Cell Proliferation

Cell Prolif., 2010, **43**, 378–384 doi: 10.1111/j.1365-2184.2010.00687.x

Expression phenotype changes of EBV-transformed lymphoblastoid cell lines during long-term subculture and its clinical significance

J.-E. Lee[1], H.-Y. Nam[1], S.-M. Shim, G.-R. Bae, B.-G. Han* and J.-P. Jeon*

Division of Biobank for Health Sciences, Center for Genome Science, Korea National Institute of Health, Korea Centers for Disease Control and Prevention, Seoul, Korea

Received 8 *September* 2009; *revision accepted* 12 *November* 2009

Abstract

Objectives: The EBV-transformed lymphoblastoid cell line (LCL) is a useful resource for population-based human genetic and pharmacogenetic studies. The principal objective here was to assess expression phenotype changes during long-term subculture of LCLs, and its clinical significance.

Materials and methods: We searched for genes that were differentially expressed in 17 LCLs at late (p161) passage compared to early passage (p4) using microarray assay, then validated them by real-time RT-PCR analysis. In addition, we estimated correlations between expression phenotypes of 20 LCL strains at early passage and 23 quantitative clinical traits from blood donors of particular LCL strains.

Results: Transcript sequences of 16 genes including nuclear factor-κB (NF-κB) pathway-related genes (such as *PTPN13*, *HERC5* and miR-146a) and carcinogenesis-related genes (such as *XAF1*, *TCL1A*, *PTPN13*, *CD38* and miR-146a) were differentially expressed (>2-fold change) in at least 15 of the 17 LCL strains. In particular, *TC2N*, *FCRL5*, *CD180*, *CD38* and miR-146a were downregulated in all 17 of the evaluated LCL strains. In addition, we identified clinical trait-associated expression phenotypes in LCLs.

Conclusion: Our results showed that LCLs acquired expression phenotype changes involving expression of NF-κB pathway- and carcinogenesis-related genes during long-term subculture. These differentially expressed genes can be considered to be a gene signature of LCL immortalization or EBV-induced carcinogenesis. Clinical trait-associated expression phenotypes should prove useful in the discovery of new candidate genes for particular traits.

*Correspondence: J.-P. Jeon and B.-G. Han, Division of Biobank for Health Sciences, Center for Genome Science, Korea National Institute of Health, Korea Centers for Disease Control and Prevention, Seoul 122-701, Korea. Tel.: 82-2-380-1531; Fax: 82-2-354-1078; E-mail: jaepiljeon@hanmail.net and bokghee@yahoo.com

[1]These authors contributed equally to this work.

Introduction

Lymphoblastoid cell lines (LCLs) have been utilized previously as biological resources for population-based human genetic or proteomic studies. For example, gene expression profiling between LCLs from autism spectrum disorder (ASD) patients and controls has been performed, using microarray techniques, to verify candidate genes for ASD diagnosis (1). LCLs from patients with mitochondrial diseases and control subjects have been assessed to identify mitochondrial disease-associated proteins, by a 2-DE procedure (2). Recently, utilization of LCLs has been extended to pharmacogenomic and pharmacogenetic research, including studies on differences in drug response according to individual genetic variation (3,4). Cytotoxicity of chemotherapeutic agents such as dexamethasone has been compared between LCLs from Down's syndrome (DS) patients and non-DS patients (4).

However, there is some concern about extensive use of LCLs due to possible genetic changes or relevant gene expression changes during LCL generation and maintenance. For example, when DNA methylation in LCLs from type 1 diabetes patients was compared with that in paired peripheral blood leucocytes, differences in DNA methylation were observed in 27 (8%) of 318 genes (5). Therefore, we have established a biological characterization of LCLs using a long-term subculture collection of 20 LCL strains, in effort to increase utility of LCLs and to provide quality-controlled LCLs for genetic and pharmacogenomic studies. In a previous study, we showed that large genomic alterations may not occur, at least in the

early stages of LCL culture (6). In addition, we reported that sustained EBV activity, as well as telomerase activity, may be required for complete LCL immortalization (7).

When LCLs proliferate up to passage number of 160, the LCL is considered 'terminally immortalized'. In this study, we have identified genes expressed differentially in 17 LCL strains at late (p161) passages compared to early passages (p4) using a microarray assay, followed by validation by real-time RT-PCR. Moreover, we assessed correlations between expression phenotypes of LCL strains at early passages (p4) and 23 quantitative clinical data of donors.

Materials and methods

Cell culture

Twenty LCL strains (n = 20) were selected randomly for long-term subculture from an LCL collection at the Korean HapMap project, as described in previous reports (7–9). These LCL strains were cultured in RPMI1640 medium (Invitrogen, Carlsbad, CA, USA) supplemented with 10% foetal bovine serum at 37 °C in 5% CO_2 humidified air. Culture medium was replaced with fresh complete medium at each passage. LCL strains were maintained in culture medium until passage number 160 to obtain terminally immortalized LCL strains. Seventeen strains among them were capable of proliferating for 160 or more passages, which is generally considered to be a critical passage number for terminal immortalization of LCLs. In contrast, three LCL strains stopped proliferating at passage numbers of 33, 44 and 48, as described in our previous study (7).

Microarray experimentation and analysis

Total RNA was isolated from LCLs using an RNeasy kit (Qiagen, Hilden, Germany), then converted into labelled cDNA and hybridized on an Affymetrix GeneChip® Human Gene 1.0ST Array containing 764 885 differential probes, in accordance with the manufacturer's recommendations. After washing the hybridized chip, chip images were scanned using Affymetrix GeneChip® Scanner 3000 7G apparatus, and were analysed using Affymetrix GCOS Software (Affymetrix, CA, USA). Expression levels of all transcripts on chips were utilized for data normalization, and differentially expressed genes (DEGs) were selected according to standards as follows: fold change >2 and P-value was <0.01.

Real-time RT-PCR

First-strand cDNA was synthesized from total RNA samples by two methods to verify differential expression levels of mRNA or miRNA transcripts. For the mRNA experiment, first-strand cDNA was synthesized with random primers using Superscript III first-strand synthesis system (Invitrogen). Subsequently, this sample was used to validate 15 genes including *PRKCH, PTPN13, CD38, CD180, FCRL5, GPR160, HERC5, IFIT1, OAS3, RASGRP3, RFTS7H, TC2N, TCL1A, XAF1 and ZNF382*. Real-time RT-PCR was conducted with a mixture (20 μl final volume) containing 100 ng of cDNA template, 2× SYBR Green Master Mix buffer (ABI), and forward and reverse primers for each gene. Primers were designed using GenBank nucleotide sequence for each gene. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was employed as internal control. Primer

Table 1. Primers used for real-time RT-PCR experiments

Gene	Forward primer	Reverse primer
CD38	5′-TTGGGAACTCAGACCGTACC-3′	5′-GTTGCTGCAGTCCTTTCTCC-3′
CD180	5′-CACCTCCTGGGATCAGATGT-3′	5′-TTGATGATGGCTTTGAAAAGTG-3′
FCRL5	5′-CCCTGTGCACTTGGATTTTT-3′	5′-CAGCGATATGCACCATTGTC-3′
GPR160	5′-GTCAAGGAAGACCCACTGGA-3′	5′-TAGGGGCTGGTTTGTTTGAC-3′
HERC5	5′-GATTGCTGGAGGGAATCAAA-3′	5′-TTGGATTTCCCTTTTTGTGC-3′
IFIT1	5′-TCTCAGAGGAGCCTGGCTAA-3′	5′-TCAGGCATTTCATCGTCATC-3′
OAS3	5′-GTCAAACCCAAGCCACAAGT-3′	5′-TGTAGGCACACCTGGTGGTA-3′
PRKCH	5′-CCAGAATCAAATCCCGAGAA-3′	5′-CTAAGGCTGATGCTGGGAAG-3′
PTPN13	5′-TTCTCTGCAGACCTCCACCT-3′	5′-TCTTCTCCACTCCCACTGCT-3′
RASGRP3	5′-TGCATTTCCCAATGATGCTA-3′	5′-AATGTTGCTGCTTTCCCAAG-3′
RFTS7H	5′-GCCTCAGTGAAGGTCTCCTG-3′	5′-CTCCATGTAGGCTGTGCTGA-3′
TC2N	5′-TCCCAGGAAGAAAACCATTG-3′	5′-GAAGGTACCGTGCCTCAAGA-3′
TCL1A	5′-TCCAGTTTCTGGCGCTTAGT-3′	5′-TCTGTCCATTCCTCCCAGAC-3′
XAF1	5′-TTCAGCTCCTGAAAGGGAAA-3′	5′-TTCAGCAGCTTGACTTGGAA-3′
ZNF382	5′-GCGCTTTACAGGGATGTGAT-3′	5′-GAGGGGTCTAGAATGCCTGTC-3′
GAPDH	5′-CAGGGCTGCTTTTAACTCTGGTAA-3′	5′-GTGGAATCATATTGGAACATGTAAACC-3′

sequences are shown in Table 1. For the miRNA experiment, first-strand cDNA samples were synthesized using TaqMan MicroRNA Reverse Transcription kit (ABI) and RT primer in TaqMan MicroRNA Assays (hsa-miR-146a), in accordance with the manufacturer's recommendations (ABI). Then the real-time RT-PCR experiment was conducted with 20 μl of PCR mixture containing 1.33 μl of RT product, TaqMan 2× Universal PCR Master Mix (No AmpErase UNG) and TaqMan MicroRNA Assay (20X). RNU6B was used as internal control.

PCR reactions were run on an ABI HT 7900 (ABI) PCR system and cycle conditions were as follows: 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s, and 60 °C for 1 min. Comparative critical threshold (C_t) value, obtained by real-time RT-PCR analysis, was used for relative quantification of gene expression. In this study, ΔC_t value means C_t value of the target gene relative to the control gene (C_t, target gene-C_t, control gene). The $\Delta\Delta C_t$ value represents ΔC_t value of LCL at late passage compared to that at early passage [$\Delta\Delta C_t = (C_t$, target gene-C_t, control gene$)_{\text{LCLs at the late passages}} - (C_t$, target gene-$C_t$, control gene$)_{\text{LCLs at the early passages}}$]. All PCR reactions were conducted in duplicate in at least two independent experiments.

Correlation between gene expression pattern and clinical data

To identify clinical trait-associated genes, correlations between microarray data from 20 LCLs at early passages and 23 quantitative clinical traits of blood donors were assessed by linear regression analysis using SPSS version 13.0 (SPSS, Chicago, IL, USA). For each clinical trait, genes with highest correlation coefficients are summarized in Table 4. Twenty blood donors were aged between 41 and 69 years (mean ± SD, 55.3 ± 8.9), and included nine women and 11 men.

Results

Microarray analysis

To identify gene expression changes during long-term LCL subculture, we conducted a microarray experiment with LCL strains at early (p4, $n = 20$) and late (p161, $n = 17$) passages. Approximately, 500–1500 transcripts per LCL strain were differentially expressed at late passages compared to early passages; around 200–700 genes per LCL strain were upregulated at late passages and approximately 300–1000 genes were downregulated (Table 2). Among them, 16 transcripts were differentially expressed with greater than 2-fold changes in at least 16 of the 17 LCL strains (Table 3). *CD38* was downregulated and *PTPN13* was upregulated in all 17 LCL strains at late passage compared to those at early passage. *CD180, FCRL5, GPR160, HERC5, IFIT1, OAS3, RASGRP3, RFTS7H, TC2N, TCL1A, XAF1, ZNF382* and miR-146a were downregulated and *PRKCH* was upregulated in 16 of 17 LCLs at the late passage.

Table 2. Numbers of differentially expressed genes (DEGs) of each LCL strain

LCL strain	No. upregulated DEGs	No. downregulated DEGs	No. total DEGs
A1	754	760	1514
A2	389	438	827
A3	575	707	1282
A4	285	693	978
A5	622	584	1206
A6	402	1051	1453
A8	230	670	900
A10	497	668	1165
K1	375	377	752
K2	325	590	915
K3	222	945	1167
K5	438	412	850
K6	230	301	531
K7	658	606	1264
K8	238	527	765
K9	230	289	519
K10	275	512	787

Approximately 1000 DEGs per each LCL strain were identified in comparison between early and late passages.

Real-time RT-PCR analysis

To validate the microarray data, we conducted a real-time RT-PCR experiment for 16 DEGs in 17 LCL strains. As shown in Fig. 1 and Table S1, 16 transcripts showed expression patterns similar to the microarray results. Among them, *PRKCH* and *PTPN13* had upregulated expression patterns in 16 LCLs at late passage, whereas 14 transcripts (*CD38, CD180, FCRL5, GPR160, HERC5, IFIT1, OAS3, RASGRP3, RFTS7H, TC2N, TCL1A, XAF1, ZNF382* and miR-146a) had downregulated expression patterns. All of 16 transcripts were up- or downregulated at late passage in at least 15 LCL strains (Table S1). Among these DEGs, downregulation of five transcripts (*CD38*, *CD180*, *FCRL5*, *TC2N* and miR-146a) was validated by qPCR in all 17 LCL strains tested in our study. The biggest difference in gene expression levels was observed for the *TC2N* gene (mean of $\Delta\Delta C_t$ values ± SD, 7.6 ± 3.22). These results suggest that these five DEGs represented passage-dependent gene expression during long-term subculture.

Table 3. Common transcripts expressed differentially in 17 LCL strains

Gene symbol	mRNA Acc. No.	Gene description	2-fold change[a]	Occurrence[b]
CD38	NM_001775	CD38 molecule	Down	17
PTPN13	NM_080685	Protein tyrosine phosphatase, non-receptor type 13 [APO-1/CD95 (Fas)-associated phosphatase]	Up	17
RFTS7H	Z34893	Immunoglobulin gamma chain variable region, rheumatoid factor	Down	16
None	hsa-miR-146a	–	Down	16
CD180	NM_005582	CD180 molecule	Down	16
FCRL5	NM_031281	Fc receptor-like 5	Down	16
GPR160	NM_014373	G protein-coupled receptor 160	Down	16
HERC5	NM_016323	Hect domain and RLD 5	Down	16
IFIT1	NM_001548	Interferon-induced protein with tetratricopeptide repeats 1	Down	16
OAS3	NM_006187	2′-5′-oligoadenylate synthetase 3, 100 kDa	Down	16
PRKCH	NM_006255	Protein kinase C, eta	Up	16
RASGRP3	NM_170672	RAS guanyl releasing protein 3 (calcium and DAG-regulated)	Down	16
TC2N	NM_152332	Tandem C2 domains, nuclear	Down	16
TCL1A	NM_021966	T-cell leukaemia/lymphoma 1A	Down	16
XAF1	NM_017523	XIAP-associated factor-1	Down	16
ZNF382	NM_032825	Zinc finger protein 382	Down	16

[a]Fold change of gene expression in LCL strains at late passage relative to early passage.
[b]Numbers of LCL strains evidencing differential expression of corresponding genes among the 17 tested LCL strains.

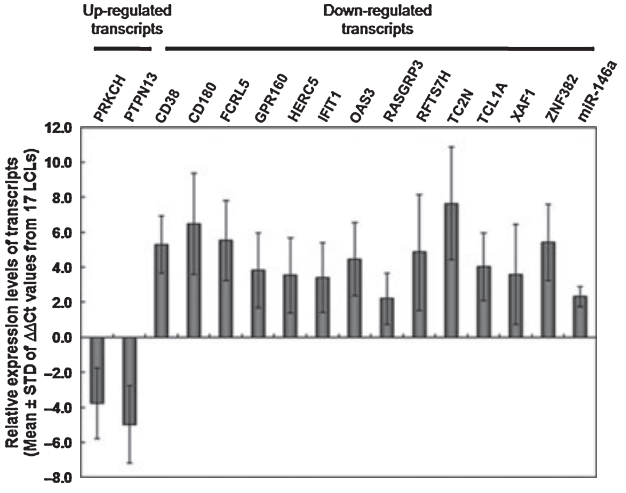

Figure 1. Real-time RT-PCR for 16 differentially expressed genes. Expression levels of 16 transcripts were assessed in 17 LCLs at late passage (p161) and early passage (p4). Results were expressed as mean ± standard deviation (STD) of $\Delta\Delta C_t$ values [$\Delta\Delta C_t$ value = ΔC_{t1}(target gene C_t − control gene C_t)$_{\text{LCL at the late passages}}$ − ΔC_{t2}(target gene C_t − control gene C_t)$_{\text{LCL at the early passages}}$].

Correlation between gene expression phenotype and clinical data

To identify clinical feature-related genes, microarray data from 20 LCLs at early passage were compared to 23 clinical parameters from donors. A gene list showing the highest positive and negative correlations ($P < 0.01$) with each clinical trait is provided in Table 4. For example, postprandial glucose level at 120 min (glucose_120) was the most positively or negatively correlated with apolipoprotein C1 (*APOC1*) or *N*-acetyltransferase 12 (*NAT12*) respectively (Table 4, Fig. S1). With regard to body mass index (BMI), we found that it was positively or negatively correlated to serpin peptidase inhibitor, clade B (ovalbumin), member 12 (*SERPINB12*) or acid phosphatase 2, lysosomal (*ACP2*) respectively (Table 4, Fig. S1). For each clinical trait, expression phenotype (expression level of the transcript) with highest correlation coefficients would provide clues for molecular targets of the corresponding trait.

In the regression analysis, the top three highest coefficients of determination (R^2) were observed in measurements of C-reactive protein, BMI and postprandial insulin levels at 120 min, which were associated with *TMSL8* ($R^2 = 0.798$), *SERPINB12* ($R^2 = 0.722$) and *ERRF11* ($R^2 = 0.713$) respectively. This finding suggests that LCLs could be used in studies of these clinical traits or their related diseases. On the other hand, correlations between gene expression levels and clinical traits in LCLs at early passage (p4) were not represented in LCLs at late passage (p161), supporting the notion that expression phenotypes changed during long-term subculture of LCLs.

Discussion

We employed a microarray approach to demonstrate gene expression changes in LCLs over long-term culture.

Table 4. Expression phenotypes correlated with 23 quantitative clinical features

Clinical data	Gene symbol[a]	Gene Accession No.	Coefficient	R^2	*P*-value
Height	*FGD4*	NM_139241	0.830	0.689	<0.001
	SQMS1	NM_147156	−0.792	0.628	<0.001
Weight	*CCNI2*	NM_006835	0.839	0.703	<0.001
	SAPS2	NM_014678	−0.691	0.478	0.001
Systolic blood pressure	*ZNF180*	NM_013256	0.708	0.501	<0.001
	STAMBPL1	NM_020799	−0.691	0.478	0.001
Diastolic blood pressure	*ST3GAL3*	NM_174963	0.736	0.541	<0.001
	LOC440087	NM_001013698	−0.706	0.499	0.001
Waist	*HTR7*	NM_019859	0.721	0.520	<0.001
	ACP2	NM_001610	−0.698	0.487	0.001
Hip	*STELLAR*	BC062480	0.814	0.662	<0.001
	HSP90B3P	NM_003130	−0.735	0.541	<0.001
Waist-to-Hip ratio	*KLHL10*	NM_152467	0.742	0.551	<0.001
	GYS2	NM_021957	−0.661	0.437	0.002
Body mass index (BMI)	*SERPINB12*	NM_080474	0.850	0.722	<0.001
	ACP2	NM_001610	−0.635	0.403	0.003
Distal radius Z	*NPC1L1*	NM_013389	0.762	0.581	<0.001
	MGC2752	BC001952	−0.803	0.645	<0.001
Total cholesterol	*RHOF*	NM_019034	0.722	0.522	<0.001
	SLC40A1	NM_014585	−0.788	0.621	<0.001
HDL-cholesterol	*NEFM*	NM_005382	0.784	0.615	<0.001
	SLC24A5	NM_205850	−0.748	0.559	<0.001
Triglyceride	*ATF5*	NM_012068	0.797	0.636	<0.001
	SNAPIN	NM_012437	−0.677	0.458	0.001
C-reactive protein	*TMSL8*	NM_021992	0.893	0.798	<0.001
	FIZ1	NM_032836	−0.752	0.566	<0.001
HbA1C	*LAIR1*	NM_002287	0.844	0.712	<0.001
	CPNE1	NM_152930	−0.780	0.608	<0.001
WBC	*TMEM185B*	NR_000034	0.749	0.561	<0.001
	ABCA10	ENST00000243451	−0.755	0.570	<0.001
RBC	*PYCR1*	NM_006907	0.840	0.706	<0.001
	CHRNA6	NM_004198	−0.740	0.548	<0.001
Haemoglobin	*PSAP1*	NM_001085382	0.752	0.565	<0.001
	DLGAP1	NM_004746	−0.768	0.590	<0.001
Haematocrit	*EIF2C2*	NM_012154	0.770	0.593	<0.001
	DLGAP1	NM_004746	−0.716	0.512	<0.001
Insulin_60 min[b]	*UBD*	NM_006398	0.749	0.561	<0.001
	WIZ	NM_021241	−0.634	0.402	0.003
Insulin_120 min[b]	*ERRF11*	NM_018948	0.845	0.713	<0.001
	ATOH8	NM_032827	−0.774	0.599	<0.001
Glucose_0 min[b]	*C21*	BC080530	0.761	0.580	<0.001
	SEC24A	NM_021982	−0.731	0.534	<0.001
Glucose_60 min[b]	*FAHD2B*	NM_199336	0.739	0.545	<0.001
	GAB3	NM_001081573	−0.778	0.605	<0.001
Glucose_120 min[b]	*APOC1*	NM_001645	0.785	0.616	<0.001
	NAT12	NM_001011713	−0.757	0.573	<0.001

[a]Genes exhibiting highest positive and negative correlations with each of the clinical factors presented.
[b]Glucose and insulin levels were measured at time points during oral glucose tolerance test. Insulin at 60 min, postprandial insulin level at 60 min; insulin at 120 min, postprandial insulin level at 120 min; glucose at 0 min, postprandial glucose level at 0 min; glucose at 60 min, postprandial glucose level at 60 min; glucose at 120 min, postprandial glucose level at 120 min.

Results demonstrated that 16 transcripts were differentially expressed, with more than 2-fold changes, being detected in at least 16 of 17 LCL strains at the late passage compared to those at early passage (Table 3). By performing the real-time RT-PCR experiment, we were able to determine that 16 transcripts showed expression patterns similar to the microarray results (Fig. 1, Table S1). All transcripts were expressed differentially in at least 15 of 17 LCL strains, thus suggesting that they may play a critical role in regulation of LCL immortalization. Nuclear

factor-κB (NF-κB) pathway-associated genes (miR-146a, *PTPN13* and *HERC5*) were included among them. It has been reported that miR-146a expression is induced by EBV-encoded LMP1 *via* NF-κB in lymphocytes (10). *PTPN13* (11), *HERC5* (12) and miR-146a expression are associated with the NF-κB pathway. *PTPN13* negatively regulates the NF-κB pathway *via* dephosphorylation of its substrate, IκBα (11), and *HERC5* is overexpressed *via* NF-κB signalling in activated endothelial cells (12). It has been demonstrated that the NF-κB pathway regulates proliferation of LCL (13,14) and transformation of EBV (11). Our data suggest that these functions may be regulated by miR-146a, *PTPN13* and *HERC5.* Furthermore, these 16 transcripts included carcinogenesis-related transcripts such as *XAF1, TCL1A, PTPN13, CD38, CD180* and miR-146a. *XAF1* has been reported as a novel tumour suppressor in colon cancer (15). The *TCL1A* oncogene has been identified as a regulator of T-cell leukaemia and EBV-positive B-cell lymphoma (16,17) and *PTPN13* can regulate cancer cells as a tumour suppressor or as tumour promoter (11). CD38 is a marker for identification of two different profiles in germinal centre B-cell lymphoma (18), and CD180 induces B-cell population growth in mice (19). miR-146a has been identified as a regulator of various cancers, including breast (20), thyroid (21,22), cervical (23) and prostate cancers (20,24). Thus, these six transcripts may possibly be involved in EBV-transformed malignancies including nasopharyngeal carcinoma, Burkitt's lymphoma and Hodgkin's lymphoma. Moreover, *TC2N, FCRL5, CD180, CD38* and miR-146a, which were downregulated in all 17 LCLs at late passage, have potential as markers for LCL immortalization or EBV-infected malignancies.

Subsequently, we determined correlations between expression phenotypes from 20 LCL strains at early passage and 23 quantitative clinical data from their donors (Table 4). In previous reports, molecular phenotype classifying tumour subtypes (25–27) and clinical features (26–28) has been identified by microarray approach. Diagnostic or prognostic biomarker candidates have also been evaluated in a variety of clinical diseases by gene expression profiling (29,30). For example, *villin1* (*VIL1*) has been identified as a diagnostic molecular marker of cervical adenocarcinoma by microarray analysis (30). Anal carcinoma was divided into two different subgroups by gene expression profiling, and upregulation of *MCM7* and *CDKN2A* (p16) was identified as a marker for one of them (27). Microarray approach has also been used to identify obesity-related genes from abdominal subcutaneous adipocytes of obese patients (31). The clinical feature-related genes identified in our study may be candidate targets for study in a variety of diseases or clinical traits. For example, it has been reported that overexpression of APOC1 influences insulin resistance in *ob/ob* mice (32,33). According to our data, expression of this gene correlated positively with postprandial glucose levels at 120 min (Table 4, Fig. S1). These results show that *APOC1* may be a candidate target for diabetes studies. In the case of BMI, we detected positive correlation with expression level of *SERPINB12* (Table 4, Fig. S1). This suggests that *SERPINB12* gene expression may be a diagnostic marker in obesity.

In conclusion, we have shown that immortalization of LCLs may be regulated by 16 transcripts (*PRKCH, PTPN13, CD38, CD180, FCRL5, GPR160, HERC5, IFIT1, OAS3, RASGRP3, RFTS7H, TC2N, TCL1A, XAF1, ZNF382* and miR-146a), *via* the NF-κB pathway. In addition, our study suggests that *XAF1, TCL1A, PTPN13, CD38* and miR-146a may play important roles in development of EBV-infected malignant tissues as well as immortalization of LCLs. Moreover, *TC2N, FCRL5, CD180, CD38* and miR-146a, which were downregulated in all tested LCLs at the late passage, may possibly function as markers for LCL immortalization or EBV-transformed malignancy.

Acknowledgements

This study was supported by intramural grant 2007-N00359-00/2910-212-207 of the Korea National Institute of Health, Korea Centers for Disease Control and Prevention.

References

1 Hu VW, Sarachana T, Kim KS, Nguyen A, Kulkarni S, Steinberg ME *et al.* (2009) Gene expression profiling differentiates autism case-controls and phenotypic variants of autism spectrum disorders: evidence for circadian rhythm dysfunction in severe autism. *Autism Res.* **2**, 78–97.

2 Xie J, Techritz S, Haebel S, Horn A, Neitzel H, Klose J *et al.* (2005) A two-dimensional electrophoretic map of human mitochondrial proteins from immortalized lymphoblastoid cell lines: a prerequisite to study mitochondrial disorders in patients. *Proteomics* **5**, 2981–2999.

3 Shukla SJ, Dolan ME (2005) Use of CEPH and non-CEPH lymphoblast cell lines in pharmacogenetic studies. *Pharmacogenomics* **6**, 303–310.

4 Valle M, Plon SE, Rabin KR (2009) Differential in vitro cytotoxicity does not explain increased host toxicities from chemotherapy in Down syndrome acute lymphoblastic leukemia. *Leuk. Res.* **33**, 336–339.

5 Brennan EP, Ehrich M, Brazil DP, Crean JK, Murphy M, Sadlier DM *et al.* (2009) Comparative analysis of DNA methylation profiles in peripheral blood leukocytes versus lymphoblastoid cell lines. *Epigenetics* **4**, 159–164.

6 Jeon JP, Shim SM, Nam HY, Baik SY, Kim JW, Han BG (2007) Copy number increase of 1p36.33 and mitochondrial henome amplification in Epstein-Barr virus-transformed lymphoblastoid cell lines. *Cancer Genet. Cytogenet.* **173**, 122–130.

7 Jeon JP, Nam HY, Shim SM, Han BG (2009) Sustained viral activity of Epstein-Barr virus contributes to cellular immortalization of lymphoblastoid cell lines. *Mol. Cells* **27**, 143–148.
8 Kim KJ, Lee HJ, Park MH, Cha SH, Kim KS, Kim HT *et al.* (2006) SNP identification, linkage disequilibrium, and haplotype analysis for a 200-kb genomic region in a Korean population. *Genomics* **88**, 535–540.
9 Yoo YK, Ke X, Hong S, Jang HY, Park K, Kim S *et al.* (2006) Fine-scale map of encyclopedia of DNA elements regions in the Korean population. *Genetics* **774**, 491–497.
10 Cameron JE, Yin Q, Fewell C, Lacey M, McBride J, Wang X *et al.* (2008) Epstein-Barr virus latent membrane protein 1 induces cellular microRNA miR-146a, a modulator of lymphocyte signaling pathways. *J. Virol.* **82**, 1946–1958.
11 Abaan OD, Toretsky JA (2008) PTPL1: a large phosphatase with a split personality. *Cancer Metastasis Rev.* **27**, 205–214.
12 Kroismayr R, Baranyi U, Stehlik C, Dorfleutner A, Binder BR, Lipp J (2004) HERC5, a HECT E3 ubiquitin ligase tightly regulated in LPS activated endothelial cells. *J. Cell Sci.* **117**, 4749–4756.
13 Forte E, Luftig MA (2009) MDM2-dependent inhibition of p53 is required for Epstein-Barr virus B-cell growth transformation and infected-cell survival. *J. Virol.* **83**, 2491–2499.
14 Faumont N, Durand-Panteix S, Schlee M, Gromminger S, Schuhmacher M, Holzel M *et al.* (2009) c-Myc and Rel/NF-kappaB are the two master transcriptional systems activated in the latency III program of Epstein-Barr virus-immortalized B cells. *J. Virol.* **83**, 5014–5027.
15 Sun Y, Qiao L, Xia HH, Lin MC, Zou B, Yuan Y *et al.* (2008) Regulation of XAF1 expression in human colon cancer cell by interferon ß: activation by the transcription regulator STAT1. *Cancer Lett.* **260**, 62–71.
16 Pekarsky Y, Hallas C, Croce CM (2001) Molecular basis of mature T-cell leukemia. *J. Am. Med. Assoc.* **286**, 2308–2314.
17 Teitell MA (2005) The TCL1 family of oncoproteins: co-activators of transformation. *Nat. Rev. Cancer* **5**, 640–648.
18 Alizadeh AA, Eisen MB, Davis RE, Ma C, Lossos IS, Rosenwald A *et al.* (2001) Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature* **403**, 503–511.
19 Miyake K, Yamashita Y, Hitoshi Y, Takatsu K, Kimoto M (1994) Murine B cell proliferation and protection from apoptosis with as antibody against a 105-kd molecule: unresponsiveness of X-linked immunodeficient B cells. *J. Exp. Med.* **180**, 1217–1224.
20 Volinia S, Calin GA, Liu CG, Ambs S, Cimmino A, Petrocca F *et al.* (2006) A microRNA expression signature of human solid tumors defines cancer gene targets. *Proc. Natl. Acad. Sci. USA* **103**, 2257–2261.
21 Jazdzewski K, Murray EL, Franssila K, Jarzab B, Schoenberg DR, de la Chapelle A (2008) Common SNP in pre-miR-146a decreases mature miR expression and predisposes to papillary thyroid carcinoma. *Proc. Natl. Acad. Sci. USA* **105**, 7269–7274.
22 He H, Jazdzewski K, Li W, Liyanarachchi S, Nagy R, Volinia S *et al.* (2005) The role of microRNA genes in papillary thyroid carcinoma. *Proc. Natl. Acad. Sci. USA* **102**, 19075–19080.
23 Wang X, Tang S, Le SY, Lu R, Rader JS, Meyers C *et al.* (2008) Aberrant expression of oncogenic and tumor-suppressive microRNAs in cervical cancer is required for cancer cell growth. *PLoS One* **3**, e2557.
24 Lin SL, Chiang A, Chang D, Ying SY (2008) Loss of mir-146a function in hormone-refractory prostate cancer. *RNA* **14**, 417–424.
25 Ishihara A, Tsuda H, Kitagawa K, Yoneda M, Shiraishi T (2009) Morphological characteristics of basal-like subtype of breast carcinoma with special reference to cytopathological features. *Breast Cancer* **16**, 179–185.
26 Tothill RW, Tinker AV, George J, Brown R, Fox SB, Lade S *et al.* (2008) Novel molecular subtypes of serous and endometrioid ovarian cancer linked to clinical outcome. *Clin. Cancer Res.* **14**, 5198–5208.
27 Bruland O, Fluge O, Immervoll H, Balteskard L, Myklebust M, Skarstein A *et al.* (2008) Gene expression reveals two distinct groups of anal carcinomas with clinical implications. *Br. J. Cancer* **98**, 1264–1273.
28 Dellas A, Jundt G, Sartorius G, Schneider M, Moch H (2009) Combined PTEN and p27kip1 protein expression patterns are associated with obesity and prognosis in endometrial carcinomas. *Clin. Cancer Res.* **15**, 2456–2462.
29 Colombo J, Fachel AA, De FreitasCalmon M, Cury PM, Fukuyama EE, Tajara EH *et al.* (2009) Gene expression profiling reveals molecular marker candidates of laryngeal squamous cell carcinoma. *Oncol. Rep.* **21**, 649–643.
30 Nakamura E, Iwakawa M, Furuta R, Ohno T, Satoh T, Nakawatari M *et al.* (2009) Villin1, a novel diagnostic marker for cervical adenocarcinoma. *Cancer Biol. Ther.* **8**, 1146–1153.
31 Dolinkova M, Dostalova I, Lacinova Z, Michalsky D, Haluzikova D, Mraz M *et al.* (2008) The endocrine profile of subcutaneous and visceral adipose tissue of obese patients. *Mol. Cell. Endocrinol.* **291**, 63–70.
32 Muurling M, van den Hoek AM, Mensink RP, Pijl H, Romijn JA, Havekes LM *et al.* (2004) Overexpression of APOC1 in obob mice leads to hepatic steatosis and severe hepatic insulin resistance. *J. Lipid Res.* **45**, 9–16.
33 Jong MC, Voshol PJ, Muurling M, Dahlmans VE, Romijn JA, Pijl H *et al.* (2001) Protection from obesity and insulin resistance in mice overexpressing human apolipoprotein C1. *Diabetes* **50**, 2779–2785.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Expression phenotypes correlated with some clinical traits. The body mass index (BMI) was positively or negatively correlated with expression levels of serpin peptidase inhibitor, clade B (ovalbumin), member 12 (*SERPINB12*) or acid phosphatase 2, lysosomal (*ACP2*), respectively. A postprandial glucose level at 120 min showed the highest positive or negative correlation with apolipoprotein C1 (*APOC1*) or N-acetyltransferase 12 (*NAT12*), respectively.

Table S1. Real-time RT-PCR experiment for 16 differentially expressed genes (DEGs).

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.