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Short communication

Genetic instability in EBV-transformed lymphoblastoid cell lines

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Abstract

Epstein Barr virus (EBV)-transformed lymphoblastoid cell lines are commonly used to provide an inexhaustible supply of DNA. We examined microsatellite instability in these cell lines in 35 individuals where DNA was available from the original blood samples and from cultured cell lines. Mutations were observed in 0.3% of the analyses, thus providing a quantitative measure of somatic mutation rate.

Keywords: EBV transformation; Lymphoblastoid cell line; Microsatellite; Population genetic; STR

Epstein Barr virus (EBV)-transformed lymphoblastoid cell lines are commonly used to provide an inexhaustible supply of DNA for phylogenetic and evolutionary studies [1]. It is, therefore, important to know to what extent the genetic patterns observed in these cell lines, some of which were established many years ago, and have been cultured for an unknown number of generations, represent the DNA from the donor's blood. Several studies have examined the stability of simple tandem repeats (STRs), or microsatellites, in father/son pairs and CEPH families [2–4]. The majority of the mutational changes consist of insertions or deletions of one or a few repeat units through replication slippage which plays an important role in microsatellite evolution and is thought to produce most of the observed allelic variation [5]. We measured the stability of STRs in EBV-transformed lymphoblastoid cell lines by examining 20 STRs on the non-recombining part of the Y chromosome in 35 Pakistani individuals, where DNA was available from the original blood samples and from cultured cell lines after 30 or 90 generations: 700 analyses in all.

Informed consent was obtained from all human subjects. Five novel Y-STRs (GenBank accession numbers BV005730;BV005731;BV005732; BV005733 and BV005734) and 15 loci, widely used in forensics and population genetics [6], were analyzed. Each Y-STR was amplified in a singleplex PCR reaction and 1% DMSO was used in the PCR master mix so as to eliminate multiple band artifacts [7]. The products were electrophoresed on an ABI Prism 377 DNA sequencer following manufacturer's instructions. Samples were loaded on alternate lanes to prevent spillover from adjacent wells. The number of repeat units, or alleles, was established by comparison with sequenced reference DNA samples [6].

Mutations, considered as a new peak with a height of ≥ 100 fluorescence units, represented 30% of the total and were observed in two cell lines. In both, mosaicism was present and the peak height of the original allele (observed in the blood sample) was higher than that of the new allele (Fig. 1). The results were highly reproducible and were seen in both individuals in 10 replications. One mutation at the DYS650 tetra-nucleotide repeat locus was a loss of two repeat units, and was observed in a cell line (MHN 061) after 30 generations, and remained similar in relative height after 90 generations. The second mutation, a gain of one repeat unit at the DYS652 penta-nucleotide repeat locus, was identified in another cell line (MHN 049) after 90 generations. The doubling time was estimated to be 25.4 ± 3.1 h (mean $\pm$ S.D.). The modal allele

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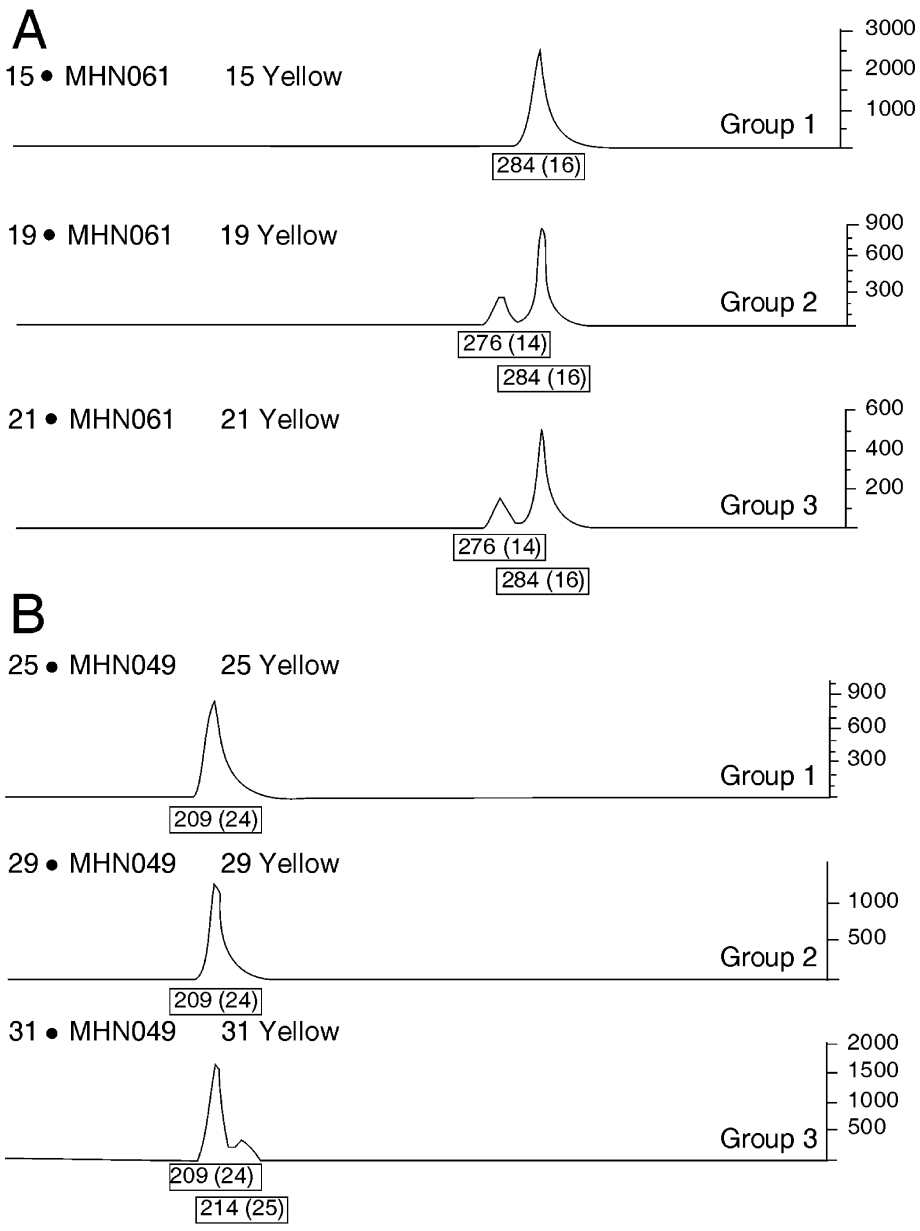

Fig 1. Y-STR mutations observed in EBV-transformed lymphoblastoid cell lines. (A) DYS650. (B) DYS652. DNA was prepared from blood samples (group 1) or cultured cell lines after 30 (group 2) or 90 generations (group 3). The fragment size in bp is given below each peak and the number of repeat units is shown in parentheses.

($\pm$ variance) was 17 ± 0.93 for DYS650 and 24 ± 1.5 for DYS652 in the population examined. The mutation rate relevant here is the proportion of analyses where a change is detectable: 2 out of 700, or about 0.3%. These results thus demonstrate significant genetic instability in long-term cultures of EBV-transformed lymphoblastoid cell lines. The mutation rates reported in this study are much higher than those reported earlier for spontaneous mutations resulting in electrophoretic variants in human lymphoblastoid cell lines [8]. Indeed, they are similar to the meiotic mutation rates reported for Y specific tetra- and pentanucleotide repeats [2,9,10] and autosomal microsatellites [4]. Although the original bands can still be identified in both cases, and scoring the highest peak would produce the correct genotype, we suggest that an error of 0.3% mistyping due to somatic mutation should conservatively be assumed when DNA from lymphoblastoid cell lines is used.

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