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Submicroscopic Deletions of Immunoglobulin Heavy Chain Gene (*IGH*) in Precursor B Lymphoblastic Leukemia with *IGH* Rearrangements

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Translocations leading to fusions between the immunoglobulin heavy chain gene (*IGH*) and various partner genes have been reported in B-cell precursor acute lymphoblastic leukemia (B-ALL). However, submicroscopic deletions within *IGH* in B-ALL have not been rigorously assessed. In this study, we investigated characteristics of *IGH* submicroscopic deletions, by FISH, in B-ALL with *IGH* rearrangements. FISH was performed by using commercially available *IGH* dual-color break-apart rearrangement probes (Abbott/Vysis, Downers Grove, IL, USA; Kreatech, Amsterdam, Netherlands). The study group included seven B-ALL patients with *IGH* rearrangements, observed by FISH. Among them, two exhibited deletion of the 5′ variable region of *IGH* by FISH. The B-ALL in these two patients included two kinds of abnormal cells; one had an *IGH* rearrangement without any *IGH* submicroscopic deletion, while the other had an *IGH* submicroscopic deletion, which showed that one normal fusion signal and one 3′ *IGH* signal were detected. Thus, submicroscopic deletion of the *IGH* 5′ variable region may have occurred in either the native or rearranged chromosome 14. These findings indicate that B-ALL with *IGH* rearrangements may be accompanied by submicroscopic deletions of the *IGH* 5′ variable region, which can be detected by FISH. The clinical significance of such deletions is unclear, but the loss of part of the *IGH* gene in B-ALL warrants further study.

Key Words: *IGH* deletion, *IGH* rearrangements, Precursor B lymphoblastic leukemia, FISH

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Chromosomal translocations involving the immunoglobulin heavy chain gene (*IGH*), located on 14q32, are associated with various mature B-cell neoplasms, and result in enhanced expression of the translocation partner genes by physical juxtaposition with enhancers within the *IGH* locus [1, 2]. Of interest, *IGH* translocations that give rise to fusions with various partner genes have also been reported in approximately 2-3% of B-cell precursor acute lymphoblastic leukemia (B-ALL) [3-5]. Among the partner genes, the cytokine receptor-like factor 2 (*CRLF2*) is the most common, followed by inhibitor of DNA binding 4 (*ID4*), erythropoietin receptor (*EPOR*), and the CCAAT enhancer-binding protein (*CEBP*) family [6]. The misregulated expression of *CRLF2*, as a consequence of *IGH* translocation may be associated with a poor prognosis in otherwise "good-risk" patients, suggesting that different *IGH* chromosomal translocations may constitute subgroups of B-ALL [6, 7].

In addition to *IGH* rearrangements, *IGH* submicroscopic deletions are observed in 14-21% of patients with multiple myeloma, and in 13-33% of patients with chronic lymphocytic leukemia [8-12]. However, *IGH* submicroscopic deletions in B-ALL have not been thoroughly investigated, and only a few studies have screened for them using Southern blot, FISH, or array comparative genomic hybridization [13-15]. In this study, we searched for *IGH* submicroscopic deletions in patients with both B-ALL

and *IGH* rearrangements by FISH using an *IGH* break-apart probe.

Seven patients with B-ALL and *IGH* rearrangements by FISH from 2011 to 2013 at the Ewha Womans University School of Medicine and Mokdong Hospital in Seoul, Korea, were enrolled. The diagnoses of B-ALL were made according to the 2008 WHO classification [1], and *IGH* rearrangements were identified by FISH. Cytogenetic studies were performed on unstimulated

Table 1. Characteristics of *IGH* rearrangements and *IGH* deletions in B precursor lymphoblastic leukemia patients

Case No.	Age (yr)	Sex	Cytogenetics	FISH									
				IGH rearrangement	*IGH* deletion	*BCR/ABL1*	*MLL*	*p16*	*ETV6*	CEP4	CEP10	*TP53*	Others
1	56	F	43,XX,-4,-7,-9,t(9;22)(q34;q11.2),-11,+der(22)t(9;22)[5]/74~75<3n>,XXX,+X,+1,+2,-4,+5,-7,+8,-9,t(9;22)(q34;q11.2)x2,-11,+15,+20,+21,+22,+der(22)t(9;22)[cp14]/46,XX[1]	Pos.* (21%)	Pos.* (22%)	Rearranged (90%)	Deletion (79%)	Deletion (79%)	Gain (19%)	Loss (70%)	Gain (18%)	Gain (23%)	
2	9	M	46,XY[20]	Pos.† (18%)	Pos.† (13%)	Neg.	Neg.	Deletion (73%)	Rearranged (82%)	Neg.	Neg.	Neg.	t(12;21): *ETV6/RUNX1* by PCR
3	24	M	46,XY,t(5;7)(p15.3;p11.2~13)[13]/46,XY[7]	Pos. (78%)	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	
4	10	F	47,XX,+21c[11]/46,XX[1]	Pos. (78%)	Neg.	Neg.	Neg.	Neg.	Neg.	Gain (14%)	Neg.	Deletion (8%)	*RUNX1*: 78%, gain
5	37	F	46,XY[10]	Pos. (9%)	Neg.	Neg.	Neg.	Neg.	Deletion (87%)	Neg.	Gain (69%)	Neg.	
6	1	M	46,XY,t(1;19)(q21;p13.3)[4]/46,XY[16]	Pos. (11%)	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	*RUNX1*: 78%, gain
7	1	F	55,XX,+X,+4,+6,+14,+15,+17,+18,+21,+21 [16]/46,XX[4]	Pos.‡ (10%)	Neg.	Neg.	Neg.	Neg.	Neg.	Gain (69%)	Neg.	Gain (78%)	6q21/6q23: 86%, gain
				IGH gain only (82%)									

*nuc ish(3′IGHx2,5′IGHx1)(3′IGH con 5′IGHx1)[43/200]/(IGHx2)(3′IGH sep 5′IGHx1)[42/200]; †nuc ish(IGHx2)(3′IGH sep 5′IGHx1)[36/200]/(3′IGHx2,5′IGHx1)(3′IGH con 5′IGHx1)[25/200]; ‡nuc ish(IGHx3)[163/200]/(IGHx3)(3′IGH sep 5′IGHx1)[19/200].
Abbreviations: IGH, immunoglobulin heavy chain gene; CEP, chromosome enumeration probe; Pos., positive; Neg., negative.

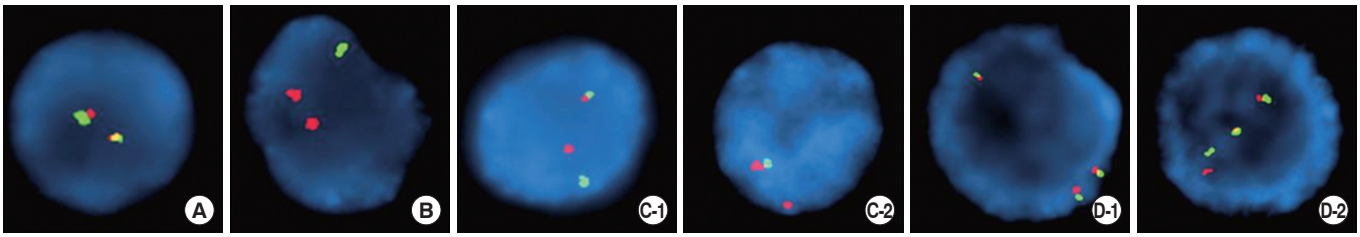

Fig. 1. Representative fluorescence in situ hybridization patterns using *IGH* break-apart probes (green signal: *IGH* 5′ variable region; red signal: *IGH* 3′ flanking probe that lies 3′ to the constant gene segments). (A) Normal pattern (2 fusion signals) (B) *IGH* rearrangement and deletion of the *IGH* 5′ variable region in the normal chromosome 14 (1 green and 2 red signals) (C-1) *IGH* rearrangement without *IGH* submicroscopic deletion (1 fusion, 1 green, and 1 red signal) (C-2) one normal *IGH* locus and submicroscopic deletion of the *IGH* 5′ variable region (1 fusion and 1 red signal). It could not be determined whether the *IGH* submicroscopic deletion developed within normal cells or cells with an *IGH* rearrangement. (D-1) gain of *IGH*, suggesting trisomy 14 (3 normal fusion signals) (D-2) gain of *IGH*, accompanied by *IGH* rearrangement (2 fusion signals, 1 green and 1 red signal).

24- and 48-hr cultures of fresh bone marrow aspirates. When possible, at least 20 metaphases per sample were analyzed, and karyotypes were determined according to the International System for Human Cytogenetic Nomenclature (ISCN, 2013) [16]. Interphase FISH was performed by using commercially available probes (Abbott/Vysis; Kreatech). The *IGH* dual-color break-apart rearrangement probe is a mixture of two fluorescent probes: 5´ *IGH* probe (green) covering the entire *IGH* variable region, and *IGH* 3´ flanking probe (red) annealing 3´ to the constant gene segments of *IGH* (Fig. 1). The cutoff for *IGH* break-apart FISH was 3%. At least 200 interphase cells were scored for each probe by two experienced independent examiners.

Among seven patients with *IGH* rearrangements, two had a submicroscopic deletion of the 5´ variable region of *IGH* by FISH (Table 1, cases 1 and 2). They also had *BCR/ABL1* and *ETV6/RUNX1* rearrangements, respectively. In regards to the *IGH* rearrangements, two kinds of abnormal cell populations were observed; one had a typical *IGH* rearrangement without *IGH* submicroscopic deletion (21% and 18% of interphase cells, in case 1 and 2, respectively) (Fig. 1C-1). The other revealed a submicroscopic deletion of the *IGH* 5´ variable region, resulting in one normal fusion signal and one 3´ *IGH* signal (Fig. 1C-2) (in 22% and 13% of interphase cells, in case 1 and 2, respectively). This finding indicated that an *IGH* submicroscopic deletion occurred in either the normal or the rearranged chromosome 14 (Fig. 1C-2); we could not distinguish by FISH, which chromosome acquired the deletion. Among the five patients with *IGH* rearrangements and without *IGH* submicroscopic deletions, four patients displayed typical *IGH* rearrangement patterns by FISH (Table 1, cases 3-6; Fig. 1C-1). One patient with hyperdiploidy (Table 1, case 7) exhibited two abnormal cell populations: the first exhibited *IGH* gain without *IGH* rearrangement, possibly signifying trisomy 14 in 82% of interphase cells (Fig. 1D-1), and the other, consisting of 10% of interphase cells, showed both *IGH* gain and *IGH* rearrangement (Fig. 1D-2).

The biologic significance of these *IGH* submicroscopic deletions in B-ALL remains to be determined. A previous study using FISH revealed that 6% of pediatric B-ALL patients had *IGH* submicroscopic deletions without *IGH* rearrangements, in more than 50% of interphase cells [13]. In the current study, two patients out of seven presented with *IGH* submicroscopic deletions in 13-22% of their interphase cells (cases 1 and 2); however, we could not distinguish whether *IGH* submicroscopic deletions developed within normal cells without *IGH* rearrangements, or in abnormal cells with *IGH* rearrangements. In most cases of mature B cell neoplasm, submicroscopic deletions of the *IGH* variable regions occur in the normal chromosome 14, and only rarely in an abnormal chromosome 14 that is involved in an *IGH* rearrangement [8]. Therefore, some studies suggest that submicroscopic deletions of the *IGH* variable regions may be a result of the DNA loss that accompanies somatic V(D)J recombination, and may not have any oncogenic role in mature B cell neoplasm [8, 10]. In contrast, one study suggested that relatively large, interstitial 14q deletions in mature B cell neoplasms might activate an unknown oncogene, by operating in a manner similar to translocations that induce fusion of genetic material [17].

This study demonstrated that B-ALL with *IGH* rearrangements might be accompanied by submicroscopic deletions of *IGH* 5´ variable regions, which can be identified by FISH. A limitation of this study is that the number of patients was relatively small, and further studies in larger cohorts are needed to investigate the incidence and clinical significance of *IGH* submicroscopic deletions in B-ALL.

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Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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