characteristics of LCLs in the process of cellular immortalization. Thus, many efforts have been made to investigate biological and genomic changes between primary B cells and transformed/immortalized LCLs [12–18].

MicroRNA (miRNA) is a small non-coding RNA molecule consisting of ~22 nucleotides in eukaryotes [19–21]. At the 5′ end of the miRNAs, 6–8 nucleotides typically have complementarity with 3′ UTRs of a target mRNA transcript forming microRNA–mRNA pairs [22–28]. This complementarity is required for the degradation of the mRNA or the translation repression in the post transcriptional gene silencing [19–21,29–31]. The microRNA-mediated gene expression is involved in control of cell differentiation [32,33], development [34], proliferation, apoptosis [35,36], and immunity [37,38]. Alterations in microRNA expression have been also associated with the progression of malignancies and other diseases [39,40]. Many studies have tried to identify the real target genes of microRNA using the computational prediction and experimental approaches including the integrated analyses of microRNA and mRNA expression profiles [1,41,42]. Target genes of microRNA are predicted by computational algorithms such as

* Corresponding author. Address: 200 Osongsaengmyung-2-ro, Osong-eup, Chungwon-gun, Chungbuk 363-951, Republic of Korea. Fax: +82 43 719 8602.
E-mail address: jaepiljeon@hanmail.net (J.-P. Jeon).
[1] These authors made equal contribution.

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.10.081

miRanda, MovingTargets, PITA, PicTar, TargetScanand DIANA-microT using the complementarity between microRNA and mRNA sequences [26,43]. These computationally predicted miRNAs are provided by public databases. For example, the miRBase database reported 1600 human microRNAs (August 2012, release 19.0).

In this study, we identified functional target genes of the microRNAs in LCLs by the integrated transcriptomic analysis mRNA and microRNA profiles differentially expressed between early passage LCLs (eLCLs) and terminally differentiated late passage LCLs (tLCL). These differentially expressed microRNAs (herein referred as DEmiRs) were used for *in silico* computational prediction analysis to select putative microRNA target genes using the miRBase::Targets database. Subsequently, biologically active target genes of the DEmiRs were identified from such putative target genes of the DEmiRs when the *in silico* predicted target genes of the DEmiRs coincided with the differentially expressed genes (herein referred as DEGs) between eLCL and tLCLs. We further analyzed the functional annotation clusters and pathways of the DEmiR-targeted DEGs. In this study, the integrated transcriptomic network analysis allowed us to identify functional networks of microRNA-mediated gene expression involved in LCL immortalization. These biological networks are possibly essential parts of the global regulatory machinery of gene expression for LCL immortalization.

2. Materials and methods

2.1. Microarray experiments

The eLCLs (passage 4–6) and tLCLs (passage 161) were used for microarray experiments. Culture conditions of LCLs and experimental methods of microarray analysis were described in supplemental information (Supplement materials).

2.2. Identification of functional DEmiR–DEG pairs

We retrieved the putative target genes of the DEmiRs in miRBase::Targets database (http://www.mirbase.org) using miRanda algorithm. Next, we selected the DEGs coincided with these putative target genes of the DEmiRs, and then identified the pairs of DEmiRs and their target DEGs (Table S1). The pair-wise coefficient correlation of microRNA–mRNA pairs was calculated by R statistical language v.2.4.1. Additionally, these putative target genes of the DEmiRs were queried for more accurate prediction to the second microRNA database, the TARGETSCAN version 5.1 (http://www.targetscan.org). In the Table S1 were highlighted these target DEGs of DEmiRs predicted by both miRBase::Targets and TARGETSCAN.

2.3. Functional enrichment analysis of DEmiR target genes

We conducted the functional enrichment analysis of genes consisting of DEmiR–DEG pairs using the Database for Annotation, Visualization and Integrated Discovery (DAVID) tool (http://david.abcc.ncifcrf.gov/). We also analyzed the over-representation of KEGG pathway, PANTHER pathway, and GO term in biological process category for the target genes and non-target genes of DEmiR by cutoff Benjamini corrected $p < 0.05$. Functional enrichment of target DEGs for each DEmiR was assessed to be over-represented for GO term in biological process category with p value <0.01. The functional annotation for each group according to the relationship of DEmiR-target DEG pairs was enriched significantly if enrichment p value (EASE scores) was less than 0.05 through functional annotation clustering tool of DAVID database.

3. Results

3.1. Integrated analysis of microRNA and mRNA expression profiles

We previously reported the mRNA and microRNA profiles differentially expressed between eLCL and tLCL [10,16]. In the present study, these two types of microarray data were used for the integrated transcriptomic analysis. When we examined both differentially expressed microRNAs (DEmiRs) and genes (DEGs) between eLCLs and tLCLs of 17 LCL strains (FDR adj-$p < 0.01$) (Fig. 1), 156 microRNA probes (34.98% of 446 microRNA probes) were found to be differentially expressed; 69 microRNA probes were up-regulated and the remaining 87 probes were down-regulated (Fig. S1, A). By contrast, we identified 2,458 mRNA probes (14.06% of 17,479 mRNA probes) as DEGs; 1,504 mRNA probes were up

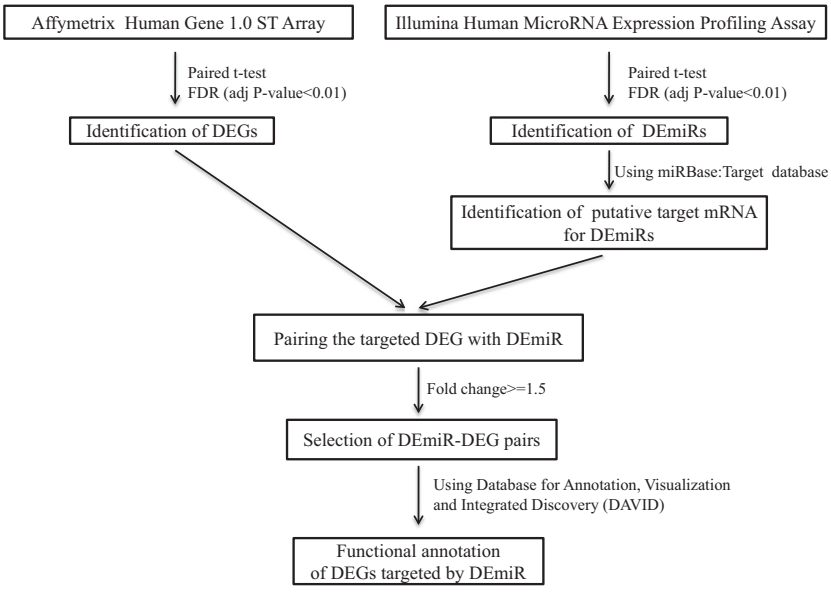

Fig. 1. Schematic flow of the integrated transcriptomic analysis of LCLs. Experimental transcriptomics data and computationally predicted miRNA target gene database were used to identify the true biological target genes of microRNAs differentially expressed during the long term subculture of LCLs.

expressed and 954 mRNA probes were down expressed between eLCL and tLCL (Fig. S1, B).

The connection with microRNAs and mRNAs were established through the miRBase::Targets database of Sanger. The miRBase database search showed that 111 out of 156 DEmiRs were hit to predict more than one putative DEmiR target genes. These putative DEmiR target genes consisted of 553 DEGs between eLCLs and tLCLs. Thus, 111 DEmiRs and 553 DEGs represented 6562 DEmiR–DEG pairs, based on the computational prediction of microRNA target genes. Among these DEmiR–DEG pairs, 1090 DEmiR–DEG pairs showed significant relationship with FDR adj-$p < 0.01$ and fold change 1.5. These significant DEmiR–DEG pairs contained 591 pairs (including 56 DEmiR and 292 DEG) with positive relationship and 507 pairs (including 55 DEmiR and 261 DEG) with negative relationship (Table 1) (Fig. 3).

Next, we estimated the proportions of the gene expression regulated by microRNAs in LCLs. Under the most stringent criteria (FDR adj $p < 0.01$ and fold change $\geqslant 2.0$), 72 (27.3%) out of 264 DEGs were found to be microRNAs targets with negative regulation while 76 DEGs (28.8%) with positive regulation (Table 1). In total, 41.6% of expressed genes (110 out of 264 DEGs) were found to be either negative or positive target genes of DEmiRs in the process of LCL immortalization, suggesting that substantial proportions of gene expression may be regulated under the control of miRNAs in LCLs. On the other hand, in the Table 2 are listed the target DEGs of individual DEmiRs. The most over-expressed microRNA, miR-205 (0.15-fold change), had 24 DEGs as the target genes in LCLs, whereas the most down-expressed microRNA, miR-28-5p (8.08-fold change), had 25 target DEGs.

3.2. Functional analysis of DEmiR-targeted genes (DEGs)

Using the DAVID tool, we conducted the functional annotation analysis of all 372 target DEGs of 56 DEmiRs (FDR $p < 0.01$ & $\geqslant 1.5$-fold) including either positive or negative DEmiR–DEG regulation patterns. The DEmiR-targeted DEGs were enriched to be associated with apoptosis signaling pathway (Benjamini $p < 0.003$) in PANTHER pathways, immune response (Benjamini $p = 4.76 \times 10^{-7}$), regulation of cell death (Benjamini $p = 2.72 \times 10^{-6}$), JAK–STAT cascade (Benjamini $p = 2.52 \times 10^{-3}$) and lymphocyte activation (Benjamini $p = 3.65 \times 10^{-3}$) in the GO biological process category (Table S2). Enrichment of the microRNA-targeted DEGs in GO and pathway were distinguished markedly from the non-targeted DEGs which were over-represented significantly in ribosome (Benjamini $p = 5.70 \times 10^{-14}$) and lysosome (Benjamini $p = 9.00 \times 10^{-3}$) of KEGG pathway, Ras pathway (Benjamini $p = 3.37 \times 10^{-2}$) and B cell activation (Benjamini $p = 2.65 \times 10^{-2}$) of PANTHER pathway, and translational elongation (Benjamini $p = 7.99 \times 10^{-14}$). This result showed that microRNA-mediated gene expression of LCLs is relatively more active on the lymphocyte-specific functions than basic cell metabolism of lymphocytes, suggesting that microRNAs may play a role of fine-tuning of gene expression for specialized functions of specific cell types such as LCLs.

Next, we classified the target DEGs of DEmiRs (FDR $p < 0.01$ & $\geqslant 1.5$-fold) into negative ($n = 261$) and positive ($n = 292$) regulation patterns, and then perform the enrichment analysis of GO and PANTHER pathway. The targeted DEGs of DEmiR with negative relationship were enriched for apoptosis signaling pathway (Benjamini $p = 0.05$) and B cell activation pathway (Benjamini $p = 0.04$) in PANTHER pathway, whereas the DEGs with positive relationship were associated with apoptosis signaling pathway (Benjamini $p = 0.02$) and JAK–STAT signaling pathway (Benjamini $p = 0.05$) (Table 3). The annotation cluster with a group enrichment score (Fisher's exact test; p) $\leqslant 0.05$ showed that the negatively associated DEGs were grouped by GO term for the regulation of cell death ($p = 5.34 \times 10^{-4}$), cell adhesion ($p = 8.46 \times 10^{-3}$), lymphocyte activation ($p = 9.26 \times 10^{-3}$), response to bacterium ($p = 2.01 \times 10^{-2}$), and cellular homeostasis ($p = 3.87 \times 10^{-2}$), whereas the positively associated DEGs were clustered with functions including the relationship of cell death ($p = 1.42 \times 10^{-4}$), lymphocyte activation ($p = 5.14 \times 10^{-3}$), JAK–STAT cascade ($p = 1.24 \times 10^{-2}$), cytokine-mediated signaling pathway ($p = 2.31 \times 10^{-2}$), cell redox homeostasis ($p = 3.47 \times 10^{-2}$), and MAPKKK cascade ($p = 4.23 \times 10^{-2}$) (Fig. 2). These results showed that the microRNA-targeted genes with both positive and negative regulation patterns were involved in with apoptosis, lymphocyte activation and basic cell metabolism. In contrast, the only negatively regulated DEGs were significantly related with cell adhesion, while the only positively regulated DEGs were significantly related with JAK–STAT and MAPKKK cascade.

3.3. Multiplicity of microRNA targets in LCL immortalization

A single microRNA has generally multiple target genes. To examine the multiplicity of DEmiR targets, the DEmiRs were listed based on number of their target DEGs, and their functional enrichment was analyzed by GO biological process category (Table S3). In the DEmiR–DEG negative relationship, miR-450a had the most multiple microRNA target genes ($n = 26$), covering 10.0% of total DEGs ($n = 262$). MicroRNAs exhibiting the next multiple target DEGs were miR-548a-3p ($n = 24$), miR-450b-5p ($n = 22$), miR-219-5p ($n = 20$), and miR-205 ($n = 19$), respectively. The target DEGs (*CCM2*, *SMAD1*, *RAPGEF2*, *MAP2K6*, *STAT1*) of miR-548a-3p were associated with protein kinase cascade ($p = 7.72 \times 10^{-4}$). The target DEGs (*CARD11*, *IGF2R*, *RIPK3*, *IL12B*, *CD24*, *FURIN*) of miR-219-5p were also associated with the positive regulation of T cell proliferation ($p = 8.30 \times 10^{-4}$) and the regulation of apoptosis ($p = 1.33 \times 10^{-3}$). In the DEmiR–DEG positive relationship, miR-20b and miR-212 targeted the most number of DEGs ($n = 26$). The target DEGs (*CFLAR*, *HSP90B1*, *BIRC3*) of miR-150 were over-represented in cellular homeostasis ($p = 2.47 \times 10^{-4}$).

On the other hand, it was reported that a single gene was regulated by multiple microRNAs [44,54]. Our result showed that some DEGs were single targets of multiple microRNAs in LCLs (Table 4). For example, erythrocyte membrane protein band 4.1 (*EPB41* gene) could be regulated by eight mulptiple microRNAs in negative relationship while putative homeodomain transcription factor 1

Table 1
Number of differentially expressed transcripts (DEGs and DEmiRs) and the DEmiR–DEG target pairs.

Criteria	No. of total DEG (probe)	No. of total DEmiR	DEmiR–DEG pairs					
			Negative regulation		Positive regulation		Total	
			DEG	DEmiR	DEG	DEmiR	DEG	DEmiR
FDR $p < 0.01$ only	1962 (2458)	156	1103	111	1089	111	1280	111
FDR $p < 0.01$ & $\geqslant 1.5$-fold	663 (779)	70	261	55	292	56	372	56
FDR $p < 0.01$ & $\geqslant 2.0$-fold	264 (309)	40	72	31	76	33	110	33

Table 2
Target DEGs of DEmiRs differentially expressed between early LCLs (p4) and late LCLs (p161).

MicroRNA	Fold change(P4/P161)	*P*-valuePaired *t*-test	Adj *P*-value (FDR-corrected)	Negative relationship		Positive relationship	
				No. of target DEGs	Target DEGs	No. of target DEGs	Target DEGs
Up-regulated				*Down-regulated*		*Up-regulated*	
hsa-miR-205	0.15	1.32E-04	1.15E-03	19	NAGK,PDIA6,ANUBL1,C13orf31,SUSD1, ACP5,SLC35B3,BTN3A1,FRK,VNN2,RAPGEF2,RASA2, CCM2,C12orf55,ATP2A3,PTK2B,FBXO16,HEY1,E2F5	5	ADAM23,WNT5A,ST3GAL6,GRAP2,CCR7
hsa-miR-125b	0.18	8.10E-06	1.67E-04	14	CD72,ZNF254,SLC2A5,PITRM1,CFLAR,FURIN, HLA-DMA,VNN2,HERC6,GOLGB1,CD84,DERL3,IKZF3,IFITM3	5	FRMD4A,EPB41,TOM1,ALKBH2,TMEM173
hsa-miR-99a	0.18	4.08E-05	5.00E-04	8	NCOA3,EPSTI1,MLKL,ITGA4,AP1S3,BLZF1,BTG2,HSP90B1	3	DTNA,RGS6,TUSC3
hsa-miR-326	0.21	2.08E-03	8.92E-03	11	ARHGAP25,ARID5B,ATP6V0D1,CD72,HLA-DMA, SLC15A2,CCM2,PDIA4,RALGPS2,PARVG,ACP2	2	DSG2,FLOT2
hsa-miR-34b[a]	0.28	3.10E-05	4.02E-04	10	CD79A,PITRM1,NEDD9,FUCA2,SLC12A8,XBP1,PARVG, CRELD2,PDE7A,CPT1A	2	SERPINB2,TMEM173
hsa-miR-34c-5p	0.30	2.85E-05	3.85E-04	16	GALM,PDIA6,HK1,ALOX5,CD79A,NEDD9,FUCA2, HERC6,GOLGB1,RALGPS2,XBP1, TRAFD1,IFI35,FBXO16,TRIM21,CPT1A	7	FRMD4A,NLRP11,HEBP2,PRLR,FLNB,FRMPD3,TMEM173
hsa-miR-100	0.34	4.79E-05	5.76E-04	13	TMEM87B,NCOA3,EPSTI1,MLKL,ITGA4,AP1S3,VNN2, CCR1,FCRL5,BLZF1,BTG2, HSP90B1,NUCB2	4	CDC42EP3,DTNA,REPS2,TUSC3
hsa-miR-548a-5p	0.35	3.34E-08	5.52E-06	14	C13orf31,CFLAR,DOCK10,CD38,LAP3,IFI44L,GBP5, RABGAP1L,LRRK2,HSP90B1, IKZF3,KIAA1618,FBXO16,LACTB2	9	DNAJC12,PARD3,SPG20,SERPINB2,PTPRG, LRIG3,DHRS13,LRP12,ADARB1
hsa-miR-26a-2[a]	0.38	1.37E-07	1.14E-05	11	BCL11A,KLF12,TM6SF1,HERC5,ALPK1,SLC12A8, GBP3,AIM2,EVI2A,PNOC,ACP2	4	HTR7,NLRP11,ADAM23,RIPK2
hsa-miR-450b-5p	0.39	6.11E-06	1.35E-04	22	PDIA6,TXNDC11,SETX,SLC35D2,ZNF790,RTTN, TM6SF1,SLC35B3,FRK,VNN2,GOLGB1, SLC15A2,PARP14,P2RY10,CDKN2C,PHTF1,BLZF1, CD69,IFI35,TMC6,FUT8,TRIM21	1	GEM
hsa-miR-143	0.42	1.56E-04	1.28E-03	16	EIF2AK3,IFIT1,MBNL2,SETDB2,ITM2B,CD79A,TCF4,SLC12A6,NEDD9, IGF2R,HERC5,CD1C, RABGAP1L,OPN3,FBXO16,RNF170	6	BAG3,LHX2,CCL20,CALN1,FLOT2,TUSC3
hsa-miR-193a-5p	0.43	1.78E-03	7.82E-03	11	NCOA3,ATP6V0D1,EHMT1,SEMA7A,CCM2,P2RY10,C1orf85, RABGAP1L,CD27,IGHV4-31,PDE7A	3	LAIR1,REPS2,FRMPD3
hsa-miR-424[a]	0.48	3.67E-04	2.38E-03	6	PHF11,SETDB2,HLA-DMA,UBE2J1,FBXO6,LACTB2	3	PRLR,PON2,LRIG3
hsa-miR-135b	0.49	6.58E-04	3.54E-03	17	RRBP1,C10orf118,SETDB2,SLC35D2,DDX58,C18orf54,ITGA4, SLC12A6,FRK,FUCA2,ATP10D,HERC5,ALPK1,FAM69A, RABGAP1L,CLEC2D,ITPR2	4	DNAJC12,PARD3,CALN1,DHRS13
hsa-miR-542-5p	0.50	3.62E-04	2.37E-03	9	5		PARD3,TLE4,HEBP2,ALDH1L1,RIPK2
hsa-miR-542-3p	0.50	2.07E-04	1.59E-03	14	PDIA6,PHF11,SETX,ACP5,NBEAL1,AP1S3,TUBB2B,CD180,ALCAM,AHR, FBXO6,CDKN2C,KIAA1618,TEP1	7	PARD3,TLE4,TNFRSF9,DTNA,FHOD3,ADAM23,PTPN13
Down-regulated				*Up-regulated*		*Down-regulated*	
hsa-miR-28-5p	8.08	2.83E-08	5.52E-06	9	CMTM3,LHX2,ZNF462,TLE4,DMKN,FHOD3,FRMD4B,EPDR1,FRMPD3	16	EXOC6B,EPSTI1,MLKL,SUSD1,ZNF254, DOCK10,KIAA1370,SEMA7A,UTRN,CD38, ALPK1,CD180,CCR1,CREB3L2,IFI44L,FCRL5
hsa-miR-151-5p	6.93	1.33E-05	2.50E-04	4	DMKN,EPDR1,FRMPD3,DHRS13	17	CLIP4,KLF12,C13orf18,EPSTI1,SUSD1,RHBDD1, SP100,SEMA7A,ISG20,CD38,IFI44L,KCNN3, ARSA,FAM18B2,SEC14L1,LGALS3BP,IFITM2
hsa-miR-20b	5.93	4.96E-05	5.79E-04	7	TLE4,IL27RA,MYO1F,PON2,EPB41,CCL5,TNFRSF10A	26	GALM,ZBP1,ZNFX1,FNDC3A,ZNF429,PITRM1, CFLAR,LRRK1,ALPK1,TNIP3,SLC12A8,EHHADH, RAPGEF5,FKBP14,FGL2,ATP7A,GLA,FBXO6,IL23R, AIM2,BLZF1,ALDH3A2,STAT3,TEP1,E2F5,SPTY2D1
hsa-miR-151-3p	5.85	9.85E-06	1.97E-04	3	FRMD4A,SERPINB10,TIMP1	10	EXOC6B,C13orf31,SLC35D2,CCDC69,CCR1,RABGAP1L, SLC16A7,LGALS3BP,RIPK3,NIN

(*continued on next page*)

Table 2 (*continued*)

MicroRNA	Fold change(P4/P161)	*P*-valuePaired *t*-test	Adj *P*-value (FDR-corrected)	Negative relationship		Positive relationship	
				No. of target DEGs	Target DEGs	No. of target DEGs	Target DEGs
hsa-miR-363	5.41	5.53E-05	6.20E-04	4	GRIN2A,GHR,EPB41,MANEAL	18	KRCC1,RNF103,GALM,ITM2B,SUSD1,ACP5,MPP1,BAZ2B, CCPG1,HIVEP1,DMXL1,IFI6,KIAA0040,CD69,HSP90B1, EVI2A,WIPI1,FUT8
hsa-miR-223[a]	4.85	8.35E-08	9.20E-06	5	INPP5F,TFAP2A,AUTS2,PLS3,PECAM1	9	RNF103,BCL11A,DOCK10,ISG20,SLC15A2,CDKN2C,GBP1, CD27,ALDH1L2
hsa-miR-20b[a]	4.46	5.74E-05	6.32E-04	2	DNAJC12,REPS2	14	EIF2AK2,EHMT1,CD72,IFIH1,MRPL44,CCPG1,CMAH, CD180,KCNN3,PARVG,CD27,WIPI1,LGALS3BP,REC8
hsa-miR-150[a]	3.26	4.87E-06	1.24E-04	3	DMKN,LRIG3,TMEM173	14	EIF2AK2,SLC2A5,ITM2C,STK38,MAP3K5,IGF2R,ANTXR2, ZBED2,CARD11,FBXO6,DERL3,SEC14L1,PNOC,LACTB2
hsa-miR-216a	3.11	2.35E-10	7.77E-08	7	ADAM23,UNC13C,MANEAL,CCL5,RGS6,TUSC3,TNFRSF10A	12	TIA1,AAK1,BLNK,BST2,MRPL44,ALPK1,GOLGB1,IL23R, IFI44L,SLC16A7,KIAA1618,SYNE2
hsa-miR-551b[a]	2.80	5.12E-05	5.83E-04	0	–	9	RSAD2,C13orf18,ATF7IP2,ZCCHC6,ITGA4,SEC24A, CRELD2,IGHV4-31,BIRC3
hsa-miR-22[a]	2.47	1.12E-06	4.37E-05	8	DNAJC12,CMTM3,TLE4,PERP,PRLR,EPB41,VCAM1,PRKCH	13	ANUBL1,PIP5K1B,ZNF790,STX7,UTRN,LNPEP,ZBTB20, RASA2,SCP2,SEMA4A,AIM2,IKZF3,NIN
hsa-miR-150	2.46	7.33E-05	7.36E-04	4	CACNA2D1,REPS2,GRAP2,CCL5	14	KRCC1,PDIA6,EGR2,KLF12,EPSTI1,ACO1,CFLAR,CCR1, FBXO6,KCNN3,IFI16,BLZF1,HSP90B1,KIAA1618
hsa-miR-212	2.30	3.05E-04	2.10E-03	4	CMTM3,TLE4,EPB41,RIPK2	26	PDIA6,SAMHD1,PHF11,C13orf18,ATP6V0D1,SLC35D2, CD72,ISG15,SP110,SP100,SLC35B3,FRK,FUCA2,GLRX, HEG1,EHHADH,PTPN12,PHTF1,SEMA4A,BTG2,STAT2, SYNE2,HEY1,E2F5,TRIB1,ACP2
hsa-miR-363[a]	2.27	2.87E-04	2.04E-03	6	TLE4,GHR,REPS2,TIMP1,TEAD4,TMEM173	9	NAGK,GPR114,EHMT1,SLC35D2,MPP1,MRPL44, SLC12A8,LGALS3BP,NCOA2
hsa-miR-335[a]	2.25	1.17E-03	5.51E-03	7	FRMD4A,ZNF462,TLE4,IL27RA,HEBP2,LRIG3,CCL5	14	ANUBL1,EHMT1,PITRM1,STX7,VNN2,CCR6, GLRX,AHR,PHTF1,C1orf85,BLZF1,RABGAP1L,KMO,LRRK2
hsa-miR-10a	2.24	2.13E-03	9.04E-03	6	IL27RA,PRLR,AUTS2,ARHGEF11,GRAP2,GIMAP8	10	SETDB2,EPSTI1,ANTXR2,HERC6,ZNF608,GOLGB1, PDIA4,P2RY10,SEMA4A,PARVG
hsa-miR-29b-2[a]	2.18	2.18E-07	1.60E-05	4	CMTM3,ZNF462,TFAP2A,EPB41	17	NCOA3,ANUBL1,MBNL2,SETDB2,SNX30,SLC35D2, DOCK8,RTTN,INPP1,CFLAR,FUCA2,IGJ,PHTF1,KCNN3, REC8,NIN,SYNE2
hsa-miR-221[a]	2.08	1.05E-03	5.02E-03	7	SLC39A10,MEIS2,PILRA,ARHGEF11,CABP1,CCL5,TMEM173	12	ITGB1,KLF12,SLC35D2,ZNF585B,SEMA7A,HLA-DMA,LAP3,ANKIB1,NUB1,RALGPS2,KIAA1618,REC8
hsa-miR-486-5p	2.04	3.20E-04	2.16E-03	5	PILRA,FRMPD3,ALKBH2,TUSC3,C11orf9	10	ZNF254,SLC12A8,CARD11,RAPGEF5,CCM2,SEMA4A, CD84,DERL3,SEC14L1

[a] The list was based on fold change ($\geqslant$2.0) of microRNA. The total list was showed in Table S1.

Table 3
Functional annotation of the DEmiR-target DEGs with negative or positive relationship.[a]

Pathway	Enrichment fold	Enrichment *P*-value	Benjamini-corrected *P* value	Number of genes	DEGs	DEmiRs
Negative relationship						
Apoptosis signaling pathway	3.82	8.40E-04	0.05	10	TNFRSF10A,CFLAR,TNFSF10,TNFRSF10D,BAG3,IGF2R,RIPK3,RIPK2,PRKCH,MAP2K6	hsa-miR-125b,hsa-miR-548a-5p,hsa-miR-30c-1*,hsa-miR-143,hsa-miR-219-5p,hsa-miR-548a-3p,hsa-miR-145,hsa-miR-551a,hsa-let-7g*,hsa-miR-22*,hsa-miR-212,hsa-miR-216a,hsa-miR-20b,hsa-miR-22
B cell activation	4.64	1.27E-03	0.04	8	PTPN6,FRK,PRKCH,IGHV4-31,CD79A,GRAP2,ITPR2,BLNK	hsa-miR-450a,hsa-miR-30c-1*,hsa-miR-143,hsa-miR-34b*,hsa-miR-34c-5p,hsa-miR-205,hsa-miR-450b-5p,hsa-miR-193a-5p,hsa-miR-135b,hsa-miR-10a,hsa-miR-150,hsa-miR-22*
Positive relationship						
Apoptosis signaling pathway	3.99	3.14E-04	0.02	11	TNFRSF10A,CFLAR,TNFSF10,MAP3K5,BAG3,IGF2R,RIPK3,RIPK2,MAPK8,BIRC3,EIF2AK2	hsa-miR-450a, hsa-miR-629*,hsa-miR-20b,hsa-miR-150,hsa-miR-130a,hsa-miR-148a*,hsa-miR-29b-2*,hsa-miR-30d,hsa-miR-30d*,hsa-miR-143,hsa-miR-150*,hsa-miR-542-5p,hsa-miR-26a-2*,hsa-miR-107,hsa-miR-551b*, hsa-miR-20b*
JAK/STAT signaling pathway	10.64	1.03E-03	0.05	5	JAK1,JAK2,STAT1,STAT3,STAT2	hsa-miR-923,hsa-miR-148a*, hsa-miR-30d*,hsa-miR-212,hsa-miR-20b,hsa-miR-130a

[a] The pathway using PANTHER category was assessed to be significant with Bejamini-corrected $p < 0.05$.

(*PHTF1*) could be regulated by nine multiple microRNAs with in positive relationship.

3.4. Genomic localization of DEmiR–DEG pairs

We examined genomic locations of DEmiRs and their target DEGs. We found that some DEmiRs were classified into four microRNA clusters of which two microRNA clusters (106a-clsuter and 424-cluster) were located in the X chromosome. All microRNAs of the 106a-cluster were down-regulated in tLCLs, compared to eLCLs. In contrast, the microRNAs of the 424-cluster were up-regulated in tLCLs. The microRNAs in the same cluster exhibited the same expression patterns (Table 5). The DEmiR–DEG pairs in the same chromosome were calculated for their distance and correlation coefficient. The distance between DEmiRs and DEGs on the same chromosome was 67.1 Mb on average, and the distance was associated with the correlation coefficient of DEmiRs and DEGs (Table S4). Interestingly, the chromosomal position of hsa-miR-548a-5p was located about 4.9 kb ahead to LRP12 with the positive correlation of expression ($r = 0.91$), suggesting that LRP12 gene is a potential target of has-miR-548a-5p in LCLs.

4. Discussion

The endogenous correlation of the microRNAs and mRNA expression has been previously studied using transcriptomic profiles of LCLs obtained at basal levels [45]. They found that microRNA-correlated genes in LCLs were associated with cell communication, signal transduction and cell cycles, representing endogenous genes targeted by miR-331, 98 and 33b. In contrast, our study focused on the identification of endogenous genes targeted directly by microRNAs in a specific condition of LCLs such as in the process of LCL immortalization for which differentially expressed miRNAs (DEmiRs) and their target genes (DEGs) between eLCLs and tLCLs were used to identify functionally active transcripts during the LCL immortalization.

Here, the present integrated transcriptomic analysis led to identify the target DEGs of DEmiRs which were related with apoptosis, protein kinase cascade and lymphocyte activation. For example, the target genes of miR-219-5p with negative correlation were involved significantly in lymphocyte proliferation, differentiation and apoptosis. Recent reports showed that the miR-219 was essential for differentiation of oligodendrocytes and suppressed directly the expression of proteins associated with proliferation of oligodendrocyte precursor cells [46,47]. Thus, our results supported that the miR-219 would be involved in proliferation and differentiation of LCL. In addition, the microRNA target genes related to B cell activation pathway were also enriched in the LCL immortalization, including miR-450a targets (*BLNK*, *FRK* and *PTPN6*), miR-135b targets (*FRK* and *ITPR2*), and miR-150 target (*GRAP2*). Lawrie et al. have investigated microRNA expression patterns in hematological cell lines including B- and T-cell lymphoma as well as LCLs [48]. They showed that mature B and T cells exhibited the over-expression of miR-150 while B- and T-cell lines exhibited the down-regulation of miR130b and miR-150 [48]. miR-150 blocked B cell development by inhibiting the conversion of the pro-B into pre-B cell stage [49], and controlled B cell differentiation by targeting c-Myb [50].

We identified the potential target genes of microRNAs with the positive and negative regulation patterns. For example, the miR-548a-5p was located 4.9 kb away from its target gene *LRP12* (low-density lipoprotein receptor-related protein 12), exhibiting the positive regulation ($r = 0.91$) of the miR-548a-5p and *LRP12* expression levels. It was reported that the intronic microRNA was co-expressed with their host gene by the same transcription factors [51]. The miR-548a-5p is located near the

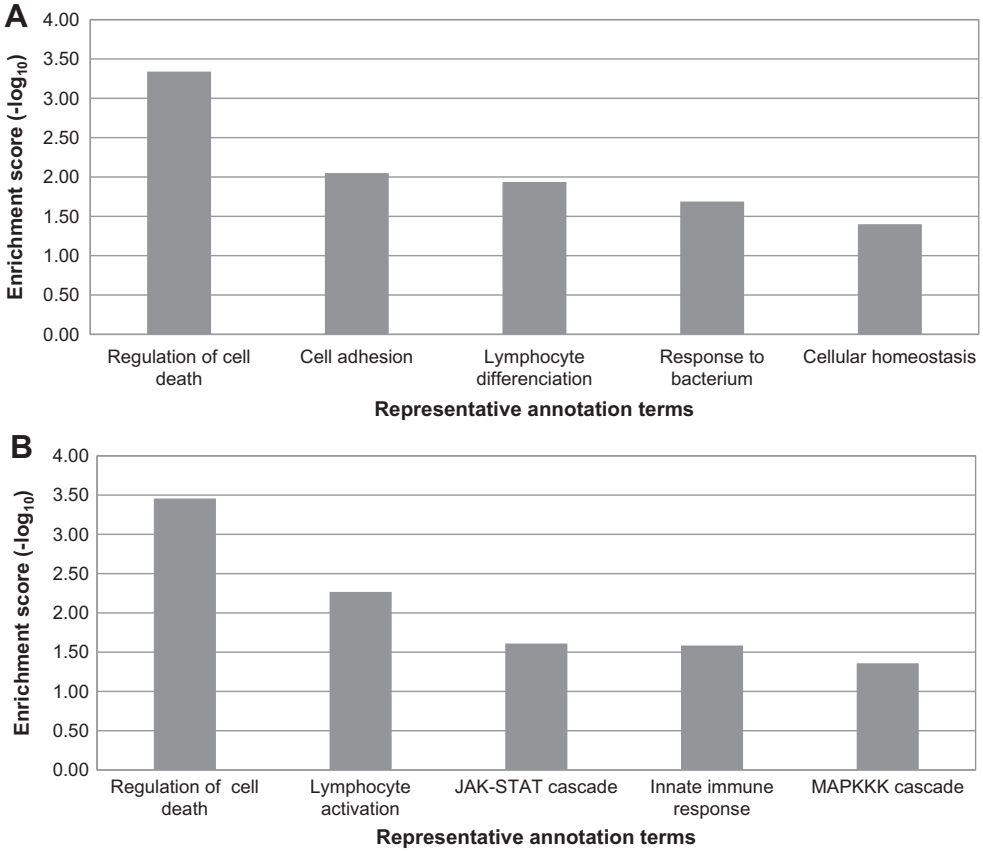

Fig. 2. Biological functions of the target genes (DEGs) of DEmiRs. The negative (A) and positive relationship (B) were shown to be differentially expressed during the long term subculture of LCLs. The functional annotation of DEmiR-target DEG pairs was enriched significantly if enrichment *p* value (EASE scores) was less than 0.05 through the functional annotation clustering tool of DAVID database.

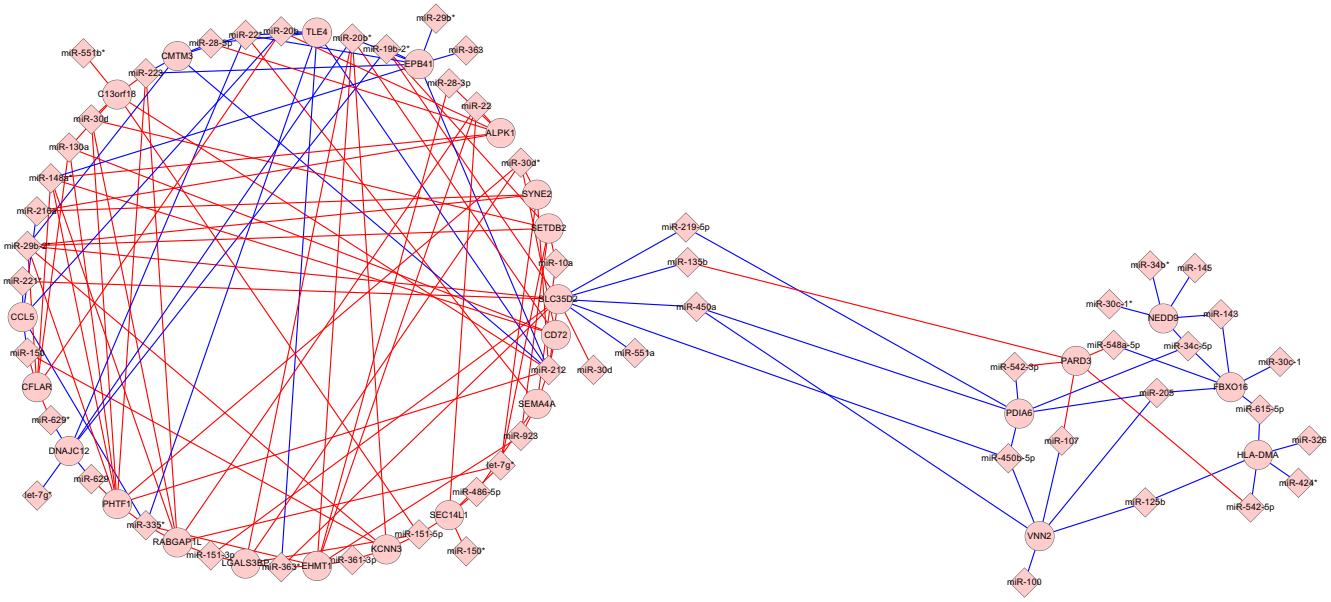

Fig. 3. Biological network of DEmiR–DEG pairs (FDR p < 0.01 & > 2.0-fold) in LCLs. The DEGs (n = 110) targeted by DEmiRs (n = 33) were selected to draw a biological network of transcriptional interactions of DEmiRs and DEGs using the Cytoscape (http://www.cytoscape.org). Blue and red lines indicate positive and negative correlations between DEmiRs (in diamonds) and DEGs (in circles), respectively(For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

promoter region of *LRP12*, suggesting that these two transcripts might be co-expressed under the control of the same transcriptional regulators in LCLs.

If the miRNAs and genes are located in the same cluster, the gene expression of the miRNA-target gene pairs might be controlled closely as in the case of the intronic microRNA and their

Table 4
List of single genes (DEGs) targeted by multiple DEmiRs.

Genes	Fold change (P4/P161)	Adj *p*-value (FDR-corrected)	No. of DEmiRs	Multiple DEmiRs	Genes	Fold change (P4/P161)	Adj *p*-value(FDR-corrected)	No. of DEmiRs	Multiple DEmiRs
Negative relationship					*Positive relationship*				
EPB41	0.58	1.04E-04	8	miR-19b-2*, miR-20b*, miR-22*, miR-29b*, miR-148a*, miR-212, miR-223, miR-363	PHTF1	1.54	3.73E-04	9	miR-29b-2*, miR-30d, miR-30d*, miR-130a, miR-148a*, miR-212miR-223, miR-335*, miR-629
DNAJC12	0.39	1.42E-04	6	miR-19b-2*, miR-20b*, miR-22*,miR-629, miR-629*, let-7g*	SLC35D2	2.87	4.12E-06	7	miR-19b-2*, miR-29b-2*, miR-30d, miR-151-3p, miR-212miR-221*, miR-363*
TLE4	0.56	1.73E-03	6	miR-20b, miR-22*, miR-28-5p, miR-212, miR-335*, miR-363*	RABGAP1L	2.36	2.77E-04	7	miR-22, miR-30d, miR-151-3p, miR-148a*, miR-223, miR-335*let-7g*
PDIA6	1.65	5.96E-04	6	miR-34c-5p, miR-205, miR-219-5p, miR-450a, miR-450b-5p, miR-542-3p,	EHMT1	1.68	2.82E-05	7	miR-20b*, miR-22, miR-28-3p, miR-335*, miR-361-3p, miR-363*miR-923
FBXO16	2.05	5.70E-04	6	miR-30c-1*, miR-34c-5p, miR-143, miR-205, miR-548a-5pmiR-615-5p	SETDB2	1.58	4.47E-03	6	miR-10a, miR-19b-2*, miR-29b-2* miR-30d, miR-923, let-7g*
VNN2	2.21	1.49E-03	6	miR-100, miR-107, miR-125b, miR-205, miR-450a, miR-450b-5p	CFLAR	2.19	2.65E-04	6	miR-20b, miR-29b-2*, miR-130a, miR-148a*, miR-150, miR-629*
CMTM3	0.47	1.91E-04	5	miR-22*, miR-28-5p, miR-29b-2*, miR-212, miR-223	C13orf18	3.08	4.78E-03	6	miR-30d, miR-130a, miR-151-5p, miR-212, miR-223, miR-551b*
CCL5	0.50	2.11E-03	5	miR-20b, miR-150, miR-216a miR-221*, miR-335*	ALPK1	1.74	7.51E-03	6	miR-20b, miR-22, miR-28-3p, miR-28-5p, miR-148a*, miR-216a
HLA-DMA	1.62	9.45E-03	5	miR-125b, miR-326, miR-424*, miR-542-5p, miR-615-5p	SYNE2	2.40	4.01E-03	5	miR-29b-2*,miR-30d*, miR-212, miR-216a, let-7g*
NEDD9	2.34	5.09E-05	5	miR-30c-1*, miR-34b*, miR-34c-5p, miR-143, miR-145	SEMA4A	2.40	5.39E-05	5	miR-10a, miR-22*, miR-212, miR-486-5p, miR-923
SLC35D2	2.87	4.12E-06	5	miR-135b, miR-219-5p, miR-450a, miR-450b-5p, miR-551a	SEC14L1	1.93	1.11E-03	5	miR-22, miR-150*, miR-151-5p, miR-486-5p, let-7g*
					PARD3	0.43	9.01E-03	5	miR-107, miR-135b, miR-542-3p, miR-542-5p, miR-548a-5p
					LGALS3BP	1.59	5.43E-03	5	miR-20b*, miR-30d*, miR-151-3p, miR-151-5p, miR-363*
					KCNN3	1.70	3.41E-03	5	miR-20b*, miR-29b-2*, miR-150, miR-151-5p, miR-361-3p
					CD72	1.59	3.03E-03	5	miR-20b*, miR-30d*, miR-130a, miR-148a*, miR-212

Table 5
The genomic location and expression pattern of DEmiR-containing microRNA clusters.

Cluster	microRNA probes	#Chr[a]	Start	End	Expression pattern[b]
143_cluster	hsa-miR-143	5	148,808,481	148,808,586	Up
	hsa-miR-145	5	148,810,209	148,810,296	
34b_cluster	hsa-miR-34b*	11	111,383,663	111,383,746	Up
	hsa-miR-34c-5p	11	111,384,164	111,384,240	
106a_cluster	hsa-miR-363	X	133,303,408	133,303,482	Down
	hsa-miR-363*				
	hsa-miR-19b-2*	X	133,303,701	133,303,796	
	hsa-miR-20b	X	133,303,839	133,303,907	
	hsa-miR-20b*				
424_cluster	hsa-miR-450b-5p	X	133,674,215	133,674,292	Up
	miR-450a-1	X	133,674,371	133,674,461	
	miR-450a-2	X	133,674,538	133,674,637	
	hsa-miR-542-3p	X	133,675,371	133,675,467	
	hsa-miR-542-5p				
	hsa-miR-424*	X	133,680,644	133,680,741	

[a] Genomic location of microRNA was referred from the miRBase database.
[b] The expression changes from early LCL (p4) to late LCL (p161).

host gene by which the mechanism may be similarly applied to the positive regulation of miRNA-target gene pairs. Yu et al. identified hematopoietic cell line-specific microRNA clusters in leukemia cell lines [52]. For examples, miR-200b and miR-29a clusters showed expression dominance in Jurkat cell lines, while the let-7a-1, let-7a-3 and mir-98 clusters were highly expressed in Raji, Jurkat and THP-1 cell lines. The clustered microRNAs were expressed by co-regulation of the same promoter, exerting closely related functions [53]. Kim et al. proved that miR-106b cluster and miR-222 cluster controlled cell cycle by suppressing Cip/Kip family proteins in gastric cancer [53]. In the present work, we identified that miR-106a cluster and miR-424 cluster in the X chromosome were dominant microRNA groups associated with many possible target genes in LCL immortalization. All of microRNAs within the

miR-106a cluster were down-regulated in the late passage of LCLs (tLCLs) compared to the early passage (eLCLs) while miR-424 cluster was up-regulated. Therefore, we suggest the miR-106a cluster and miR-424 cluster might be LCL-specific microRNA cluster for LCL immortalization.

It has been well known that microRNAs have multiple target genes. Recently, it has been also suggested that a single gene is targeted by multiple microRNAs [44,54]. They reported that 28 microRNAs including the clustered microRNAs could target the p21Cip1/Waf1. In this view, we presented the mRNAs expected to be regulated by multiple microRNAs during the LCL immortalization or maintenance.

Acknowledgments

This work was supported by intramural grants (2010-N74001-00, 2013-NG74001-00) of Korea Centers for Disease Control and Prevention.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.10.081.

References

[1] J.C. Huang, T. Babak, T.W. Corson, et al., Using expression profiling data to identify human microRNA targets, Nat. Methods 4 (2007) 1045–1049.
[2] L. Li, B. Fridley, K. Kalari, et al., Gemcitabine and cytosine arabinoside cytotoxicity: association with lymphoblastoid cell expression, Cancer Res. 68 (2008) 7050–7058.
[3] H. Chen, N. Wang, M. Burmeister, et al., MicroRNA expression changes in lymphoblastoid cell lines in response to lithium treatment, Int. J. Neuropsychopharmacol. 12 (2009) 975–981.
[4] A. Morag, J. Kirchheiner, M. Rehavi, et al., Human lymphoblastoid cell line panels: novel tools for assessing shared drug pathways, Pharmacogenomics 11 (2010) 327–340.
[5] J.E. Lee, H.Y. Nam, S.M. Shim, et al., Expression phenotype changes of EBV-transformed lymphoblastoid cell lines during long-term subculture and its clinical significance, Cell Prolif. 43 (2010) 378–384.
[6] Z. Talebizadeh, M.G. Bulter, M.F. Theodoro, Feasibility and relevance of examining cell lines to study role of microRNAs in autism, Autism Res. 1 (2008) 240–250.
[7] T. Sarachana, R. Zhou, G. Chen, et al., Investigation of post-transcriptiona; gene regulatory networks associated with autism spectrum disorders by microRNA expression profiling of lymphoblastoid cell lines, Genome Med. 2 (2010) 23.
[8] J.L. Min, J.M. Taylor, J.B. Richards, et al., The use of genome-wide eQTL associations in lymphoblastoid cell lines to identify novel genetic pathways involved in complex traits, PLoS ONE 6 (2011) e22070.
[9] E. Forte, M.A. Luftig, Use of viral systems to study miRNA-mediated regulation of gene expression in human cells, Methods Mol. Biol. 936 (2013) 143–156.
[10] S.M. Shim, H.Y. Nam, J.E. Lee, et al., MicroRNAs in human lymphoblastoid cell lines, Crit. Rev. Eukaryot. Gene Expr. 22 (2012) 189–196.
[11] R.L. Skalsky, D.L. Corcoran, E. Gottwein, et al., The viral and cellular microRNA targetome in lymphoblastoid cell lines, PLoS Pathog. 8 (2012) e1002484.
[12] A. Mohyuddin, Q. Ayub, S. Siddiqi, et al., Genetic instability in EBV-tansformed lymphoblastoid cell line, Biochim. Biophys. Acta 1670 (2004) 81–83.
[13] R. Redon, S. Ishikawa, K.R. Fitch, et al., Global variation in copy number in the human genome, Nature 444 (2006) 444–454.
[14] J.P. Jeon, S.M. Shim, H.Y. Nam, et al., Copy number increase of 1p36.33 and mitochondrial genome amplification in Epstein–Barr virus-transformed lymphoblastoid cell lines, Cancer Genet. Cytogenet. 173 (2007) 122–130.
[15] J.P. Jeon, J.W. Kim, B. Park, et al., Identification of tumor necrosis factor signaling-related proteins during Epstein–Barr virus-induced B cell transformation, Acta Virol. 52 (2008) 151–159.
[16] E.P. Brennan, M. Ehrich, D.P. Brazil, et al., Comparative analysis of DNA methylation profiles in peripheral blood leukocytes versus lymphoblastoid cell lines, Epigenetics 4 (2009) 159–164.
[17] J.T. Herbeck, G.S. Gottlieb, K. Wong, et al., Fidelity of SNP array genotyping using Epstein Barr virus-transformed B-lymphocyte cell lines: implications for genome-wide association studies, PLoS ONE 4 (2009) e6915.
[18] D. Grafodatskaya, S. Choufani, J.C. Ferreira, et al., EBV transformation and cell culturing destabilizes DNA methylation in human lymphoblastoid cell line, Genomics 95 (2010) 75–83.
[19] L. He, G.J. Hannon, MicroRNAs: small RNAs with a big role in gene regulation, Nat. Rev. Genet. 5 (2004) 522–531.
[20] J.T. Mendell, MicroRNAs: critical regulators of development, cellular physiology and malignancy, Cell Cycle 4 (2005) 1179–1184.
[21] M. Esteller, Non-coding RNAs in human disease, Nat. Rev. Genet. 12 (2011) 861–874.
[22] G. Hutvagner, P.D. Zamore, A microRNA in a multiple-turnover RNAi enzyme complex, Science 297 (2002) 2056–2060.
[23] B.P. Lewis, I.H. Shih, M.W. Jones-Rhoades, Prediction of mammalian microRNA targets, Cell 115 (2003) 787–798.
[24] A. Krek, D. Grun, M.N. Poy, et al., Combinatorial microRNA target predictions, Nat. Genet. 37 (2005) 495–500.
[25] A. Grimson, K.K. Farh, W.K. Johnston, et al., MicroRNA targeting specificity in mammals: determinants beyond seed pairing, Mol. Cell 27 (2007) 91–105.
[26] D.P. Bartel, MicroRNAs: target recognition and regulatory functions, Cell 136 (2009) 215–233.
[27] H. Siomi, M.C. Siomi, Posttranscriptional regulation of microRNA biogenesis in animals, Mol. Cell 38 (2010) 323–332.
[28] J.E. Lee, E.J. Hong, H.Y. Nam, et al., MicroRNA signatures associated with immortalization of EBV-transformed lymphoblastoid cell lines and clinical traits, Cell Prolif. 44 (2011) 59–66.
[29] E.C. Lai, MicroRNAs are complementary to 30 UTR sequence motifs that mediate negative post-transcriptional regulation, Nat. Genet. 30 (2002) 363–364.
[30] L.P. Lim, N.C. Lau, P. Garrett-Engele, et al., Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs, Nature 433 (2005) 769–773.
[31] R.S. Pillai, MicroRNA function: multiple mechanisms for a tiny RNA?, RNA 11 (2005) 1753–1761
[32] Y. Tay, J. Zhang, A.M. Thomson, et al., MicroRNAs to Nanog, Oct4 and Sox2 coding regions modulate embryonic stem cell differentiation, Nature 455 (2008) 1124–1128.
[33] J. Ren, P. Jin, E. Wang, et al., MicroRNA and gene expression patterns in the differentiation of human embryomic stem cells, J. Transl. Med. 7 (2009) 20.
[34] R. Yi, M.N. Poy, M. Stoffel, E. Fuchs, A skin microRNA promotes differentiation by repressing "stemness", Nature 452 (2008) 225–229.
[35] D.C. Corney, A. Flesken-Nikitin, A.K. Godwin, et al., MicroRNA-34b and MicroRNA-34c are targets of p53 and cooperate in control of cell proliferation and adhesion-independent growth, Cancer Res. 67 (2007) 8433–8438.
[36] M.J. Bueno, I.P. Castro, M. Malumbres, Control of cell proliferation pathways by micro RNAs, Cell Cycle 7 (2008) 3143–3148.
[37] A. Rodriguez, E. Vigorito, S. Clare, et al., Requirement of bic/microRNA-155 for normal immune function, Science 316 (2007) 608–611.
[38] E. Tsitsiou, M.A. Lindsay, MicroRNAs and the immune response, Curr. Opin. Pharmacol. 9 (2009) 514–520.
[39] W. Filipowicz, S.N. Bhattacharyya, N. Sonenberg, Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight?, Nat Rev. Genet. 9 (2008) 102–114.
[40] M. Guerau-de-Arellano, H. Alder, H.G. Ozer, et al., MiRNA profiling for biomarker discovery in multiple sclerosis: from microarray to deep sequencing, J. Neuroimmunol. 248 (2012) 32–39.
[41] V.A. Gennarino, M. Sardiello, R. Avellino, et al., MicroRNA target prediction by expression analysis of host genes, Genome Res. 19 (2009) 481–490.
[42] G. Sales, A. Coppe, S. Bicciato, et al., Impact of probe annotation on the integration of miRNA–mRNA expression profiles for miRNA target detection, Nucleic Acids Res. 38 (2010) e97.
[43] N.D. Mendes, A.T. Freitas, M.F. Sagot, Current tools for the identification of miRNA gene and their targets, Nucleic Acids Res. 3 (2009) 2419–2433.
[44] M.E. Peter, Targeting of mRNAs by multiple miRNAs: the next step, Oncogene 29 (2010) 2161–2164.
[45] L. Wang, A.L. Oberg, Y.W. Asmann, et al., Genome-wide transcriptional profiling reveals microRNA-correlated genes and biological processes in human lymphoblastoid cell lines, PLoS ONE 4 (2010) e5878.
[46] C. Jason, J.C. Dugas, T.L. Cuellar, et al., Dicer1 and miR-219 are required for normal oligodendrocyte differentiation and myelination, Neuron 65 (2010) 597–611.
[47] X. Zhao, X. He, X. Han, et al., MicroRNA-mediated control of oligodendrocyte differentiation, Neuron 65 (2010) 612–626.
[48] C.H. Lawrie, N.J. Saunders, S. Soneji, et al., MicroRNA expression in lymphocyte development and malignancy, Leukemia 22 (2008) 1440–1446.
[49] B. Zhou, S. Wang, C. Mayr, et al., MiR-150, a microRNA expressed in mature B and T cells, blocks early B cell development when expressed prematurely, Proc. Natl. Acad. Sci. USA 104 (2007) 7080–7085.
[50] C. Xiao, D.P. Calado, G. Galler, et al., MiR-150 controls B cell differentiation by targeting the transcription factor c-Myb, Cell 131 (2007) 146–159.
[51] J. Friedman, P. Jones, MicroRNAs: critical mediators of differentiation, development and disease, Swiss Med. Weekly 139 (2009) 466–472.
[52] J. Yu, F. Wang, G.H. Yang, et al., Human microRNA clusters: genomic organization and expression profile in leukemia cell lines, Biochem. Biophys. Res. Commun. 349 (2006) 59–68.
[53] Y.K. Kim, J. Yu, T.S. Han, et al., Functional links between clustered microRNA: suppression of cell-cycle inhibitors by microRNA clusters in gastric cancer, Nucleic Acids Res. 37 (2009) 1672–1681.
[54] S. Wu, S. Huang, J. Ding, et al., Multiple microRNAs modulate p21Cip1/Waf1 expression by directly targeting its 3′untranslated region, Oncogene 29 (2010) 2302–2308.