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ORIGINAL ARTICLE

Instability at Short Tandem Repeats in Lymphoblastoid Cell Lines

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Abstract
Objectives: Epstein Barr virus (EBV)-transformed lymphoblastoid cell lines (LCLs) are a useful biological resource, however, genomic variations can happen during the generation and immortalization processes of LCLs. The purpose of this study was to identify genomic variations in LCL DNA compared with matched blood DNA using short tandem repeats (STRs) analysis.
Methods: We analyzed 15 STRs with blood DNA and their matched LCL DNA samples from 6645 unrelated healthy individuals.
Results: Mutations (such as repeat variations and triallelic patterns) of 15 STR loci were detected in 612 LCL DNAs (9.2% of total) without mutations in their matched blood DNA. The repeat variations of 15 STRs were detected in 526 LCL DNAs (mutation rate = 0.0792) and triallelic patterns were identified in 123 (mutation rate = 0.0185). Among 15 STRs, the most common repeat variations (n = 214, mutation rate = 0.0322) and triallelic patterns (n = 17, mutation rate = 0.0026) were found at FGA locus.
Conclusion: Our study shows that mutations in STRs can occur during generation and immortalization of LCLs.

1. Introduction

Epstein–Barr virus (EBV)-transformed lymphoblastoid cell lines (LCLs) are a biological resource that is widely used in various research fields such as human genetics, immunology, pharmacogenomics, and toxicogenomics. Screening for toxicological effects of environmental toxicants and drugs using animal models or humans has concerns including high cost burden, time-consuming tasks, bioethics, and safety [1]. For this reason, many animal cell models have been widely used for pharmacological and toxicological research. LCLs are a model system that can assess various toxicants and drug-induced toxicity, as well as study the genetics of

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response to these. LCLs also have the advantage of being able to provide an unlimited DNA or RNA source for identification of disease-associated genetic factors [2]. For example, LCL samples from Parkinson's disease patients were used to identify mutation of parkin (PRKN) [3] and DJ-1 [4] genes. With the development of next-generation sequencing technology, whole genome and exome sequencing has been carried out using a large number of LCL samples [5].

LCLs are generated by a transformation process by which human B lymphocytes are infected with EBV. Thus, genomic alterations can occur in LCLs during their generation and immortalization processes, but little is known about the genomic instability in LCLs. Previous studies showed genomic variations in LCLs compared to their matched blood samples through genome-wide single nucleotide polymorphism (SNP) analysis [6,7]. Copy number variation was observed in LCLs [8]. Genomic alterations in LCLs are minimal, however, these can influence the results of genome-wide association studies [6].

In this study, we identified genomic alterations in LCLs compared with their matched blood samples, using short tandem repeats (STRs) analysis.

2. Materials and Methods

2.1. Population

The National Biobank of Korea has collected a large number of DNA samples and performed STR analysis for quality control of DNA samples. For this study, we used STR data of blood DNA and matched LCL DNA from 6645 unrelated healthy individuals of the Ansan and Ansung cohort in Korea.

2.2. DNA extraction

Genomic DNA was isolated with blood and LCL samples from 6645 individuals using Gentra Puregene Blood kit (Qiagen, Chatsworth, CA, USA) in accordance with the manufacturer's instructions.

2.3. STR analysis

Multiplex polymerase chain reaction for 15 STR loci (CSF1P0, D2S1338, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D19S433, D21S11, FGA, TH01, TPOX, and vWA) and amelogenin, the gender marker, was performed with genomic DNA using the AmpF*l*STR Identifiler (Applied Biosystems, Foster City, CA, USA) commercial kit, following the user's manual. PCR amplicons were separated and genotyped using the ABI PRISM 3730 DNA Analyzer (Applied Biosystems) and all allele fragment sizes were determined using GeneMapper ID 3.2 software.

3. Results

We analyzed 15 STRs (CSF1P0, D2S1338, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D19S433, D21S11, FGA, TH01, TPOX, and vWA) in blood DNA and matched LCL DNA from 6645 individuals. Through comparative analysis of STR data in blood and LCL DNA, we identified mutations of 15 STR loci in 612 LCL DNAs (9.2% of total) without mutations in their matched blood DNA. These mutations included repeat variations (such as single- or multi-repeat changes and repeat gains or losses) and triallelic patterns of STRs. The repeat

Table 1. Mutation data of STRs analyzed from blood and LCL DNA samples of 6645 individuals

	Repeat variations at STRs		Triallelic patterns at STRs	
STR markers	Number of LCLs with mutations	Mutation rate	Number of LCLs with mutations	Mutation rate
D8S1179	165	0.0248	14	0.0021
D21S11	159	0.0239	12	0.0018
D7S820	186	0.0280	6	0.0009
CSF1PO	163	0.0245	10	0.0015
D3S1358	147	0.0221	2	0.0003
TH01	150	0.0226	1	0.0002
D13S317	182	0.0274	3	0.0005
D16S539	174	0.0262	4	0.0006
D2S1338	182	0.0274	6	0.0009
D5S818	161	0.0242	8	0.0012
FGA	214	0.0322	17	0.0026
D19S433	196	0.0295	8	0.0012
vWA	170	0.0256	16	0.0024
TPOX	124	0.0187	5	0.0008
D18S51	194	0.0292	17	0.0026
Total	526	0.0792	123	0.0185

LCL = lymphoblastoid cell line; STR = single tandem repeat.

variations were observed in 526 LCL DNAs (mutation rate = 0.0792) and triallelic patterns were identified in 123 (mutation rate = 0.0185) (Table 1). Thirty-seven of all LCL DNAs with triallelic patterns also showed the repeat variations. STR data of 86 LCL DNAs showed triallelic patterns without repeat variations. Among 15 STRs, the most common repeat variations (n = 214, mutation rate = 0.0322) and triallelic patterns (n = 17, mutation rate = 0.0026) were found at FGA locus.

4. Discussion

Instability of genomic DNA can occur in LCLs through EBV transformation during generation and immortalization. Genomic alterations in LCLs have been confirmed using various genome analysis tools such as SNP genotyping and copy number variation analysis [6−8]. Chromosomal abnormalities were identified in 30 of 268 LCLs from the HapMap project using karyotyping and SNP genotyping [8]. Chromosomes 9, 12, and X displayed a tendency toward trisomy, in particular. Furthermore, somatic deletions were detected in LCLs from father/mother−child pairs when SNP genotyping was performed after removing these abnormal chromosomes.

In this study, we identified mutations (including single- or multi-repeat changes, repeat gains or losses, and triallelic patterns) of 15 STR loci in 612 of 6645 LCL DNAs (9.2%) via comparative analysis with STR data of blood DNA and matched LCL DNA from 6645 unrelated healthy individuals. In previous, somatic mutations were investigated with DNA samples from 1,730−1,764 father-son pairs which confirmed in paternity by various DNA markers via Y-STRs analysis [9]. The 84 mutations were identified in all 29,792 17 Y-STRs data of father−son pairs (mutation rate = 0.0028). Triallelic patterns of STRs were detected at D21S11 and FGA loci of patients with Down syndrome [10] and oral cancer [11], respectively. This showed that mutations of STRs can happen in cell transformation processes, although the mechanism is unknown. In conclusion, our data showed that mutations of STRs can occur during generation and immortalization of LCLs.

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