Indian J Med Res 135, June 2012, pp 803-805

Commentary

Novel lymphoblastoid cell lines from primary neoplasms of the upper aero-digestive tract

A cell line is a permanently established cell culture that will proliferate indefinitely when provided with appropriate nutrients and space. For decades, immortal human cancer cell lines have served as easily accessible, *in vitro* biological models that were used to unveil novel signalling pathways in cancer and for investigating the efficacy of various anti-cancer agents. A large bank of well characterized cell lines is required to reflect the diversity of tumour phenotypes and provide adequate models for studying tumour heterogeneity.

The quest for establishing the cell lines began in 1951 at John Hopkins University, USA, when Gey *et al*[1] established the first continuously growing human cell line (HeLa) from carcinoma of the human uterine cervix. The HeLa cell line and most other human cell lines that were subsequently established from various solid tumours adhered to the culture vessel and grew as monolayers. In 1963, Pulvertaft[2] at the University of Ibadan, Nigeria, established the first continuous human hematopoietic cell lines derived from Burkitt's lymphoma patients that grew in suspension cultures in the nutrient medium.

Electron microscopic examination of these and subsequent Burkitt's lymphoma derived cell lines led to the identification of herpes-type virus particles, that were later designated as Epstein-Barr virus (EBV)[3]. The first leukaemia-derived cell line RPMI 6410, established from a patient with acute myeloid leukaemia also contained similar herpes-type virus particles in the cells, however, it was later shown that the cell line was derived by the spontaneous immortalization of normal bystander B-cells by EBV infection and not from the leukaemia cells[4].

Hundreds of lymphoblastoid cell lines (LCLs) had been derived from the peripheral blood of patients with leukaemias, lymphomas, other malignant tumours and even from many healthy individuals, however, EBV was detected in every cell line irrespective of the blood donor's health status. Thereafter, LCLs have been generated by transformation of the B-lymphocyte component of the peripheral blood lymphocyte population by EBV. This method has been used successfully for over two decades. The ease of maintenance and a somatic mutation rate of 0.3 per cent make lymphoblastoid cells the preferred choice of storage of patient's genetic material. The effect of LCLs generation process on genome level was first examined by Simon-Sanchez *et al*[5] where they had analysed 408,804 SNPs in 276 DNA samples extracted from EBV immortalized LCLs. It was observed that 9.5 per cent of DNA samples displayed extended homozygosity and 340 structural alterations were observed in 66.9 per cent samples. When similar analyses were performed on DNA extracted from blood of 30 subjects, they observed all instances of extended homozygosity, 75 per cent of structural genomic alterations <5 Mb in size while 13 per cent were >5 Mb in size. It is, therefore, concluded that structural genomic variations are common in the general population. Although a proportion of this variability might be caused or its relative abundance altered by LCLs creation process, this could have a minor effect on genotype frequencies[5].

RNAs and proteomes from LCLs have been used for detecting splice mutations and for performing differential proteome analyses in various studies. LCLs are also suitable for molecular and functional analyses, as the gene expression in LCLs encompasses a wide range of metabolic pathways that are specific to individuals from where the cells have originated[6]. Recently, Londin *et al*[7] had performed whole exome sequencing on a tetrad family using DNA derived from peripheral blood mononuclear cells and LCLs from

each individual. Ninety nine per cent concordance was obtained between DNA sequences derived from the samples obtained from two sources. Sanger's sequencing on the subset of discordant variants suggested that EBV transformation of LCLs could result in generation of *de novo* mutations. Though this study revealed 99 per cent concordance between paired samples, the approach only focused on the exome, therefore, essentially examining only 1.22 per cent of genome, while additional mutations may still exist outside these regions. The study also undermined the effects of additional cell passaging may have on the genome, as the cells used were in pre-immortal state. However, after repeated population doublings (typically 160), LCLs reach a proliferative crisis in which pre-immortal cells die and post-immortal cells survive. These cells are often aneuploid and are able to differentiate indefinitely. Additional changes, such as DNA copy number changes and loss-of-heterozygosity may be present in LCL-derived DNA; neither of which have been examined and, therefore, the early passage EBV-transformed cells for genome wide association studies are recommended[7]. All these changes require that DNA derived from LCLs and peripheral blood mononuclear cells (PBMCs) from the same individual be extensively examined to ascertain the fidelity of the genome represented in LCLs[7,8].

Besides genetic alterations, an array of phenotypic features had been observed in LCLs, such as variation in shape, growth in clumps in stationary cultures, expression of normal characteristics of B-lymphocytes and most particularly the synthesis of immunoglobulin (Ig) which was the unique feature by which the B-cell origin of these lymphoblastoid cell lines was ascertained. On the contrary, a number of unusual LCLs have been obtained with markedly different growth characteristics and cell morphology than those displayed by the common LCLs of the B-cell type. These unusual cell lines also contained the EBV genome, but immunoglobulin synthesis was not demonstrable in any of these[9].

In this issue, Hussain *et al*[10] have derived and characterized novel LCLs from patients with multiple primary neoplasms of upper aero-digestive tract. They observed that the cells displayed a rosette morphology while growing in clusters. Cellular characterization by immunophenotyping was performed by assessing the expression of typical B-cell marker CD 19, absence of expression of the T-cell marker CD 3 and NK-cells marker CD 56. The established LCLs were studied for contribution of DNA damage repair *in vitro* in patients with primary neoplams of upper aero-digestive tract and to elucidate the mechanism involved. Hence, it was necessary to ensure that the process of EBV transformation did not affect expression and activity of DNA repair gene *ATM*. No difference in the expression or protein activity of ATM protein was observed in LCLs in comparison to peripheral B-lymphocytes from the same individual, therefore, the cells in cell lines ascertained were maintaining a similarity with the parent lymphocyte population. This characteristic makes LCLs useful for genotypic and phenotypic assays.

LCLs have a number of advantages, as they are derived from the tissue material that can be easily obtained from patients. EBV transformed lines exhibit chromosomal stability up to high passages and high resolution chromosome preparations can be easily performed by cell synchronization in contrast to fibroblasts. LCLs can be grown in suspension and can be cultivated in high cell density without an expense on labour and costs. Repeated DNA preparations can be obtained without much effort thereby making LCLs the ideal source for molecular studies in humans. The creation of LCLs from normal individuals and patients have a large potential use in biomedical research projects that require large amounts of biological material. Genome wide association studies[11,12], functional genomics[13], proteomics[14] and pharmacogenomics[15,16] would particularly benefit from such cell lines resources. These cell lines can also be used in cell hybridization experiments giving rise to inter- and intra-specific somatic cell hybrids[17]. The wide importance of LCLs is now being recognized and with appropriate infrastructure, LCLs will be an important resource for genetic and functional research on various diseases.

Sanjay Katiyar*, Sneha Balani & Virendra Singh
Department of Zoology
University of Delhi
Chaatra Marg, Delhi 110 007, India
**For correspondence*:
skatiyar@zoology.du.ac.in

References

1. Gey GO, Coffman WD, Kubicek MT. Tissue culture studies of the proliferative capacity of cervical carcinoma and normal epithelium. *Cancer Res* 1952; *12* : 264-5.

2. Pulvertaft JV. Cytology of Burkitt's tumour (African lymphoma). *Lancet* 1964; *1* : 238-40.
3. Epstein MA, Barr YM. Characteristics and mode of growth of tissue culture strain (EB1) of human lymphoblasts from Burkitt's lymphoma. *J Natl Cancer Inst* 1965; *34* : 231-40.
4. Iwakata S, Grace JT Jr. Cultivation *in vitro* of myeloblasts from human leukemia. *NY State J Med* 1964; *64* : 2279-82.
5. Simon-Sanchez J, Scholz S, Fung HC, Matarin M, Hernandez D, Gibbs JR, *et al*. Genome-wide SNP assay reveals structural genomic variation, extended homozygosity and cellline induced alterations in normal individuals. *Hum Mol Genet* 2007; *16* : 1-14.
6. Sie L, Loong S, Tan EK. Utility of lymphoblastoid cell lines. *J Neurosci Res* 2009; *87* : 1953-9.
7. Londin ER, Keller MA, D'Andrea MR, Delgrosso K, Ertel A, Surrey S, *et al*. Whole-exome sequencing of DNA from peripheral blood mononuclear cells (PBMC) and EBV-transformed lymphocytes from the same donor. *BMC Genomics* 2011; *12* : 464.
8. Jeon JP, Shim SM, Nam HY, Baik SY, Kim JW, Han BG. Copy number increase of 1p36.33 and mitochondrial genome amplification in Epstein-Barr virus-transformed lymphoblastoid cell lines. *Cancer Genet Cytogenet* 2007; *173* : 122-30.
9. Fu SM, Hurley JN. Human cell lines containing Epstein-Barr virus but distinct from the common B cell lymphoblastoid lines. *Proc Natl Acad Sci USA* 1979; *76* : 6637-40.
10. Hussain T, Kotnis A, Sarin R, Mulherkar R. Establishment & characterization of lymphoblastoid cell lines from patients with multiple primary neoplasms in the upper aero-digestive tract & healthy individuals. *Indian J Med Res* 2012; *135* : 820-9.
11. Latourelle JC, Pankratz N, Dumitriu A, Wilk JB, Goldwurm S, Pezzoli G, *et al.* Genomewide association study for onset age in Parkinson disease. *BMC Med Genet* 2009; *10* : 98.
12. Simon-Sanchez J, Schulte C, Bras JM, Sharma M, Gibbs JR, Berg D, *et al.* Genome-wide association study reveals genetic risk underlying Parkinson's disease. *Nat Genet* 2009; *41* : 1308-12.
13. Ding J, Gudjonsson JE, Liang L, Stuart PE, Li Y, Chen W, *et al.* Gene expression in skin and lymphoblastoid cells: Refined statistical method reveals extensive overlap in cis-eQTL signals. *Am J Hum Genet* 2010; *87* : 779-89.
14. Toda T, Sugimoto M. Proteome analysis of Epstein-Barr virus-transformed B-lymphoblasts and the proteome database. *J Chromatogr B Analyt Technol Biomed Life Sci* 2003; *787* : 197-206.
15. Pratt VM, Zehnbauer B, Wilson JA, Baak R, Babic N, Bettinotti M, *et al.* Characterization of 107 genomic DNA reference materials for CYP2D6, CYP2C19, CYP2C9, VKORC1, and UGT1A1: a GeT-RM and Association for Molecular Pathology collaborative project. *J Mol Diagn* 2010; *12* : 835-46.
16. Stark AL, Zhang W, Zhou T, O'Donnell PH, Beiswanger CM, Huang RS, *et al.* Population differences in the rate of proliferation of international HapMap cell lines. *Am J Hum Genet* 2010; *87* : 829-33.
17. Neitzel H. A routine method for the establishment of permanent growing lymphoblastoid cell lines. *Hum Genet* 1986; *73* : 320-6.