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Molecular Cloning of Immunoglobulin Heavy Chain Gene Translocations by Long Distance Inverse PCR

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1. Introduction

B-cell functions as a key player in the humoral immunity producing immunoglobulin protein in mammalian. To produce wide-ranged and antigen-specific antibody, the B-cell undergoes genetic rearrangement of immunoglobulin genes during its maturation process. The genetic rearrangements in immunoglobulin genes require unstable steps for genome; breakage and re-ligation of the double strand DNA. In the unstable steps, misconduct can be occurred; leading chromosome translocations involving immunoglobulin genes.

Chromosome translocations involving the immunoglobulin heavy chain (IGH) locus on chromosome 14q32 are found in various B-lymphoid malignancies, including B-cell precursor lymphoblastic leukemia (BCP-ALL), B-cell non-Hodgkin's lymphoma (B-NHL), and myeloma. Chromosome translocations involving IGH locus are often associated to disease entities among the B-lymphoid malignancies; thus, identification of certain translocation is clinically important information for definitive diagnosis. Moreover, molecular cloning of the IGH translocation breakpoint allows the identification of genes of physiologically or pathologically importance in B-cells (Willis and Dyer, 2000; Küppers and Dalla-Favera, 2001; Siebert et. al., 2001). The consequence of IGH translocations is the deregulated expression of the target gene controlled by potent B-cell-specific transcriptional enhancers within the IGH locus, resulting from physically close apposition with the IGH locus. The vast majority of target genes of IGH translocations play a fundamental role in B-cell biology, such as cell growth, differentiation, apoptosis and signal transduction (Wills and Dyer, 2000). Thus, deregulated expression of those genes alters B-cell fate and may initiate malignant transformation of the affected B-cell. To date, many IGH translocation breakpoints have been molecularly cloned and the target genes have been identified; however, several recurrent breakpoints remain to be cloned (Heim and Miltelman, 1995). Molecular cloning of IGH translocation breakpoints would reveal the involvement of either genes of unknown biological functions or the unsuspected oncogenic potential of known genes. Moreover, some target genes of IGH translocation have been found to be deregulated by gene amplification or unknown genetic mechanisms rather than IGH translocation, thereby contributing to disease development or progression (Dyer et. al, 2010).

2. Structure of IGH and its genetic modifications during B-cell differentiation

The human IGH locus consists of 51 functional variable (V) segments, 27 diverse (D) segments, six joining (J) segments and nine constant (C) segments in noncontiguous fashion starting from the telomeric end of 14q32 and spans 1.4 megabases (Mb) (Honjo and Alt, 1995) (Fig. 1). Each C segment, except Cδ, is preceded by repetitive DNA sequences named switch (S) segments, which play a role in class switch recombination, as discussed below. During the B-cell maturation process, the IGH gene undergoes three major genetic modifications: VDJ recombination, somatic hypermutation, and class switch recombination. Generally, the former occurs in the bone marrow, whereas the latter two occur in the germinal center of the lymph nodes. As a consequence of these genetic modifications, the IGH gene can produce part of the immunoglobulin protein (antibody), which can bind to a specific antigen with distinct effecter functions.

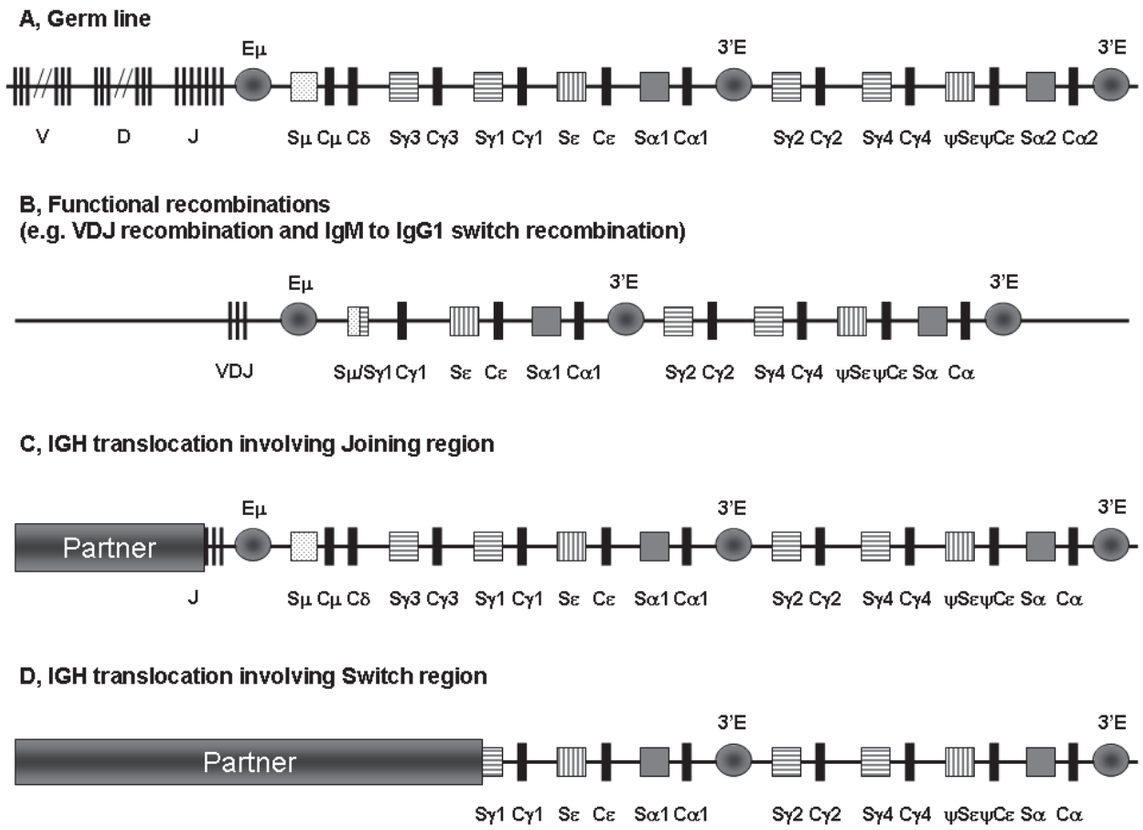


Fig. 1. A, Schematic diagram of human IGH structure. The coding exons lie on 14q32 as non-contiguous fashion. There are three potent enhancers within the IGH locus (Eμ and two 3'E). B, Schematic diagram of functionally rearranged IGH allele. In this schema, VDJ recombination and switch recombination of IgM to IgG1 are shown. Note Sμ and Sγ1 are combined. C, Schematic diagram of IGH translocation involving Joining region. The target gene on the partner chromosome is deregulated by Eμ enhancer. D, Schematic diagram of IGH translocation involving Switch region. The target gene on the partner chromosome is deregulated by 3'E enhancer.

Each B-progenitor cell forms a unique VDJ complex by selecting one V, one D, and one J segment randomly, and this process is called VDJ recombination. The VDJ complex therefore encodes part of the variable domain of immunoglobulin heavy chain protein. After encountering an antigen in the germinal center of a lymph node, VDJ complex is further introduced by nucleotide substitution, deletion or duplication, a process called somatic hypermutation, resulting in increased affinity with the respective antigen. In the germinal center, some B-cells exchange constant segments from Cμ to others by the class switch recombination process, which results in the exchange effecter domain of immunoglobulin protein (Fc-domain) retaining the antigen specific binding domain. This process involves the recombination of S regions preceding the Cμ segment and other C segment, and the intervening DNA segment is looped out. When class switching from IgM to IgG1 occurs, for example, Sμ and Sγ1 are recombined with deletion of the intervening DNA, thereafter, Cγ1 segment positions at 3′ flanking the VDJ complex thus produce VDJ-Cγ1 transcript (Figure 1). All VDJ recombination, somatic hypermutation and class switch recombination involve DNA double-strand cleavage and re-ligation; errors in each process may result in chromosome translocation targeted to the IGH locus (Küppers and Dalla-Favera, 2001). The IGH translocations seen in B-cell malignancies commonly take place in either the joining (IGHJ) or the switch (IGHS) segments.

3. Disease entities and IGH translocations

IGH translocation is a common genetic aberration in B-cell malignancies; however, the incidence various according to the disease entity. Precursor B-cell acute lymphoblastic leukemia shows a low frequency of IGH translocation, but recent studies have revealed diverse target genes of IGH translocation in this disease (Dyer et. al., 2010). Examples include cytokine receptor genes (Russell et.. al., 2009), the CCAAT enhancer-binding protein gene family (Chapiro et. al. 2006; Akasaka et. al., 2007), and microRNA 125b-1 (Sonoki et. al 2005; Chapiro et al, 2010, Tassano et al., 2010). Non-Hodgkin lymphoma often shows specific IGH translocations corresponding to the disease entity. t(14;18)(q32;q21) is seen in ~85% of follicular lymphoma and t(8;14)(q24;q32) is seen in ~90% of Burkitt lymphoma. Myeloma, which arises from terminal differentiated B-cells, shows extensive IGH translocation; however, the IGH translocation seen in myeloma defines distinct molecular subtypes of myeloma and is associated with clinical behavior (Bergsagel and Kuehl, 2005).

The vast majority of IGH translocation breakpoints are clustered in joining or switch regions. Since most IGH translocation results from physiological genetic modification errors, the breakpoints correspond to the differentiation stage of the tumor cells. IGH translocation breakpoints seen in B-cell precursor acute lymphoblastic leukemia are clustered in the joining region, whereas those seen in myeloma are clustered in the switch region. In B-NHL, IGH translations are seen in joining as well as switch regions. Follicular lymphoma and Mantle cell lymphoma show breakpoints in the joining region of IGH breakpoints suggesting that IGH translocations occur in the early stage of B-cell differentiation in bone marrow. Diffuse large B-cell lymphoma shows switch region breakpoints, suggesting that the translocation occurs in the late stage of B-cell differentiation in lymph nodes. Table 1 summarizes known partner genes of IGH translocation seen in B-cell malignancies.

Disease entity	Chromosome translocation	Involved gene	Biological function in B-cell
Precursor B-ALL	t(14;X)(q32;p22) or t(14;Y)(q32;p11)	CRFL2*	Cytokine Receptor
	t(5;14)(q31;q32)	IL3	Cytokine
	t(1;14)(q21;q32)	BCL9*	Modification of WNT signaling
	t(6;14)(p21;q32)	ID4*	Unkown
	t(8;14)(q11;q32)	CEBPD*	Transcription factor
	t(14;19)(q32;q13)	CEBPA*	Transcription factor
	t(14;14)(q11;q32)	CEBPE*	Transcription factor
	t(14;20)(q32;q13)	CEBPB*	Transcription factor
	t(14;17)(q32;q21)	IGF2BP1*	unkown
	t(11;14)(q24;q32)	microRNA125 b-1*	Anti-apotosis
Follicular lymphoma	t(14;18)(q32;q21)	BCL2	Anti-apotosis
Mantle cell lymphoma	t(11;14)(q13;q32)	Cyclin D1	Regulation of cell cycle
Burkitt Lymphoma	t(8;14)(q24;q32)	C-MYC	Cell proliferation and metabolism
Diffuse Large B-cell lymphoma	t(3;14)(q27;q32)	BCL6	Transcriptional repressor
	t(1;14)(q21;q32)	ITRA	B-cell signaling
	t(11;14)(p13:q32)	CD44	Cell migration or proliferation
Marginal cell lymphoma	t(6;14)(p21;q32)	Cyclin D3*	Regulation of cell cycle
B-CLL	t(2;14)(p13;q32)	BCL11A*	Unkown
MALT	t(1;14)(p21;q32)	BCL10*	Modification of NF-kB signaling
	t(14;18)(q32;q21)	MALT1	Modification of NF-kB signaling
Lymphoplasmacytic lymphoma	t(9;14)(p13;q32)	PAX-5	Transcription factor

Disease entity	Chromosome translocation	Involved gene	Biological function in B-cell
Myeloma	t(11;14)(q13;q32)	Cyclin D1	Regulation of cell cycle
	t(4;14)(p16;q32)	FGFR3/MMSET	Unkown
	t(8;14)(q24;q32)	C-MYC	Cell proliferation and metabolism
	t(14;16)(q32;q23)	C-MAF	Unkown
	t(6;14)(p21;q32)	Cyclin D3	Regulation of cell cycle
	t(14;20)(q32;q12)	MAFB	Unkown

Table 1. Disease entity and IGH chromosome translocation. *, Genes identified by LDI-PCR.

4. Molecular cloning of IGH translocation breakpoints using long distance inverse-PCR

Inverse PCR is a useful method to define unknown nucleotide alignments flanking known nucleotide sequences, such as retroviral DNA integration sites into host chromosome DNA (Takemoto et. al. 1994). The principle of inverse PCR uses self-ligated circular DNA as a PCR template and primers setting in a known sequence in the opposite direction. Inverse PCR is easily applied for determination of unknown partner sequence combined with IGH resulting from chromosome translocation. After thermostable DNA polymerase that can amplify long target DNA is available, the combination of inverse PCR and long target thermostable DNA polymerase (long distance inverse PCR; LDI-PCR) is a powerful tool for molecular cloning of an unknown sequence from IGH. Molecular cloning by LDI-PCR requires a small amount of genomic DNA and only couple of days to determine the translocation breakpoints involving IGH translocation. Using this method, many novel partner genes have been identified as listed in Table 1.

Since PCR may yield non-specific products, Southern blot analysis using IGH-specific probes is informative to interpret LDI-PCR results. The LDI-PCR product can be estimated by rearranging the band detected in Southern blot analysis; however, cloning of IGHJ translocations is simple and clear, and can be performed without reference to Southern blot data (Willis et al. 1997; Akasaka et al., 2007). In contrast, IGHS might undergo complex rearrangements, including insertion, deletion, and inversion (Bergsagel et. al. 1999) To distinguish translocation or other rearrangements, Southern blot assays using several probes are required. Thus, it is necessary to have large amounts of high-molecular-weight DNA available to allow Southern blot analysis before performing LDI-PCR for switch regions (Sonoki et al, 2001).

The detailed protocol of LDI-PCR for molecular cloning of the immunoglobulin heavy chain gene has been published elsewhere (Karran et al. 2005). A brief principle is shown in Figure 2. After complete digestion of high molecular genomic DNA with the restriction enzyme, the DNA is reacted with T4-ligase at low DNA concentration to form self-ligated

circular DNA. The circular DNA is then purified by a silica-based column and subjected to PCR using primer pairs set at the IGH sequence. When the PCR product of interest is obtained, the nucleotide alignment can be determined directly. Although genomic DNA can be isolated from paraffin-embedded materials, the DNA is fragmented, and thus is not suitable for LDI-PCR.

LDI-PCR allows the rapid cloning of vast majority of translocations targeting IGH in B-cell malignancies. The benefits of LDI-PCR are first the speed and ease with which this cloning can now be performed and second that cloning can be performed when only small amounts of material are available (Willis TG, et al. 1997). The principle for LDI-PCR can be applied for other cloning purpose than IGH translocations.

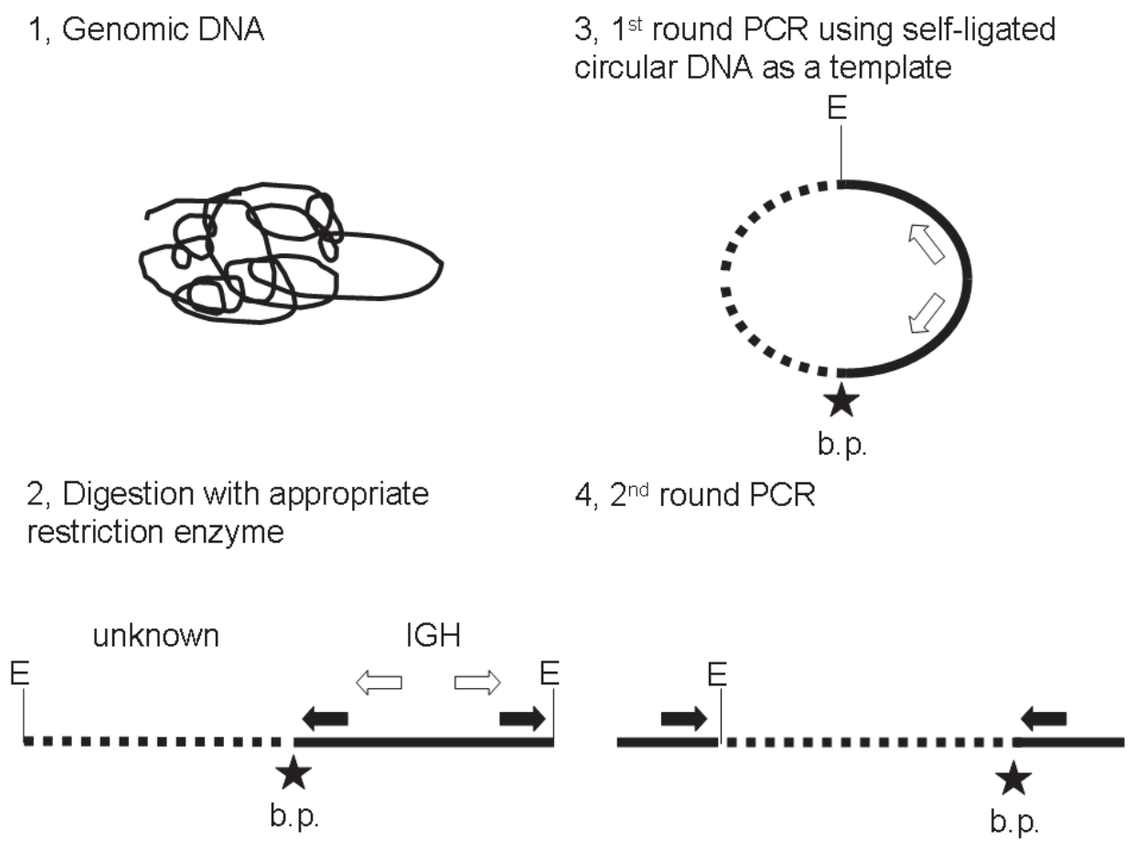


Fig. 2. Schematic diagram of LDI-PCR for cloning IGH translocation breakpoint. Using long distant DNA polymerase, 4~5 kb PCR products are able to be amplified routinely. E; restriction enzyme site, star; translocation breakpoint.

5. References

Akasaka T, Balasas T, Russell LJ, Sugimoto KJ, Majid A, Walewska R, Karran EL, Brown DG, Cain K, Harder L, Gesk S, Martin-Subero JI, Atherton MG, Bruggemann M, Calasanz MJ, Davies T, Haas OA, Hagemeijer A, Kempski H, Lessard M, Lillington DM, Moore S, Nguyen-Khac F, Radford-Weiss I, Schoch C, Struski S, Talley P, Welham MJ, Worley H, Strefford JC, Harrison CJ, Siebert R, Dyer MJ. Five members of the CEBP transcription factor family are targeted by recurrent IGH translocations in B-cell precursor acute lymphoblastic leukemia (BCP-ALL). Blood. 2007 Apr 15;109(8):3451-61.

Chapiro E, Russell L, Radford-Weiss I, Bastard C, Lessard M, Struski S, Cave H, Fert-Ferrer S, Barin C, Maarek O, Della-Valle V, Strefford JC, Berger R, Harrison CJ, Bernard OA, Nguyen-Khac F. Overexpression of CEBPA resulting from the translocation t(14;19)(q32;q13) of human precursor B acute lymphoblastic leukemia. Blood. 2006 Nov 15;108(10):3560-3.

Chapiro, E., L. Russell, S. Struski, H. Cave, I. Radford-Weiss, V. Valle, J. Lachenaud, P. Brousset, O. Bernard, C. Harrison, and F. Nguyen-Khac. A new recurrent translocation t(11;14)(q24;q32) involving IGH@ and miR-125b-1 in B-cell progenitor acute lymphoblastic leukemia. Leukemia 2010 24:1362-1364.

Dyer MJ, Akasaka T, Capasso M, Dusanjh P, Lee YF, Karran EL, Nagel I, Vater I, Cario G, Siebert R. Immunoglobulin heavy chain locus chromosomal translocations in B-cell precursor acute lymphoblastic leukemia: rare clinical curios or potent genetic drivers? Blood. 2010 Feb 25;115(8):1490-9.

Heim S and Mitelman F Cancer Cytogenetics, The second edition, Wiley-Liss, 1995

Honjo T and Frederick WA ed. Immunoglobulin genes, The second edition., Academic Press, 1995

Karran EL, Sonoki T, Dyer MJ. Cloning of immunoglobulin chromosomal translocations by long-distance inverse polymerase chain reaction. Methods Mol Med. 2005;115:217-30.

Küppers R, Dalla-Favera R. Mechanisms of chromosomal translocations in B cell lymphomas. Oncogene. 2001 Sep 10;20(40):5580-94.

Russell LJ, Capasso M, Vater I, Akasaka T, Bernard OA, Calasanz MJ, Chandrasekaran T, Chapiro E, Gesk S, Griffiths M, Guttery DS, Haferlach C, Harder L, Heidenreich O, Irving J, Kearney L, Nguyen-Khac F, Machado L, Minto L, Majid A, Moorman AV, Morrison H, Rand V, Strefford JC, Schwab C, Tonnies H, Dyer MJ, Siebert R, Harrison CJ. Deregulated expression of cytokine receptor gene, CRLF2, is involved in lymphoid transformation in B-cell precursor acute lymphoblastic leukemia. Blood. 2009 Sep 24;114(13):2688-98.

Siebert R, Rosenwald A, Staudt LM, Morris SW. Molecular features of B-cell lymphoma. Curr Opin Oncol. 2001 Sep;13(5):316-24.

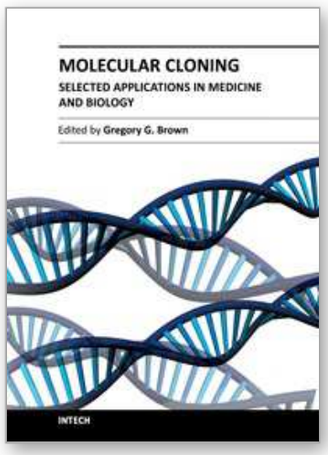
Sonoki T, Willis TG, Oscier DG, Karran EL, Siebert R, Dyer MJ. Rapid amplification of immunoglobulin heavy chain switch (IGHS) translocation breakpoints using long-distance inverse PCR. Leukemia. 2004 Dec;18(12):2026-31.

Takemoto S, Matsuoka M, Yamaguchi K, Takatsuki K. A novel diagnostic method of adult T-cell leukemia: monoclonal integration of human T-cell lymphotropic virus type I provirus DNA detected by inverse polymerase chain reaction. Blood. 1994 Nov 1;84(9):3080-5.

Tassano E, Acquila M, Tavella E, Micalizzi C, Panarello C, Morerio C. MicroRNA-125b-1 and BLID upregulation resulting from a novel IGH translocation in childhood B-Cell precursor acute lymphoblastic leukemia. Genes Chromosomes Cancer. 2010 Aug;49(8):682-7.

Willis TG, Dyer MJ. The role of immunoglobulin translocations in the pathogenesis of B-cell malignancies. Blood. 2000 Aug 1;96(3):808-22.

Willis TG, Jadayel DM, Coignet LJ, Abdul-Rauf M, Treleaven JG, Catovsky D, Dyer MJ. Rapid molecular cloning of rearrangements of the IGHJ locus using long-distance inverse polymerase chain reaction. Blood. 1997 Sep 15;90(6):2456-64.



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The development of molecular cloning technology in the early 1970s created a revolution in the biological and biomedical sciences that extends to this day. The contributions in this book provide the reader with a perspective on how pervasive the applications of molecular cloning have become. The contributions are organized in sections based on application, and range from cancer biology and immunology to plant and evolutionary biology. The chapters also cover a wide range of technical approaches, such as positional cloning and cutting edge tools for recombinant protein expression. This book should appeal to many researchers, who should find its information useful for advancing their fields.

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