A triple translocation in childhood acute lymphoblastic Leukemia

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انتقال كروموسومي ثلاثي في ابيضاض الدم اللمفاوي الحاد لدى الأطفال
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الخلاصة:
إن للشذوذ الكروموسومي في حالات ابيضاض الدم اللمفاوي الحاد عند الأطفال أهمية كبيرة تتعلق بتشخيص وإدارة ومتابعة سير المرض.أستخدم تحليل التهجين ألتألقي في الموضع في هذه الدراسة باستعمال مجسات الكروموسوم البكتيري ألصنعي في هذه الدراسة للتحري عن انتقال كروموسومي غير مألوف في حالة لابيضاض الدم اللمفاوي الحاد.اظهر استعمال المجس Rp11-111312 انتقال كروموسومي ثلاثي للكروموسومات9 و 22 و 3.كما اظهر استعمال المجس نفسه إشارات على كروموسوم 22 ونظير كروموسوم 3. واظهر استعمال المجس Rp-11465M18 إشارة على كروموسوم 9 .تؤكد هذه الدراسة حدوث انتقال كروموسومي ثلاثي بين الكروموسومات 9 و22و 3 كشذوذ كروموسومي غير مألوف في ابيضاض الدم اللمفاوي الحاد لدى الأطفال.

Abstract
Chromosomal abnormality in childhood acute lymphoblastic leukemia(ALL) has an important role in diagnosis ,management and prognosis. Fluorescence *in situ* hybridization(FISH) analysis using BAC probes was performed to detect a novel translocation in childhood ALL. The BAC probe RP11-111312 revealed a triple translocation involving chromosome 9,22 and 3.This BAC probe showed signals on normal chromosome 22 and derivative chromosome 3. RP11-465M18 showing only one signal on normal chromosome 9. This study confirms the triple translocation comprising chromosome 9,22,3 as a novel chromosomal abnormality in childhood ALL.

Introduction
Acute lymphoblastic leukemia (ALL) ,the most common hematologic malignancy of children, account for one for one forth of all cases of childhood cancer(1).Many types of leukemia are

associated with specific chromosomal rearrangements. These a aberrations may play a pivotal role in the development of neoplasm.Oncogenes have been identified which are located near the breakpoint sites. If an oncogene is disturbed, either by rearrangement or by having a foreign gene in juxtaposition ,alteration in the regulation of the gene may result ,and this leads to altered structure and function or over-production of its gene product(2).We report the occurrence of a triple translocation in a case report of childhood ALL.

Materials and Methods

A 10-year-old girl with acute lymphoblastic leukemia was a case report in this study. Fluorescence *in situ* hybridization (FISH) analysis using bacterial artificial chromosome(BAC) probes was performed to detect a novel translocation in childhood ALL. In brief:, chromosomal preparations from bone marrow cells were hybridized in situ with 1 µg of probe labeled by nick translation (Table 1). Hybridization was performed at 37°C in 2X SSC, 50% (vol/vol) formamide, 10% (wt/vol) dextran sulfate, 5 µg COT1 DNA (Bethesda Research Laboratories, Gaithersburg, MD, USA), and 3 µg sonicated salmon sperm DNA in a volume of 10 µL. Post-hybridization washings were performed three times at 60°C in 0.1X SSC . In cohybridization experiments, the probes were directly labeled with Fluorescein, Cy3 and Cy5. Chromosomes were identified by DAPI staining. Digital images were obtained using a Leica DMRXA epifluorescence microscope equipped with a cooled CCD camera (Princeton Instruments, Boston, MA). Cy3 (red; New England Nuclear, Boston, MA, USA), fluorescein (green; Fermentas Life Sciences, Milan, IT), Cy5 (IR; New England Nuclear, Boston, MA, USA) and DAPI (blue) fluorescence signals, which were detected using specific filters, were recorded separately as gray-scale images. Pseudocoloring and merging of images were performed with Adobe Photoshop software(3).

Table 1: List of probes used in FISH experiments.

CHR	BAC PROBE	BAND	UCSC 2006	FISH RESULTS	GENE
9	RP11-42D9	9p21.3	chr9:20,560,274-20,736,915		
	RP11-243F8	9p13.2	chr9:36,834,935-37,023,204		
	RP11-465M18	9p13.2	chr9:36,965,063-37,199,364	9	PAX5
	RP11-652D9	9p13.2	chr9:36,976,566-37,179,379		
	RP11-1150N16	9p13.1	chr9:38,985,367-39,118,143		

	RP11-120F8	**9q34.13**	**chr9:133,533,399-133,687,048**	**9, Ph**	
	RP11-188C12	**9q34.3**	**chr9:139,801,943-139,802,960**	**9, Ph**	
3	**RP11-95E11**	**3p26.3**	**chr3:3,134,219-3,304,869**		
	RP11-1016H17	**3p25.3**	**chr3:10,177,039-10,177,516**	**3, der(3)**	
	RP11-264H11	**3p25.2**	**chr3:11,757,820-11,901,653**	**3, der(3)**	
	RP11-1113I2	**22q13.33**	**chr22:48,805,612-48,969,515**	**22, der(3)**	

Results and Discussion

In this study,using FISH analysis ,the BAC probe RP11-111312 revealed a triple translocation involving chromosome 9,22 and 3.This BAC probe showed signals on normal chromosome 22 and derivative chromosome 3,where as RP11-465M18 showing only one signal on normal chromosome 9. The occurrence of *BCR/ABL1* fusion gene was confirmed by FISH experiment using RP11-164N13 probe [specific for *BCR* gene (chr22:21,852,552-21,990,224)] that showed a splitting signal on both derivative chromosome 22 (Ph) and 9 (Figure1). The deletion on the short arm of chromosome 9 was confirmed by FISH with RP11-465M18 clone showed only one fluorescent signal on normal chromosome 9 in Ph+ metaphases. Indeed, we used RP11-164N13 BAC in order to distinguish between Ph+ cells from Ph- ones.

Chromosomal abnormality in childhood ALL has an important role in diagnosis ,management and prognosis.Understanding of leukemogenesis is enhanced by identification of specific chromomosomal alteration, which pinpoint to sites for molecular studies to identify genes involved in the transformation and proliferation of leukemic cells(4).The chromosomal abnormalities in ALL cases were mostly presented in chromosome 9,11,22, followed by 5,7,12 and 17.Chromosome 9 was found to be the

most commonly involved through the course of disease(5). Other chromosomal abnormalities in ALL comprising chromosome 1,10,8 ,20 and X(6).Moreover, frequency of loss is highest in chromosome 22, but other reports in chromosome 20(7,8).

In this study, probably the telomeric region of the long arm of chromosome 22 was translocated with the telomeric region of the short arm of chromosome 3 after the occurrence of the

translocation t(9;22).The BAC probes used in our study;RP11-1061H1 and RP11-246H11 were retained on derivative chromosome 3,the breakpoint region maps more telomerically.

Authors reported different novel translocation in ALL. A novel balanced t(2;11)(q11.2;p15.1)translocation found as the sole cytogenetic abnormality in the bone marrow of childhood ALL patients(9).Recurrent t(9;15)(p13;q24) in two cases of childhood ALL was also reported which results in an in-frame fusion of *PAX5* to the promyelocytic leukemia(PML)gene (10).Using M-FISH analysis ,the presence of t(3;12) was also present (11). Two possible mechanisms for variant translocation formation were suggested. The first is a single event rearrangement via the simultaneous breakage of several chromosomes followed by mismatching joining (12).Nacheva and his colleagues (13) proposed a classical Ph translocation followed by a further translocation event between chromosome 19 and 22 plus a third chromosome.

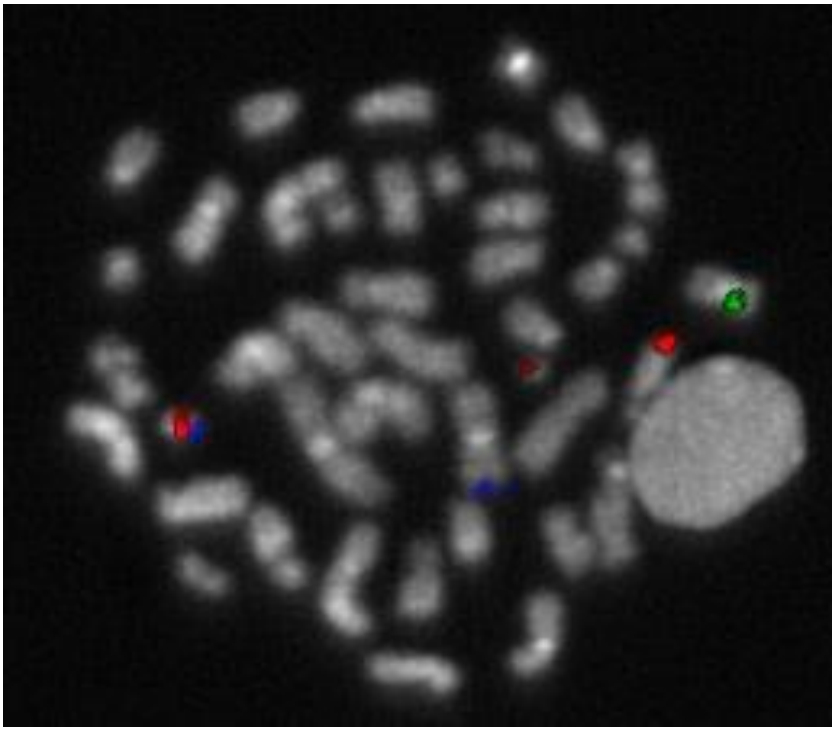

Figure 1. FISH co-hybridization with BACs RP11-164N13 Cy3-labeled (red) (showing spot on normal chromosome 22 and splitting signal on derivative chromosomes 22 and 9), RP11-465M18 Fluorescein-labeled (green) (showing only one signal on normal chromosome 9) and RP11-1113I2 Cy5-labeled (blue) (showing 2 signals on normal chromosome 22 and derivative

chromosome 3).

The mechanism of formation of a variant Ph translocation may have prognostic importance in that a two event mechanism represents clonal evolution , whereas a variant translocation occurring via a single genomic rearrangement may confer a similar prognosis to the classical Ph translocation(14).

This study documents the triple translocation comprising chromosome 9,22,3 as a novel chromosomal abnormality in childhood ALL.

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