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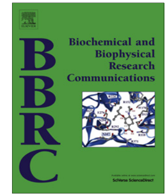

Network signatures of cellular immortalization in human lymphoblastoid cell lines

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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.

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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

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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

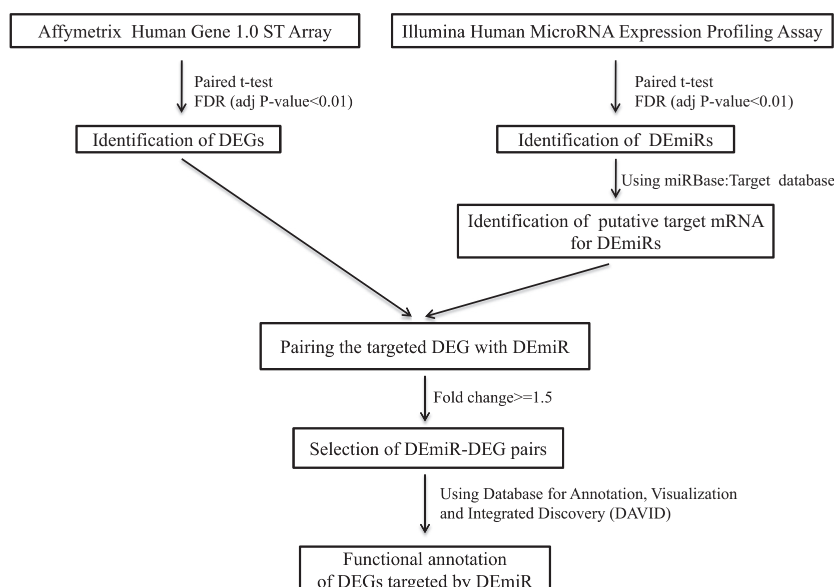

Fig. 1. Schematic flow of the integrated transcriptomic analysis of LCLs. Experimental transcriptomics data and computationally predicted miRNA target gene database were used to identify the true biological target genes of microRNAs differentially expressed during the long term subculture of LCLs.

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