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The DG75 B-cell lymphoma line exhibits biclonal immunoglobulin gene rearrangement

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Abstract. Immunoglobulin (Ig) and T-cell receptor (TCR) gene rearrangement (GR) studies have been successfully employed to investigate the clonality and cell lineage of various lymphoid malignancies. Several lymphoma cell lines, such as BJAB, RAJI, DG75 and Jurkat cell lines, were often used as the positive controls in GR detection assays. Of those, the DG75 B-cell lymphoma line was found to exhibit biclonality [two or more homoduplex and heteroduplex bands in a polymerase chain reaction (PCR) product of clonality assay] in the PCR of GR detection assays. To further explore these characteristics of the biclonal phenomenon, the PCR products were purified and cloned into a pEGM-T clone vector. The sequences were analyzed using DNA analysis software. The results demonstrated that the two bands originated from two forms of GR of DG75 cell lines, i.e., DG75 is a biclonal cell line in Ig GRs, which has not been reported before.

Introduction

Immunoglobulin (Ig) and T-cell receptor (TCR) gene rearrangement (GR) studies have been successfully employed to investigate the clonality and cell lineage of various lymphoid malignancies (1). By being simple, highly efficient, cost-effective with wide fitness, polymerase chain reaction (PCR)-based techniques have been extensively used in the detection of GRs.

According to the numbers of PCR primer pairs, PCR-based methods of detecting B-cell clonality may be divided into two groups: the one using one or two pairs of primers in the PCR amplification, potentially termed the 'classical method' (2,3) and the other using multiple primers in one PCR tube, designated as the 'BIOMED-2 method' (4,5). However, oligoclonality (more than two homoduplex and heteroduplex bands in a PCR product) sometimes appears in the routine detection using either the classical or the BIOMED-2 methods. In previous studies (6), we found that the DG75 cell line, used as one of the positive control cells in GR detection, exhibited biclonality in the assay. To further explore their characteristics, we purified, cloned and sequenced these bands, and analyzed them using DNA software.

Materials and methods

Cell lines and DNA extraction. DG75, BJAB, RAJI, L428, and Jurkat cell lines (kindly provided by Dr Ren Song, Department of Molecular and Medical Pharmacology, the David Geffen School of Medicine at the University of California, LA, USA) were employed in our study. The cell lines were maintained in a humidified 37˚C atmosphere containing 5% CO_2. Of these, DG75 cell lines were established from the pleural effusion of a 10-year-old boy with Burkitt's lymphoma in 1975 (7). The Germany DSMZ cell bank sequence number of the DG75 cell line is ACC83. Cell DNA was extracted using the DNAzol reagent, according to the manufacturer's protocol.

PCR conditions and DNA sequencing. In the classical method of immunoglobulin heavy (IgH) GR assay, two pairs of FR3 region primers were used, as previously reported (6). In the BIOMED-2 assay method, however, Tube C primers were used (4) (Table I). The PCR program was performed using rTaq (Takara, Dalian, China), beginning with initial denaturation at 94˚C for 5 min, followed by 35 cycles (94˚C for 30 sec, 55˚C for 40 sec and 72˚C for 60 sec) of amplification, with a final extension at 72˚C for 10 min. Products were visualized and photographed with GeneSnap (Gene Co, Chicago, IL, USA) after electrophoresis in 2.0% agarose. PCR products were purified, cloned into the pGEM-T vector, and sequenced at the Invitrogen Biotech Co., Ltd. (Shanghai, China). DNA sequences were analyzed using the ClustalW 2.1 multiple sequence alignment software, accessed at http://www.ebi.ac.uk/Tools/msa/clustalw2/.

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Table I. Primers used in the detection of immunoglobulin heavy gene rearrangement.

Regions	Primer names	Primers (5'-3')
FR3	FR3 (up)	ACGGC (C/T) GTGTATTACTGTGC
Classical method	FR33 (up)	CTGTCGACACGGCCGTGTATTACTG
	JH1 (down)	ACCTGAGGAGAC(G/A)GTGACC
FR3	VH1-FR3	TGGAGCTGAGCAGCCTGAGATCTGA
BIOMED-2 method	VH2-FR3	CAATGACCAACATGGACCCTGTGGA
	VH3-FR3	TCTGCAAATGAACAGCCTGAGAGCC
	VH4-FR3	GAGCTCTGTGACCGCCGCGGACACG
	VH5-FR3	CAGCACCGCCTACCTGCAGTGGAGC
	VH6-FR3	GTTCTCCCTGCAGCTGAACTCTGTG
	VH7-FR3	CAGCACGGCATATCTGCAGATCAG
	JH2 (down)	CTTACCTGAGGAGACGGTGACC

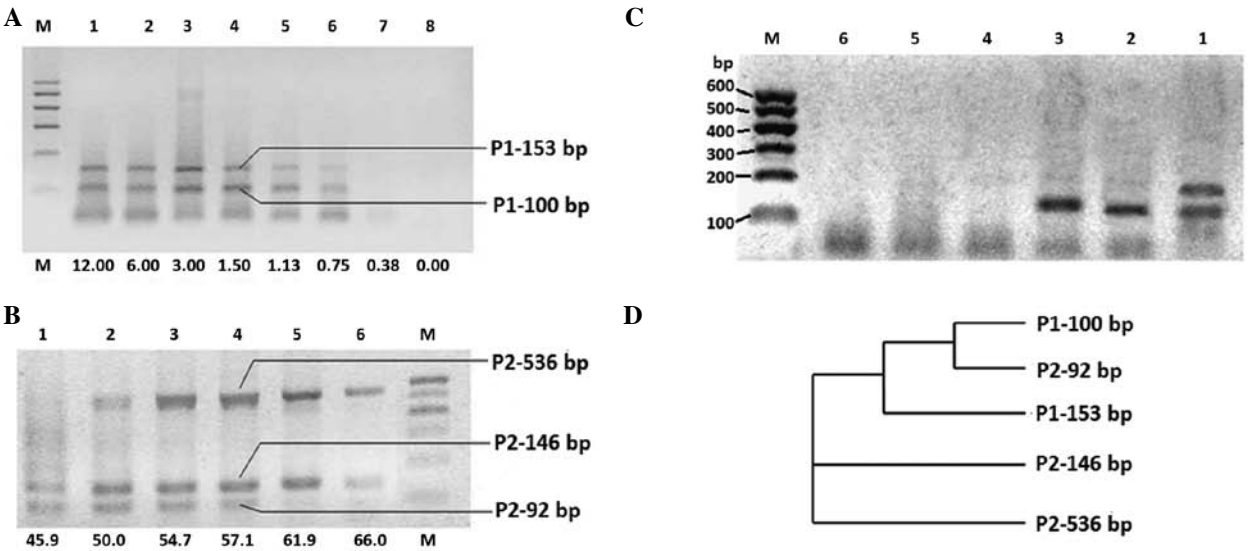

Figure 1. PCR products by the primers of immunoglobulin heavy (IgH) gene rearrangement with the DNA of DG75 cell lines are shown. (M: 100-600 bp ladder marker). (A) Electrophoresis of 2% agarose gel with the primer of IgH (classical primer pair FR3-JH1) primer on different Mg^{2+} concentration (μM). (B) Electrophoresis of 2% agarose gel with the primer of IgH (classical primer pair FR33-JH1) primer at different annealing temperatures (˚C). (C) Electrophoresis of 2% agarose gel with the primer of BIOMED-2 Tube C (VH1-VH7 -FR3-JH2) detected five lymphoma cell lines. M, marker; lane 1, DG-75 cell line; lane 2, BJAB cell line; lane 3, Raji cell line; lane 4, Jurkat cell line; lane 5, L428 cell line and lane 6, the negative control. (D) The phylogram results of DNA sequences of five bands (showed in Fig. 1A and B) using the ClustalW2 software.

Results

PCR amplification of DG75 cell DNA by the primer of IgH GR. PCR conditions were initially optimized using various concentrations of $MgCl_2$, without modifying any other parameters. The results showed two clear bands in the first primer pair (IgH FR3-JH1) PCR products. One band was ~100 base pair (designated as the P1-100 bp band after DNA sequencing), while the other was ~150 bp size (designated as P1-153 bp) (Fig. 1A).

The results also showed that there were three clear bands in the second PCR primer pair amplifications, of ~100 (designated as P2-92 bp), 150 (designated as P2-146 bp) and 500 bp (designated as P2-536 bp) size, respectively (Fig 1B).

The IgH GR statuses of five lymphoma cell lines were also assayed using the primer of Tube C of the BiOMED-2 method. Of these five lines, only DG-75 cell lines exhibited two clear bands in the detection (Fig. 1C). The DNA sequence resulting from the two bands was almost identical to that resulting from the FR3-JH1 primer pair. We also used seven single primer pairs from the multiple PCR of Tube C. The results demonstrated that there were two pairs of primers that demonstrated clear bands: the VH3-JH and VH4 -JH primer pairs (data not shown).

DNA sequences. DNA sequences of these bands were analyzed using the ClustalW2 software. The alignment results are shown in Table II, while the phylogram results are shown in Fig 1D. These results demonstrated that the P1-100 bp and P2-92 bp bands were derived from the same form of GR, while the P1-153 bp, P2-146 bp and P2-536 bp from another form of GR.

Table II. Result of ClustalW 2.1 multiple sequence alignment (abbreviated).

Name - Size (bp)	Primer sequence	Sequence no.
P2-146	------ACGGCTGTGTATTACTGTGCGAGAGACTTTCCATATTGTGGTGGTGACTGCTAC	54
P2-536	------ACGGGTGTGTATTACTGTGCGACAGACTTTCCATATTGTGGTGGTGACTGCTAC	54
P1-153	GTCGACACGGCCGTGTATTACTGTGCGAGAGACTTTCCATATTGTGGTGGTGACTGCTAC	60
P1-100	GTCGACACGGCCGTGTATTACTGTGCGAC----------TTG-GATTATGACTACCAT	47
P2-92	-------CGGCTGTGTATTACTGTGCGAC----------TTG-GATTATGACTACCAT	40
	*** ********** *** * * ***** * *	
P2-146	TCCGCGATGGATTACTATGATAGTAGTGGTTATCACTCCCTTATTAGTTTGACTAGTGGG	114
P2-536	TCCGCGATGGATTACTATGATAGTAGTGGTTATCACTCCCTTATTAGTTTGACTAGTGGG	114
P1-153	TCCGCGATGGATTACTATGATAGTAGTGGTTATCACTCCCTTATTAGTTTGACTAGTGGG	120
P1-100	----------ACGCCCTT------TTGACTACTGGG	67
P2-92	----------ACGCCCTT------TTGACTACTGGG	60
	** ***** ******* ****	
P2-146	GCCAGGGAACCCTGGTCACCGTCTCCTCAGGT----------	146
P2-536	GCCAGGGAACCCTGGTCACCGTCTCCTCAGGTGAGTCCTCAGAACGTCTCTCCTGCTTTA	174
P1-153	GCCAGGGAACCCTGGTCACCGTCTCCTCTGGAG----------	153
P1-100	GCCAGGGAACCCTGGTCACCGTCTCCTCTGCAG----------	100
P2-92	GCCAGGGAACCCTGGTCACCGTCTCCTCAGGT----------	92
	********** *	
P2-146	----------	
P2-536	ACTCTGAAGGGTTTTGCTGCATTTTTGGGGGGAAATAAGGGTGCTGGGTCTCCTGCCAAG	234
P1-153	----------	
P1-100	----------	
P2-92	----------	
P2-146	----------	
P2-536	AGAGCCCCGGAGCAGCCTGGGGGGCTCAGGAGGAT----------	536
P1-153	----------	
P1-100	----------	
P2-92	----------	

(*), same bases in the columns; (-), different bases in the columns.

Discussion

By definition, the bi/oligoclonal pattern indicates the presence of two or more bands, following PCR and electrophoretic resolution. The explanation for the difference in oligoclonality findings may be due to the somatic mutation of the VH genes (8,9). Another explanation for bi/oligoclonality may be the presence of two cell populations in the bone marrow, due to two separate events or the formation of sub-clones (10,11).

In general, the tumor cell line is a stable cell population that is relatively homogeneous in cell morphology, proliferation and biological traits. There are several B-cell lymphoma cell lines potentially employed as the positive control in detecting GR, such as Raji, Nam, Daudi, BJAB and DG75 cell lines. Of the B-cell lymphoma cell lines we used, the DG75 cell line demonstrated two clear bands in the PCR amplifications. DNA sequencing results showed that these two bands are distinct in the N region of amino sequence, showing at least two different forms of IgH GR. One form is the sequences of P2-92 bp or P1-100 bp, which are almost identical to the form of VH3-JH2 of the primer in the BIOMED-2 primer sets. Another form is the P2-146 bp or P1-153 bp, identical to the form of VH4-JH2 of the primer in the BIOMED-2 primer sets.

Of note, the agarose electrophoresis results also showed that there was a clear band in ~500 bp (P2-536 bp) in the PCR amplification of the classical primer pair FR33-JH1 (P2) of IgH primer (Table I). Based on their location, the DNA sequencing of P2-536 bp may be divided into two parts: the one almost overlapped with the sequence of P2-146 bp fragment, while the latter part is also associated with the sequence of Ig sequences. Thus, we hypothesize that the P2-536 bp DNA fragments, amplified by the P2 PCR primer pair, are also the non-specific amplification of P2-146 fragment of Ig sequences.

In conclusion, we occasionally found that the DG75 cell line, a B-cell lymphoma cell line with the potential to be used as a positive control in the detection of Ig heavy chain GR, was a bioclonal in GR, reported for the first time in the present study.

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