

Epstein-Barr virus-transformation of B-cell lines in ovarian cancer patients: feasibility of genomic storage for unlimited use

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Objective: The aim of the current study is to test whether immortalized B-lymphocyte cell line via Epstein-Barr virus (EBV) transformation is feasible and can be an unlimited source of genome wide study.

Methods: We obtained peripheral whole blood from 5 ovarian cancer patients and immortalized the B-cell lines using EBV transformation. The success rate was analyzed and the bio-identity of the genome was performed using human leukocyte antigen (HLA) identity test.

Results: EBV transformation was successful in all 5 cases (95% confidence interval, 46.3% to 100%). After cryopreservation of EBV-transformed B-cell lines and subsequent thawing, we observed that all cell lines were viable and proliferative. To check bio-identity, HLA-A, B, and DR were tested between the genome of the original samples and the transformed samples. The HLA typing revealed that all observed HLA-A, B, and DR type was identical in 5 cases before and after EBV-transformation.

Conclusion: The current results suggest that EBV-transformation of peripheral blood is an efficient tool in genome banking. The EBV-transformed B-cell lines may be a valuable resource of genome in multi-center translational research by the Korean Gynecologic Oncology Group.

Key Words: Genomics, Epidemiology, DNA storage, EBV transformation, Cryopreservation

INTRODUCTION

The rapid improvement of genomics research has unveiled the secrets of the human genome, which enabled us to get access the cause of disease more efficiently.¹⁻⁴ The progress along with the development of high-throughput analytic techniques forced many researchers to perform more large-scale genomic association studies.⁵⁻⁸ Consequently, there has been an increasing need of human genome data, especially from the individuals with diseases of interest. In cancer research, many genomic association studies revealed the association between the human genome and cancer susceptibility, drug response, and novel targets of anti-cancer strategy.^{3-5,7,8} Because more genomic data sample usually means more probability of obtain-

ing meaningful results, researchers have made an effort to insure large genomic dataset with greater clinical information. Especially, the genomic samples obtained from the prospective clinical trial data is regarded as the best resource of many association tests and many clinical trial groups have developed their own genomic banks to promote translational research within the groups.

There are several types of specimens for DNA banking for genomic association studies.⁹ First, blood spots or buccal cells can be used because the sample collection is easy. However, the major caveat of the specimen types is low DNA yield. Another specimen type is whole blood or buffy coat. Although they have merits of low-cost storage and relatively high DNA yield, it is obvious that the genomic DNA from whole blood or buffy coat is limited. To overcome this limitation, the Epstein-Barr virus (EBV) transformation has been developed. Although it has a disadvantage of high cost, it can provide unlimited, renewable source of genomic DNA. Here we tested the feasibility and the bio-identity of EBV-transformed B-cell lines from patients with epithelial ovarian cancer.

MATERIALS AND METHODS

1. Blood sample preparation and separation of lymphocytes

From 5 ovarian cancer patients, 5 ml of whole blood samples

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Table 1. Comparison of human leukocyte antigen (HLA)-A, B, DR typing between pre- and post-transformed cells

Sample	HLA-A	HLA-B	HLA-DR
01 (pre)	HLA-A*01 & 02	HLA-B*37 & 51	HLA-DRB1*04 & 10; DRB4*
01 (post)	HLA-A*01 & 02	HLA-B*37 & 51	HLA-DRB1*04 & 10; DRB4*
02 (pre)	HLA-A*11	HLA-B*07 & 15	HLA-DRB1*01 & 04; DRB4*
02 (post)	HLA-A*11	HLA-B*07 & 15	HLA-DRB1*01 & 04; DRB4*
03 (pre)	HLA-A*11	HLA-B*35 & 54	HLA-DRB1*12 & 15; DRB3*; DRB5*
03 (post)	HLA-A*11	HLA-B*35 & 54	HLA-DRB1*12 & 15; DRB3*; DRB5*
04 (pre)	HLA-A*11 & 24	HLA-B*51	HLA-DRB1*09 & 11; DRB3*; DRB4*
04 (post)	HLA-A*11 & 24	HLA-B*51	HLA-DRB1*09 & 11; DRB3*; DRB4*
05 (pre)	HLA-A*24 & 33	HLA-B*40 & 59	HLA-DRB1*13 & 14; DRB3*
05 (post)	HLA-A*24 & 33	HLA-B*40 & 59	HLA-DRB1*13 & 14; DRB3*

were obtained using ACD tubes. The samples were maintained at room temperature. After mounting on histopaque, whole blood samples were centrifuged at 3,000 rpm for 15 minutes. The buffy coat was pipetted into the PBS tube for washing. After removal of the supernatant, it was centrifuged again at 1,500 rpm for 5 minutes.

2. Culture of EBV

After thawing of cryo-preserved B95-8 cell line in 37°C water bath, 1 ml of thawed cell line was mixed with 10% growth media. The mixture was centrifuged at 1,500 rpm for 5 minutes and the supernatant was removed. Then the cell pellet was suspended in media and cultured in T-flask for 2-3 days. The supernatant and the Trypsin-EDTA treated cells were centrifuged at 1,500 rpm for 5 minutes. After discarding the supernatant, cells were counted and 10^8 cells/ml were cultured. After 3 days, growth media was collected and the supernatant was filtered with a 0.45 μ m syringe filter. Finally, the filtered cells were stored at -20°C.

3. Primary culture and EBV transformation of lymphocyte

The separated lymphocytes were mixed with 0.4% trypan blue dye solution and counted to provide 10^8 cells/ml. Then the prepared EBV was added. After 24 hours incubation in a CO₂ incubator, 0.5 μ g/ml cyclosporine A was added and cultured for 3 weeks. After 1 week, cell line formation was confirmed. After 2 weeks, adequate transformation was confirmed by phase microscopy. Finally, transformed cells were retrieved and centrifuged at 1,500 rpm for 5 minutes. After removal of the supernatant, 10 ml of PBS was added and cells were counted. After centrifugation at 1,500 rpm for 5 minutes, the cell pellet without supernatant was mixed into 40% freezing media (40% FBS, 10% DMSO). Then the transformed cells were cryo-preserved at -190°C.

4. HLA typing

To compare the identity of the genome, we selected the HLA-typing as an indicator.^{10,11} Using Dynal RELI SSO DNA typing kit (Dynal Biotech, Warral, UK), we compared HLA-A, B, and DR types between the pre- and post-transformed

B-lymphocyte. The typing process was performed according to the manual provided by supplier.

RESULTS

All samples were negative for HBs Ag and antibodies to HIV and HCV. Overall, successful B-cell transformation was achieved in 5 out of 5 samples (100%; 95% CI, 46.3 to 100). All the successfully transformed samples were transformed within a week from the collection. All samples processed were eventually cryo-preserved.

After thawing, all transformed cells were assessed as to their potential to EBV transformation. All samples exhibited clear features of EBV cell line transformation and expansion. The number of transformed cells was sufficient to maintain and expand the culture in all 5 samples. No samples were identified as having bacterial or fungal contamination after 3 days.

A Bio-identity check was done via the HLA phenotype test. The HLA phenotypes of the 5 original samples and the transformed samples were all unique and correctly matched (Table 1). The PCR-SSCP results confirmed HLA phenotype concordance in all samples when tested before EBV transformation and after each expansion of the culture.

DISCUSSION

The current data showed that the EBV-transformation of B-cell line may be used for banking genomic DNA. Our data showed that the immortalized B-cell line can be cryo-preserved and the genomic information may be identical before and after expansion of cryo-preserved cells.

Especially in the situation of multi-institutional cooperative genomic banking, the most intriguing problem is to enhance the available supply of DNA because there may be a conflict between investigators in use of stored DNA. The EBV transformed cells made it possible to harvest virtually unlimited quantities of DNA from the study subjects. According to a review,¹² it is not the only benefit of EBV transformation. Further, it enables detailed biochemical and molecular studies in living cells. In addition, another advantage is for

high-risk groups of patients from whom additional blood samples would be difficult or impossible to obtain, including those at high risk of dying.

However, the EBV transformation of B-cells also has limitations. First, it is expensive and requires cooperation of a skilled laboratory. Therefore, in large-scale genomic-epidemiologic studies such as the PLCO trial,¹³ it is often impossible or impractical to use standard separation techniques for long-term storage of viable cells. However, in a study of biochemical characteristics or genomic/pharmacogenomic data from cancer individuals, the required number of archived samples would be much smaller and the technique can be useful. However, when a large number of stored samples is expected, other techniques such as cryopreservation of whole blood or whole genome amplification would be suitable.

The reported rate of successful EBV transformation is varied among past studies. While the study of Penno et al.¹⁴ reported an 83% success rate, after a decade, Hayes et al.¹³ reported 92.5% success rate in the PLCO study samples. In the current study, we reported a 100% success rate. Because of the limited sample numbers, the confidence interval was wide and it is difficult to conclude that the actual success rate is 100%. However, we speculated that the improved techniques and instruments may be the reason of enhanced rate of successful EBV transformation.

The current study has several limitations. As we mentioned earlier, the small number of transformed samples made it difficult to estimate the actual success rate. Therefore, the current study should be regarded as a preliminary data to initiate the sample collection using EBV transformation. Another limitation is that we did not check the identity of whole genomic data. Because it is almost impossible to compare whole genomic sequence of original samples to the samples after EBV transformation, many researchers use HLA sequence as a unique surrogate indicator of identity of genomic data. However, the new technique such as SNP array may be able to test genomic identity more precisely.

In summary, here we report the preliminary data of EBV-transformed lymphocyte from patients with ovarian cancer. We believe this technique is the most useful because it can prevent the possible conflict among investigators that originates from limited specimens. The technique is feasible and has high success rate. Although it requires high cost and a skilled laboratory, we believe that it would not be practical and may be cost-efficient. Moreover, we can establish the

strategy of cryopreservation of whole blood and selective EBV transformation. However, the accurate success rate and the data of bio-identity should be estimated in future studies with greater number of cases of EBV-transformation. The EBV-transformed B-cell lines may be a valuable resource of genome in multi-center translational research by Korean Gynecologic Oncology Group.

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