Ⅲ．　研究成果の刊行に関する一覧

研究成果の刊行に関する一覧表(論文)

発表者氏名	論文タイトル名	発表誌名	巻名	ページ	出版年
Nakagawa K, Kanda Y, yamashita H, Hosoi Y, Oshima K, Ohtomo K, Ban N, Yamakawa S, Nakagawa S, and Chiba S	Preservation of ovarian function by ovarian shielding when undergoing total body irradiation for hematopoietic stem cell transplantation:a report of two successful cases	Bone Marrow Transplantation	37	583-587	2006
Hosoya N, Sanada M, Nannya Y, Nakazaki K, Lili Wang, hangaishi A, Kurokawa M, Chiba S, Ogawa S	Genomewide Screening of DNA Copy Number Changes in Chronic Myelogenous Leukemia with the Use of High-Resolution Array-Based Comparative Genomic Hybridization	Genes,Chromosomes&Cancer	45	482-494	2006
Mori-Asano Y, kanda Y, Oshima K, Watanabe T, Shoda E, Motokura T, Kurokawa M, Chiba S	Pharmacokinetics of ganciclovir in haematopoietic stem cell transplantation recipients with or without renal impairment	Journal of Antimicrobial Chemotheraphy	57	1004-1007	2006
Yamashita H, izutsu K, Nakamura N, Shiraishi K, Chiba S, Kurokawa M, Tago M, Igaki H, Ohtomo K, Nakagawa K	Treatment results of chemoradiation therapy for localized aggressive lymphomas: a retrospective 20-year study	Ann Hematol	85	523-529	2006
Haraguchi K, Takahashi T, nakahara F, Matsumoto A, Kurokawa M, Ogawa S, Oda H, Hirai H, Chiba S	CD1d espression level in tumor cells is an important determinant for anti-tumor immunity by natural killer T cells	Leukemia& Lymphoma	47	2218-2223	2006
Suzuki T, Yokoyama Y, Kumano K, Takanashi M, Kozuma S, Takato T, Nakahata T, Nishikawa M, Sakano S, Kurokawa M, Ogawa S and Chiba S	Highly Efficient Ex Vivo Expansion of Human Hematopoietic Stem Cells Using Delta1-Fc Chimeric Protein	Stem Cells	24	2456-2465	2006
Nakagawa M, Ichikawa M, Kumano K, Goyama S, Kawazu M, Asai T, Ogawa S, Kurokawa M, and Chiba S	AML1/Runx1 rescues Notch1-null mutation-induced deficiency of para-aortic splanchnopleural hematopoiesis	blood	108	3329-3334	2006

研究成果の刊行に関する一覧表(論文)

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Oshima K, kanda Y, nakahara F, Shoda E,Suzuki T,Imai Y, Watanabe T, Asai T,Izutsu K, Ogawa S, motokura T,Chiba S, Kurokawa M	Pharmacokinetics of Alemtuzumab after Haploidentical HLA-mismatched Hematopoietic Stem Cell Transplantation Using in Vivo Alemtuzumab With of Without CD52-Positive Malignancies	American Journal of Hematology	81	875-879	2006
Nitta E, Izutsu K, Sato T, Ota Y, Takeuchi K, Kamijo A, Takahashi K, Oshima K, Kanda Y, Chiba S,Motokura T, Kurokawa M	A high incidence of late-onset neutropenia following rituximab-containing chemotherapy as a primary treatment of CD20-positive B-cell lymphoma: a single-institution study	Annals of Oncoloby	18	364-369	2007
Chiba S	Concise Review: Notch Signaling in Stem Cell Systems	Stem Cells	24	2437-2447	2006

Ⅳ. 研究成果の刊行物・別刷

Bone Marrow Transplantation (2006) 37, 583–587

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ORIGINAL ARTICLE

Preservation of ovarian function by ovarian shielding when undergoing total body irradiation for hematopoietic stem cell transplantation: a report of two successful cases

K Nakagawa[1], Y Kanda[2], H Yamashita[1], Y Hosoi[1], K Oshima [2], K Ohtomo[1], N Ban[3], S Yamakawa[1], S Nakagawa[4] and S Chiba[2]

[1]*Department of Radiology, University of Tokyo Hospital, Tokyo, Japan;* [2]*Department of Cell Therapy & Transplantation Medicine, University of Tokyo Hospital, Tokyo, Japan;* [3]*Department of Health Sciences, Oita University of Nursing and Health Sciences, Oita, Japan and* [4]*Department of Gynecology, University of Tokyo Hospital, Tokyo, Japan*

The purpose of this study was to evaluate the possibility of preserving ovarian function by ovarian shielding to reduce the irradiation dose in total body irradiation (TBI). The subjects in the study were females aged less than 40 years, who were undergoing allogeneic hematopoietic stem cell transplantation using a TBI-based regimen and who desired to have children after transplantation. For ovarian shielding, abdominal computed tomography (CT) and skin marking were performed in both the supine and prone positions, prior to the TBI. A pair of columnar blocks was placed just above the patient's body. Thus far three patients have been treated. The serum estradiol level decreased to an undetectable level (<8.5 pg/ml) after transplantation and the follicle-stimulating hormone (FSH) level increased above 90 mIU/ml in all patients and they became amenorrheic. However, regular menstruation recovered in patients no. 1 and 2 about 800 and 370 days after transplantation, respectively, with a decrease in the serum FSH level. Menstruation did not recover in patient no. 3, and serum estradiol was transiently detected above 20 pg/ml. The preservation of ovarian function was made possible by ovarian shielding. However, a longer follow-up is needed to know if normal pregnancy and delivery can occur.

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Keywords: ovarian function; total body irradiation; ovarian shielding;stem cell transplantation

Correspondence: Dr K Nakagawa, Department of Radiology, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113, Japan.
E-mail: nakagawa-rad@umin.ac.jp
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Introduction

The conditioning regimen before allogeneic hematopoietic stem cell transplantation is intended to eradicate tumor cells and to promote immunosuppression to prevent graft rejection. A combination of cyclophosphamide and total body irradiation (TBI) is the most widely used regimen in transplantation for leukemia. However, this regimen causes severe germ cell injury and infertility.[1,12] On the other hand, patients who have received cyclophosphamide alone for aplastic anemia frequently recover ovarian function after transplantation. Considering that the dose of cyclophosphamide in transplantation for aplastic anemia is usually higher than that in transplantation for leukemia (200 vs 120 mg/kg), we explored the possibility of preserving ovarian function by reducing the irradiation dose by ovarian shielding.

Patients and methods

Patients

Three female patients aged less than 40 years, who were undergoing allogeneic hematopoietic stem cell transplantation using a TBI-based regimen and who desired to have children after transplantation, were the subjects of this study. The study was approved by the Ethics committee of the University of Tokyo Hospital and all patients gave informed consent to participate in this study.

Transplantation procedure

The preparative regimen was a combination of cyclophosphamide at 60 mg/kg/day for 2 days and TBI at 2 Gy twice daily for 3 days. In patient no. 3, the dose of cyclophosphamide was reduced to 40 mg/kg/day for 1 day and etoposide at 20 mg/kg/day for 2 days was added instead, because of impaired cardiac function before transplantation. Cyclosporin A was administered as a continuous infusion at a dose of 3 mg/kg/day combined with short-term methotrexate (10–15 mg/m^2 on day 1 and 7–10 mg/m^2 on days 3 and 6, and optionally on day 11) to prevent GVHD. Patient no. 3, who underwent transplantation from a

two-locus-mismatched sibling donor, received alemtuzumab at 0.2 mg/kg/day from day −8 to day −3.[13] Methylprednisolone at 1–2 mg/kg/day was added for patients who developed grade II–IV GVHD. Prophylaxis against bacterial, fungal and *Pneumocystis carinii* infection consisted of fluconazole, ciplofloxacin, and sulfamethoxazole/trimethoprim. For prophylaxis against herpes simplex virus infection, acyclovir was given 750 mg/day intravenously or 1000 mg/day orally from days −7 to 35, followed by long-term low-dose (400 mg/day) oral administration until the end of immunosuppressive therapy. A cytomegalovirus antigenemia assay using C10/C11 antibody was performed at least once a week after engraftment. Ganciclovir was started when more than two positive cells were detected on two slides.

TBI and ovarian shielding

Patients were treated in a mobile box made of 10 mm thick polymethyl methacrylate 600 mm wide by 2000 mm long by 400 mm high. The box is capable of moving up to 250 cm forward and backward on the rails with a constant speed. Beam intensity and moving velocity defined dose rate in TBI.[14] Normally, beam opening of the linac is $400 \times 10\,cm^2$. Leukemia patients were usually treated in the supine position for three fractions in the morning and in the prone position for three fractions in the evening.

The center of the mobile box was selected to be a reference point to attain the prescribed dose. Beam intensity and moving velocity were determined based on the measurement of the doses in Mix-DP slab phantoms with an ionization chamber, but no corrections for patient body size were required due to the use of the mobile box.

In TBI for the leukemia patients, most commonly, a pair of customized metal blocks was placed on the mobile box for lung shielding. The blocks were fabricated according to the lung shape, which was obtained by use of the X-ray film taken in the box. Lung shielding was performed in a fraction of TBI out of six fractions for three consecutive days in most cases.

For ovarian shielding, abdominal magnetic resonance imaging (MRI) and computed tomography (CT) were performed prior to the TBI. Position of the ovaries was checked with T2-weighted image of MRI and was projected and marked onto the patient's skin. Trans-abdominal ultrasound on the day of treatment was performed for the accurate positioning of the shields. As the ovarian shielding was performed in all six fractions, CT scan and skin marking were performed both in supine and prone positions. A pair of columnar blocks (8 cm in height and 5 cm in diameter) was placed just above the patient's body, as demonstrated in Figures 1 and 2. For ovarian shielding, beam opening was $40 \times 2\,cm^2$ to decrease penumbra. Figure 3 shows a portal image taken during an actual TBI with ovarian shielding.

Actual measurement for humanoid phantom

Actual doses to the ovary were measured with glass dosimeters within a humanoid phantom. Doses of 2 Gy in the supine position and 2 Gy in the prone position were given for total body with the tracking technique. Twelve glass dosimeters were placed at the ovarian position of the humanoid phantom under shielding (Figure 4).

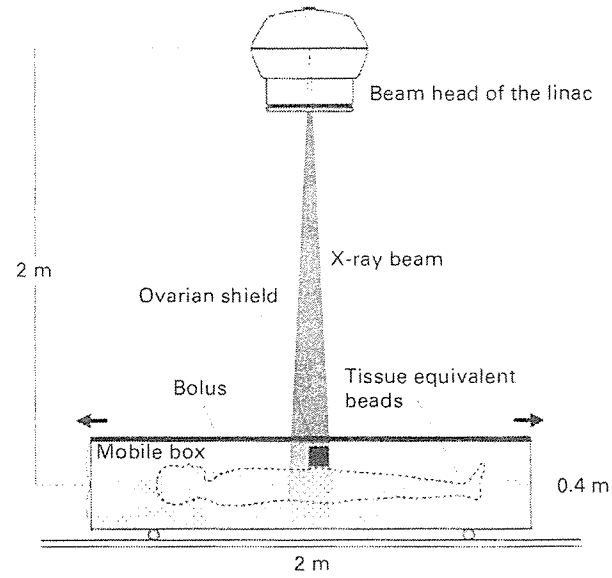

Figure 1 A schematic illustration of ovarian shielding in TBI.

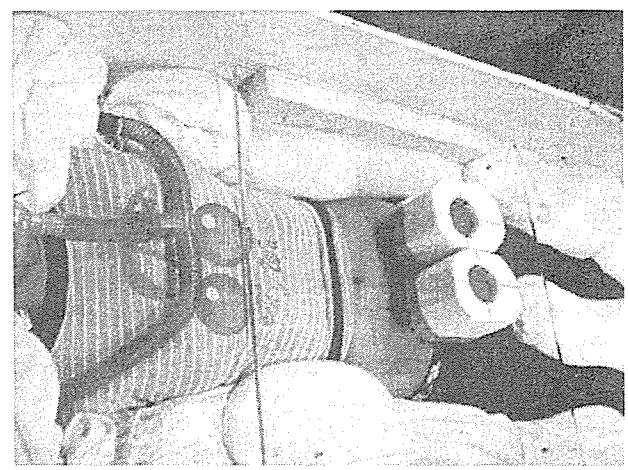

Figure 2 A pair of columnar blocks with dimensions of 8 cm in height and 5 cm in diameter. It was placed just above the patient's body.

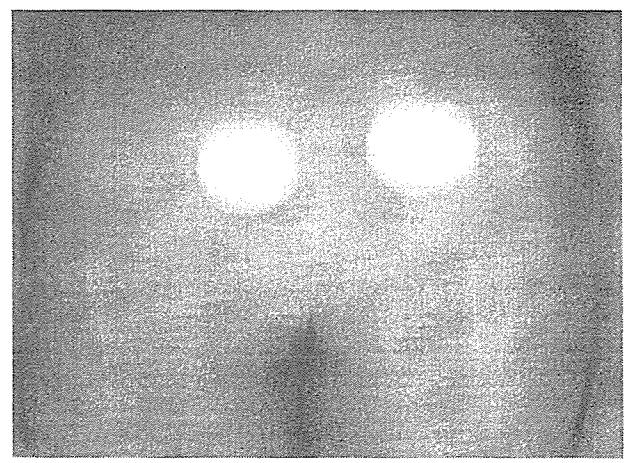

Figure 3 A portal image taken during an actual TBI with ovarian shielding.

Results

Patients

Thus far, three patients have been treated (Table 1). Two had chronic myelogenous leukemia in first chronic phase and had not received intravenous of antineoplastic agents before transplantation. The other patient had acute lymphoblastic leukemia in second remission and had received multiple courses of intensive chemotherapy. The donors were a matched unrelated donor, an HLA-identical sibling donor, and a two-locus-mismatched sibling donor in patients no. 1, 2, and 3, respectively. Patients no. 1 and 2 had regular menstruation before transplantation, but patient no. 3 already had chemotherapy-induced amenorrhea.

Transplantation outcome

All three patients had donor cell engraftment between days 15 and day 31 after transplantation. Acute GVHD was observed in only patient no. 1. She developed grade II acute GVHD limited to the skin, which was followed by extensive chronic GVHD. Patients no. 1 and 2 are alive without leukemia on days 1163 and 1055 after transplantation, respectively. However, patient no. 3 had a relapse of leukemia on day 223 and died on day 522.

Ovarian function after transplantation

The serum estradiol level decreased to an undetectable level (<8.5 pg/ml) after transplantation and the follicle-stimulating hormone (FSH) level increased above 90 mIU/ml in all patients and they became amenorrheic (Figure 5). However, patients no. 1 and 2 recovered regular menstruation about 800 and 370 days after transplantation, respectively, with a decrease in serum FSH level. In patient

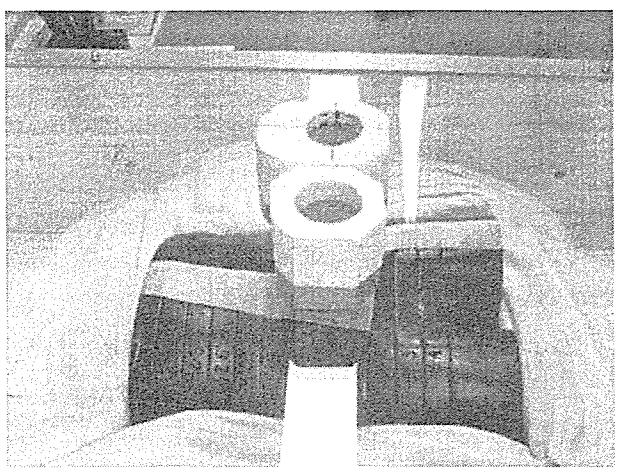

Figure 4 Humanoid phantom experiment.

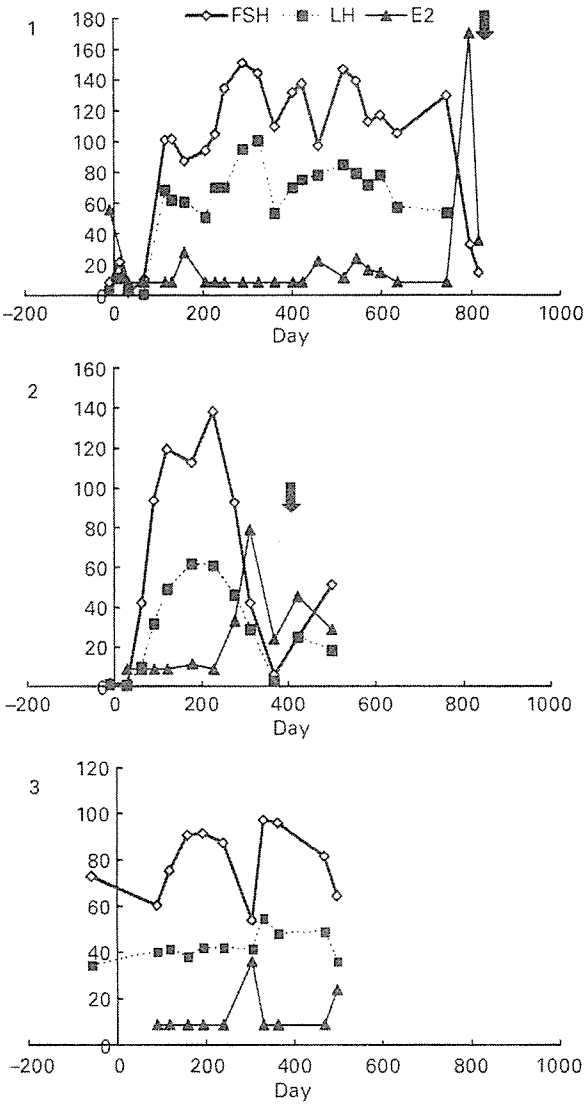

Figure 5 Ovarian function after transplantation. Arrows indicate the day of menstruation recovery.

Table 1 Patient characteristics

	Age[a]	*Diagnosis*	*Duration*[b]	*Prior Tx*	*Regimen*	*TBI dose (Gy)*	*Donor*
1	20	CML	7 years	HU, IFN	Cy/TBI	12	MUD
2	25	CML	6 months	HU	Cy/TBI	12	ISD
3	27	ALL	6 years	CCT	ETP/Cy/TBI	12	PMRD

[a]Age at transplantation.
[b]Duration form diagnosis to transplantation.
CML = chronic myelogenous leukemia; ALL = acute lymphoblastic leukemia; HU = hydroxyurea; IFN = interferon alpha; CCT = multiple courses of combined chemotherapy; Cy = cyclophosphamide; TBI = total body irradiation; ETP = etoposide; MUD = matched unrelated donor; MSD = HLA-identical sibling donor; PRMD = partially mismatched related donor.

no. 3, serum estradiol was transiently detected above 20 pg/ml, but she did not resume menstruating.

Assessment of basal body temperature and monitoring of follicle growth by sonohysterography would be useful to assess ovarian function of patients undergoing ovarian shielding in TBI.

Actual measurement for phantom

The mean and median actual doses measured by means of the glass dosimeters, which were inserted in the position of the ovaries in the humanoid phantom were between 1.041 and 1.042 Gy, respectively with a prescribed dose of 4 Gy. The range was 9.98–1.096 Gy. The results meant that the average total dose of the ovary was reduced from 12 to 3.123 Gy (74% less).

Discussion

The dose-limiting toxicity of TBI is interstitial pneumonia. Although the incidence of interstitial pneumonia has been significantly reduced by the use of fractionated irradiation compared to single dose irradiation,[13] 15% of patients still develop interstitial pneumonia after fractionated TBI. Therefore, lung shielding has been investigated to decrease lung toxicity of TBI. In a small nonrandomized study, the incidence of interstitial pneumonia was lower in patients who underwent TBI with lung shielding than in those who did not have shielding.[14] TBI may also affect renal function after transplantation. Therefore, Lawton *et al.*[15] attempted to protect renal function by renal shielding decreasing the total dose to the kidneys from 14 to 12 Gy, and the incidence of late renal dysfunction decreased from 26 to 6%.

The ovary is an organ sensitive to irradiation and the number of antral follicles per ovary has been shown to be reduced by ovarian irradiation in long-term survivors of childhood cancer.[16] Also, Shuck *et al.*[17] reported that all patients who received irradiation to the ovaries at greater than 15 Gy developed hormone failure. The radiation doses that cause 5 and 50% complications to the ovaries are about 3 and 10 Gy, respectively.[18] In this study, the irradiation dose to the ovaries was decreased by 75% by ovarian shielding and the total dose to the ovaries was estimated at about 3 Gy. Considering that recovery of ovarian function is frequently observed after a conditioning regimen of cyclophosphamide at 200 mg/kg only, the combination of cyclophosphamide at 120 mg/kg and TBI at 12 Gy with ovarian shielding should be reasonably protective to the ovaries.

Although patients who have received a conditioning regimen of cyclophosphamide and TBI may have spontaneous recovery of ovarian function long after transplantation, the incidence is less than 15% and it takes a median of 5 years for recovery of ovarian function after transplantation.[19] In this study, regular menstruation recovered in two of the three patients within 2 years after transplantation, showing the protective effect of ovarian shielding. However, spontaneous recovery of ovarian function is rarely seen after a combination of busulfan and cyclophosphamide, another major conditioning regimen for leukemia.[19 22] The risk of persistent alopecia is also more frequent after a busulfan-containing regimen.[23] Therefore, the combination of busulfan and cyclophosphamide should be avoided in young female patients, unless the patient has a condition that precludes the use of TBI, such as previous high-dose irradiation to a major organ.

It remains to be seen whether the recovery of ovarian function in these patients will allow a normal pregnancy and normal live birth. Recently, Carter *et al.*[24] analyzed pregnancy outcomes of female recipients and female partners of male recipients after hematopoietic stem cell transplantation. Seven females reported 13 pregnancies and 21 males reported 34 pregnancies. Most pregnancies were uncomplicated and resulted in 40 live births. Pregnancy outcomes were compared with those of their nearest-age siblings. The incidence of miscarriage or stillbirth was similar between the two groups. However, a larger study from the European Group for Blood and Marrow Transplantation[25] showed that the incidences of caesarean section, preterm deliverly, and low birthweight singleton birth offspring were higher compared to those in the normal population. Therefore, pregnancies in transplant recipients should be treated as high risks for maternal and fetal complications. In addition, the freezing of ovarian tissues or embryos might have a role as a back-up method of fertility treatment for the patient with ovarian failure after TBI.

We have shown that ovarian function could be preserved by ovarian shielding. However, a longer follow-up is needed to know whether this will allow normal pregnancy and delivery. Also needed is a larger study to evaluate the possible risk of increased relapse of leukemia after transplantation. In addition, the freezing of ovarian tissues or embryos might have a role as a back-up method of fertility preservation for patients who undergo hematopoietic stem cell transplantation and should be evaluated in the future.

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GENES, CHROMOSOMES & CANCER 45:482–494 (2006)

Genomewide Screening of DNA Copy Number Changes in Chronic Myelogenous Leukemia with the Use of High-Resolution Array-Based Comparative Genomic Hybridization

Noriko Hosoya,[1,2] Masashi Sanada,[1] Yasuhito Nannya,[1] Kumi Nakazaki,[1] Lili Wang,[1] Akira Hangaishi,[1] Mineo Kurokawa,[1] Shigeru Chiba,[1,2] and Seishi Ogawa[1,3,4]*

[1]Department of Hematology and Oncology, Graduate School of Medicine, University of Tokyo, Tokyo, Japan
[2]Department of Cell Therapy and Transplantation Medicine, University of Tokyo Hospital, University of Tokyo, Tokyo, Japan
[3]Department of Regeneration Medicine for Hematopoiesis, Graduate School of Medicine, University of Tokyo, Tokyo, Japan
[4]Core Research for Evolutional Science and Technology, Japan Science and Technology Corporation, Saitama, Japan

Chronic myelogenous leukemia (CML) evolves from an indolent chronic phase (CP) characterized by the Philadelphia chromosome. Without effective therapy, it progresses to an accelerated phase (AP) and eventually to a fatal blast crisis (BC). To identify the genes involved in stage progression in CML, we performed a genomewide screening of DNA copy number changes in a total of 55 CML patients in different stages with the use of the high-resolution array-based comparative genomic hybridization (array CGH) technique. We constructed Human 1M arrays that contained 3,151 bacterial artificial chromosome (BAC) DNAs, allowing for an average resolution of 1.0 Mb across the entire genome. In addition to common chromosomal abnormalities, array CGH analysis unveiled a number of novel copy number changes. These alterations included losses in 2q26.2–q37.3, 5q23.1–q23.3, 5q31.2–q32, 7p21.3–p11.2, 7q31.1–q31.33, 8pter-p12(p11.2), 9p, and 22q13.1–q13.31 and gains in 3q26.2–q29, 6p22.3, 7p15.2–p14.3, 8p12, 8p21.3, 8p23.2, 8q24.13–q24.21, 9q, 19p13.2–p12, and 22q13.1–q13.32 and occurred at a higher frequency in AP and BC. Minimal copy number changes affecting even a single BAC locus were also identified. Our data suggests that at least a proportion of CML patients carry still-unknown cryptic genomic alterations that could affect a gene or genes of importance in the disease progression of CML. This article contains Supplementary Material available at http://www.interscience.wiley.com/jpages/1045-2257/suppmat.

INTRODUCTION

Chronic myelogenous leukemia (CML) is a clonal disorder originating from pluripotent hematopoietic stem cells that is characterized by the Philadelphia (Ph) chromosome generated by the t(9;22)(q34;q11) (Rowley, 1973; Melo et al., 2003). CML typically shows 3 clinical stages: the initial indolent chronic phase (CP), followed by the intermediate accelerated phase (AP), and then the terminal fatal stage, blast crisis (BC). The prognosis of patients in BC is still very poor, with a median survival of only a few months (Calabretta and Perrotti, 2004). At present, no promising curative therapeutic options are available for patients in BC. The recent development of imatinib mesylate, which selectively inhibits enhanced tyrosine kinase activity of the chimeric BCR–ABL oncoprotein generated by the Ph chromosome, produced impressive therapeutic effects on patients in CP. However, the benefits from this drug seem short-lived once patients progressed to BC (Calabretta and Perrotti, 2004). Thus, to develop new therapeutic approaches for patients in BC, it is essential to identify molecular targets of blastic transformation.

The BC stage of CML is commonly associated with nonrandom secondary chromosomal changes that, in addition to the t(9;22), include +Ph, +8, i(17q), +19, t(3;21)(q26;q22), and t(7;11)(p15;p15) (Prigogina et al., 1978; Alimena et al., 1987; Blick et al., 1987; Melo et al., 2003), or with mutations in

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*Correspondence to: Seishi Ogawa, Department of Hematology and Oncology, Department of Regeneration Medicine for Hematopoiesis, Graduate School of Medicine, University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo 113-8655, Japan.
E-mail: sogawa-tky@umin.ac.jp

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the *TP53*, *CDKN2A*, *RB1*, or *RAS* genes (Ahuja et al., 1989; Kelman et al., 1989; LeMaistre et al., 1989; Feinstein et al., 1991; Nakai et al., 1992, 1994; Mitani et al., 1994; Nakai and Misawa, 1995; Sill et al., 1995; Nakamura et al., 1996; Fioretos et al., 1999; Beck et al., 2000). However, the molecular mechanisms responsible for disease progression in CML have not been fully understood. Array-based comparative genomic hybridization (array CGH) is a robust technology in which a large number of genomic clones are spotted on a glass slide and comparatively hybridized to differentially labeled tumor and reference DNA to enable high-resolution analysis of copy number changes in cancer genomes (Pinkel et al., 1998). Although the array CGH technique has been drawing increasing attention as a tool for studying alterations of genomes in various tumors (Albertson and Pinkel, 2003), it had not been applied to the analysis of patients with CML.

In the present study, to identify genes underlying stage progression in CML, we manufactured Human 1M arrays containing 3,151 bacterial artificial chromosome (BAC) DNAs and performed CGH analysis in 55 primary CML samples in different stages using these arrays. A number of previously unrecognized small cryptic genomic regions were identified.

MATERIALS AND METHODS

PATIENTS AND SAMPLES

After obtaining informed consent, bone marrow or peripheral-blood samples were obtained from 55 Japanese patients diagnosed with CML. Twenty-five of the patients were in the CP stage, 4 were in the AP stage, and 26 were in the BC stage. Clinical details are summarized in Table 1. After approval by the ethical committee at the University of Tokyo, all the samples were subjected to extraction of genomic DNA and anonymized to be used for further analysis according to the regulation of the Japanese government.

Array Fabrication

We constructed Human 1M arrays containing a subset of the FISH (fluorescence in situ hybridization) Mapped Clones V1.3 collection, which were obtained from BACPAC Resources Center (Children's Hospital Oakland Research Institute, Oakland, CA). After excluding clones missing mapping information, a total of 3,151 clones were finally selected for fabrication of Human 1M arrays (Supplementary Table 1; Supplementary material for this article can be found at http://www.interscience.wiley.com/jpages/1045-2257/suppmat), which could be used for genomewide copy number detection at an average resolution of approximately 1.0 Mb. Each BAC DNA was amplified with degenerated oligonucleotide-primed PCR (DOP-PCR) according to the protocol published by Fiegler et al. (2003), with the minor modification of an equimolar combination of DOP 1, 2, and 3 primers being used in the first PCR cycles. Amplified DNA was spotted in duplicate onto GAPS™ II coated slides (Corning, International K.K., Tokyo, Japan), using an Affymetrix 419 Arrayer (Affymetrix, Santa Clara, CA). Before hybridization, array slides were briefly rehydrated over steam and immediately dried on a 75 C heat block. After being baked in a drying oven at 65 C for 3 h and UV-crosslinked at 60 mJ, the slides were rinsed with 0.2× standard saline citrate (SSC) and then with distilled water. The reactive moieties of amino-silane remaining on the glass surface were inactivated for 20 min by gently shaking arrays in a blocking solution, which was freshly prepared by dissolving 4.15 g of succinic anhydride in 245 ml of 1-methyl-2-pyrrolidinone and then adding 22.5 ml of sodium borate (1M, pH 8.0). The slides were briefly rinsed with distilled water and preserved in a desiccator at room temperature, and immediately before hybridization, they were treated in boiling water for 2 min, placed in 100% cold ethanol, and then dried by centrifugation.

DNA Labeling and Hybridization to BAC Arrays

Genomic DNA was extracted from mononuclear cells of the bone marrow or peripheral blood of normal individuals using a PUREGENE™ DNA Isolation Kit (Gentra Systems, Minneapolis, MN). One microgram each of normal reference genomic (male or female) and test DNA were labeled with Cy3-dUTP and Cy5-dUTP, respectively, using a BioPrime® Array CGH Genomic Labeling System (Invitrogen, Carlsbad, CA). After overnight incubation at 37 C, unincorporated nucleotides were removed by use of a BioPrime™ Array CGH Purification Module (Invitrogen, Carlsbad, CA). The labeled test and reference DNA were ethanol-precipitated together with 80 μg of human Cot-1 DNA (Invitrogen, Carlsbad, CA) and 100 μg of yeast tRNA (Roche, Basel, Switzerland), redissolved in a hybridization mix [50% formamide, 5% dextran sulfate, 2× SSC, 5% Tris (pH 7.4), 0.1% Tween 20], and denatured at 75 C for 15 min. After incubation at 37 C for 30 min, the mixture was

TABLE 1. Patient Characteristics, Cytogenetic Description of Their Karyotypes, and Presence of *BCR/ABL* Confirmed by FISH or RT-PCR

Case No.	Sex	Age	Stage	Phenotype	Blast(%)	Karyotype	Methods of *BCR/ABL* detection
AP1	M	51	CML AP		6	46,XY,t(9;22)(q34;q11)(20/20)	NS
AP2	F	56	CML AP		2	50,XX,t(9;22)(q34;q11)+13, +19,+21,+22(8/15) 51,XX,t(9;22)(q34;q11), +t(9;22),+13,+19,+21, +22(4/15) 52,XX,+8,t(9;22)(q34;q11), +t(9;22),+13,+19,+21, +22(2/15) 47,XX,t(9;22)(q34;q11), +19,−20,−21,+der(22) t(9;22),+mar(1/15)	FISH and RT-PCR
AP3	M	37	CML AP		3.3	46,XY,t(9;22)(q34;q11)(18/20) 47,XY,+8,t(9;22)(q34;q11) (2/20)	RT-PCR
AP4	M	74	CML AP		9	46,XY,t(9;22)(q34;q11)(14/20) 45,XY,−21,t(9;22)(q34;q11) (5/20) 45,XY,−17,t(9;22)(q34;q11) (1/20)	RT-PCR
BC1	M	78	CML BC	ND	30	46,XY,t(9;22)(q34;q11)	NS
BC2	M		CML BC	ND	72	46,XY,t(9;22)(q34;q12)	NS
BC3	M	65	CML BC	lymphoid	90	46,XY,t(9;22)(q34;q11)	NS
BC4	M	33	CML BC	lymphoid	70	NA	NS
BC5	M		CML BC	lymphoid	65	NA	NS
BC6	F	48	CML BC	lymphoid	56	46,XX,t(9;22)(q34;q11)	NS
BC7	F	42	CML BC	lymphoid	60	46,XX,t(9;22)(q34;q11)	NS
BC8	F	42	CML BC	lymphoid	70	46,XX,t(9;22)(q34;q11)	NS
BC9	F	60	CML BC	myeloid	98	46,XX,t(9;22)(q34;q11)	NS
BC10	M	62	CML BC	myeloid	90	46,XY,t(9;22)(q34;q11)	NS
BC11	F		CML BC	myeloid	60	NA	NS
BC12	F	53	CML BC	myeloid	20	NA	NS
BC13	F		CML BC	myeloid	88	NA	NS
BC14	M		CML BC	myeloid	75	NA	NS
BC15	M	46	CML BC	myeloid	70	46,XY,t(9;22)(q34;q11)	NS
BC16	M	67	CML BC	myeloid	73	48,XY,t(3;21;18)(q21;q22;p11), +8,t(9;22)(q34;q11), +12(20/20)	FISH and RT-PCR
BC17	F	57	CML BC	myeloid	39	46,XY,t(9;22)(q34;q11)(10/10)	NS
BC18	M	51	CML BC	myeloid	86	46,XY,t(9;22)(q34;q11)(20/20)	FISH and RT-PCR

(Continued)

TABLE 1. Patient Characteristics, Cytogenetic Description of Their Karyotypes, and Presence of *BCR/ABL* Confirmed by FISH or RT-PCR (Continued)

Case No.	Sex	Age	Stage	Phenotype	Blast(%)	Karyotype	Methods of *BCR/ABL* detection
BC19	M	54	CML BC	myeloid	13	NA	FISH and RT-PCR
BC20	M	69	CML BC	myeloid	35	46,XY,t(9;22)(q34;q11)(13/20) 46,XY,t(9;22)(q34;q11),der(12)t(1;12)(q12;q24)(3/20) 46,XY,t(9;22)(q34;q11),der(19)t(1;19)(q12;p13)(4/20)	FISH and RT-PCR
BC21	M	71	CML BC	lymphoid	59	48,XY,11q+,+19,22q−,+22q−(20/20)	RT-PCR
BC22	M	62	CML BC	myeloid	61	46,XY,t(20;22)(p13;q11)(8/20) 47,XY,t(20;22)(p13;q11),+der(22)t(20;22)(p13;q11)(5/20) 45,XY,del(4)(q31),add(6)(p21),der(8;17)(q10;q10),+i(8)(q10),+add(9)(p22),−13,−16,t(20;22)(p13;q11),der(22)t(20;22),inc(1/20) 44,X,−Y,add(6)(p21),der(8;17)(q10;q10),+i(8)(q10),add(9)(p22),−13,−16,t(20;22)(p13;q11),inc(1/20) 74–87, ND, including add(6)(p21),der(8;17)(q10;q10),add(9)(p22),t(20;22)(p13;q11)(5/20)	RT-PCR
BC23	M	28	CML BC	myeloid	36	46,XY,t(9;22)(q34;q11)(18/20) 46,XY(2/20)	FISH and RT-PCR
BC24	M	60	CML BC	myeloid	44	48,XY,+8,t(9;22)(q34;q11),+der(22)t(9;22)(q34;q11)(19/20) 50,XY,+8,+8,t(9;22)(q34;q11),+21,+der(22)t(9;22)(q34;q11)(1/20)	FISH and RT-PCR
BC25	M	37	CML BC	myeloid	28	46,XY,t(9;22)(q34;q11)(12/20) 46,XY(8/20)	FISH and RT-PCR
BC26	M	64	CML BC	myeloid	85	45,XY,add(5)(q15),der(9)t(9;22)(q34;q11),add(12)(p11),del(17)(p11),add(19)(q13),−21,der(22)add(22)(p11)t(9;22)(17/20)	FISH and RT-PCR

(Continued)

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TABLE 1. Patient Characteristics, Cytogenetic Description of Their Karyotypes, and Presence of *BCR/ABL* Confirmed by FISH or RT-PCR (Continued)

Case No.	Sex	Age	Stage	Phenotype	Blast(%)	Karyotype	Methods of *BCR/ABL* detection
						45,XY,der(5)t(5;21)(q31;q11), der(9)t(9;22)(q34;q11), del(17)(p11),add(19)(q13), −21,der(22)add(22)(p11) t(9;22)(1/20)	
						48,XY,dup(3)(q21q27),add(4) (q21),der(5)t(5;21)(q31;q11), t(9;22)(q34;q11),add(13)(p11), del(17)(p11),+19,−21, +der(22)t(9;22),+mar(1/20)	
						48,XY,dup(3)(q21q27), 4, der(5)t(5;21)(q31;q11), t(9;22)(q34;q11),add(13)(p11), del(17)(p11),+19, 21, +der(22)t(9;22),+mar(1/20)	
CP1	M	40	CML CP		0	46,XY,t(9;22)(q34;q11)	NS
CP2	M	28	CML CP		0	NA	NS
CP3	F	60	CML CP		0	46,XX,t(9;22)(q34;q11)	NS
CP4	M	62	CML CP		0	46,XY,t(9;22)(q34;q11)	NS
CP5	M	38	CML CP		5	46,XY,t(9;22)(q34;q11)	NS
CP6	M	35	CML CP		5	46,XY,t(9;22)(q34;q11)	NS
CP7	M	54	CML CP		0	46,XY,t(9;22)(q34;q11)	NS
CP8	F		CML CP		0	NA	NS
CP9	M		CML CP		0	46,XY,t(9;22)(q34;q11)	NS
CP10	M		CML CP		1	46,XY,t(9;22)(q34;q11)	NS
CP11	F	32	CML CP		0	46,XX,t(9;22)(q34;q11)	NS
CP12	F	59	CML CP		0	46,XX,t(9;22)(q34;q11)	NS
CP13	M	51	CML CP		0	46,XY,t(9;22)(q34;q11)	NS
CP14	F		CML CP		0	46,XX,t(9;22)(q34;q11)	NS
CP15	M	46	CML CP		0	46,XY,t(9;22)(q34;q11)	NS
CP16	M	58	CML CP		0	46,XY,t(9;22)(q34;q11)	NS
CP17	F	58	CML CP		0	46,XX,t(9;22)(q34;q11)	NS
CP18	F	74	CML CP		0	46,XX,t(9;22)(q34;q11)	NS
CP19	F	54	CML CP		0	46,XX,t(9;22)(q34;q11)	NS
CP20	M		CML CP		0.5	46,XY,t(9;22)(q34;q11)	NS
CP21	M	71	CML CP		0	46,XY,t(9;22)(q34;q11)(20/20)	NS
CP22	M	40	CML CP		0	46,XY,t(9;22)(q34;q11)(20/20)	FISH and RT-PCR
CP23	M	43	CML CP		1.5	46,XY(20/20)	FISH and RT-PCR
CP24	F	55	CML CP		2	46,XX,t(9;22)(q34;q11)(20/20)	NS
CP25	M	75	CML CP		0	46,XY,t(9;22)(q34;q11)	NS

ND: not determined; NA: information not available; NE: not examined; NS: not specified in clinical records; Ph chr: Philadelphia chromosome; chr: chromosome; RT-PCR: reverse-transcriptase-polymerase-chain-reaction; The t(20;22)(p13;q11) in case BC26 is a variant Ph translocation.

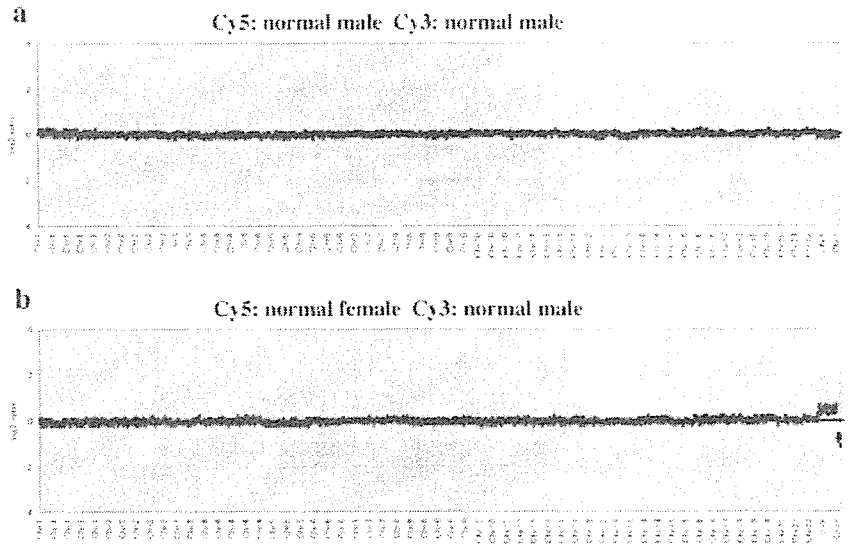

Figure 1. Representative array CGH results obtained from reference-versus-reference control hybridization. Clones are ordered from chromosomes 1 to 22, X, and Y and within each chromosome according to the UCSC mapping position (http://genome.ucsc.edu/; May 2004 draft). Each spot represents an average $\log_2$ signal ratio for each BAC locus. (a) For all loci, $\log_2$ ratios were within the thresholds −0.2 and 0.2 in the male-versus-male control experiment. (b) Gain in chromosome X (0.435 ± 0.124) and loss in chromosome Y (−0.807 ± 0.167) were clearly visualized in the female-versus-male control experiment (Cy5 and Cy3, respectively).

applied to an array slide placed in a MAUI® Mixer AO Hybridization Chamber Lid (BioMicro Systems, Salt Lake City, UT) and incubated at 37°C for 60–66 h using a MAUI Hybridization System (BioMicro Systems). After hybridization, the slides were washed once in a solution of 50% formamide and 2× SSC for 15 min at 50 C and once in 2× SSC for 15 min at room temperature. Slides were rinsed briefly with 0.2× SSC and dried immediately by centrifugation.

Image Analysis and Processing

After hybridization, the arrays were scanned by an Affymetrix 428® Array Scanner (Affymetrix, Santa Clara, CA). The scanned image was analyzed by an ImaGene v4.2 (BioDiscovery, Inc., Marina Del Rey, CA) in order to extract Cy3 and Cy5 signals for each spot, and after local background signals were subtracted, test/reference $\log_2$ ratios of the test and reference signals were calculated for all spots. The $\log_2$ ratios were normalized so that the average $\log_2$ ratio of all spots became zero. A spot was eliminated from the analysis if the signal intensity after the background subtraction in either Cy5 or Cy3 was less than −18 decibels or the duplicated signals differed by more than 0.4 in the $\log_2$ ratios. The average $\log_2$ ratios of the two replicate spots were calculated for the remaining spots. An experiment was not adopted if less than 90% of all spots met the above-mentioned criteria or if the standard deviation (SD) of all spots was larger than 0.25. Thresholds for copy number gain and loss were defined as $\log_2$ ratios of +2 SD and −2 SD, respectively. The reproducibility of the data was confirmed in two independent experiments for each tumor sample. For two representative cases, the consistency of the CGH results was confirmed by dye-swap experiments, in which tumor and reference DNA were inversely labeled with Cy3 and Cy5, respectively.

FISH Analysis

Interphase FISH experiments were performed as previously described (Wang et al., 2003).

RESULTS

Quality Test of BAC Array

Prior to the analysis of CML samples, control experiments were performed to evaluate the quality of the Human 1M array, in which DNA from normal individuals was used as a test sample. In the male-versus-male control hybridizations, $\log_2$ ratios for all spots were within the thresholds of −0.2 and 0.2 (Fig. 1a), whereas in the female-versus-male hybridizations, copy number gain of the whole chromosome X and copy number loss of the whole chromosome Y were detected successfully (Fig. 1b). In the latter experiments, the mean $\log_2$ ratios of the clones on the X and Y chromosomes were 0.435 ± 0.124 and −0.807 ± 0.167, respectively, compared to the mean $\log_2$ ratio of −0.008 ± 0.083 for all clones from autosomal chromosomes.

Higher Frequency of DNA Copy Number Changes in CML in BC and AP

A total of 55 CML samples in different stages were analyzed for copy number alterations by array CGH using Human 1M arrays. Table 2 lists the copy number alterations detected in individual

TABLE 2. Gains and Losses Detected by Array CGH

Case No.	Regions and clones that showed copy number gains	Regions and clones that showed copy number losses
AP1	3q26.2–q29 (RP11-91A17~RP11-233N20), 7p15.2–p14.3 (RP11-81F15~RP11-89N17)	22q13.2–q13.31 (RP11-81N15~RP11-66M5)
AP2	9p21.2 (RP11-81B19)-qter, Chromosome13, Chromosome19, Chromosome21, Chromosome22, ***22q11.1–q11.22 and 9q34.13–qter*** 22q13.1–q13.32 (RP11-4H24-RP11-133P21)	**8p23.1 (RP11-287P18)**
AP3	**5p15.1 (RP11-88L18, RP11-90B23), 19p13.2 (RP11-79F15)**	none
AP4	**8q21.2 (RP11-90G23)**	none
BC1	none	none
BC2	none	none
BC3	**4p15.33 (RP11-143120), 5p15.1 (RP11-88L18)** 8p12 (RP11-274F14-RP11-100B16), 9q, **19p13.2 (RP11-79F15)**, ***22q11.1–q11.22 and 9q34.13–qter***	**1q25.1 (RP11-177M16)**, 5q23.1–q23.3 (RP11-47L19-RP11-89G4), 5q31.2–q32 (RP11-11514~RP11-88H2), 7q31.1–q31.33 (RP11-79G19~RP11-90C13), 8pter–p12 (RP11-91P13), 9p
BC4	**8p23.1 (RP11-287P18), 22q11.21 (RP11-278E23)**	none
BC5	**8p23.1 (RP11-287P18), 17p13.3 (RP11-582C6), 19p13.2 (RP11-79F15)**	**17q21.31 (RP11-52N13)**
BC6	none	**5p15.1 (RP11-88L18)**
BC7	Chromosome8	none
BC8	none	none
BC9	none	**21q22.12 (RP11-17O20)**
BC10	**8p23.1 (RP11-287P18), 17p13.3 (RP11-582C6)**	none
BC11	none	none
BC12	**8p23.1 (RP11-287P18), 17p13.3 (RP11-582C6)**	**5p15.1 (RP11-88L18)**
BC13	none	Chromosomes 4 and 13
BC14	Chromosome8, **8q21.2 (RP11-90G23)**	none
BC15	**8p23.1 (RP11-287P18)**	none
BC16	Chromosome8*, **8p23.1 (RP11-287P18)**, Chromosome12*, **17p13.3 (RP11-582C6)**, ***22q11.1–q11.2 and 9q34.13–qter***	2q36.2–q37.3 (RP11-68H19~RP11-90E11*), 18pter–q11.2 (RP11-79F3)*
BC17	none	**1q25.3 (RP11-196B7), 17q21.31 (RP11-52N13)**
BC18	none	**1q25.3 (RP11-173E24), 1q25.3–q31.1 (RP11-162L13)**
BC19	none	**5p15.1 (RP11-88L18)**, 7p21.3–p11.2 (RP11-79O21~RP11-90N11)
BC20	none	**9q22.32 (RP11-223A21)**
BC21	**5p15.1 (RP11-88L18)**, Chromosome19*, ***22q11.1–q11.2 and 9q34.13–qter***	none
BC22	6p22.3 (RP11-43B4~RP11-288M24), 8p21.3 (RP11-89O4~RP11-274M9), 8p11.21 (RP11-282J24)-qter	8pter–p11.2 (RP11-284J3)
BC23	**5p15.1 (RP11-88L18)**	none
BC24	Chromosome8*, **17p13.3 (RP11-582C6), 17q22 (RP11-143M4)** ***22q11.1–q11.2 and 9q34.13–qter***	**5p15.1 (RP11-88L18), 7q11.21 (RP11-90C3)**
BC25	**5p15.1 (RP11-88L18), 19p13.2 (RP11-79F15)**	none
BC26	8q24.13–q24.21 (RP11-229L23-RP11-237F24), 19p13.2–p12 (RP11-84C17~RP11-91L5), ***22q11.1–q11.2 and 9q34.13–qter***	none
CP1	**8p23.1 (RP11-287P18), 17p13.3 (RP11-582C6)**	**1q25.1 (RP11-177M16), 1q25.3 (RP11-173E24), 5p15.1 (RP11-88L18)**
CP2	**17q21.31 (RP11-52N13)**	**1q25.1 (RP11-177M16)**
CP3	**17p13.3 (RP11-582C6), 17q12(CTD-2019C10)**	**5p15.1 (RP11-88L18), 17q25.2 (RP11-145C11)**

(Continued)

TABLE 2. Gains and Losses Detected by Array CGH (Continued)

Case No.	Regions and clones that showed copy number gains	Regions and clones that showed copy number losses
CP4	**5p15.1 (RP11-88L18), 19p13.2 (RP11-79F15)**	**1q25.1 (RP11-177M16), 17q21.31 (RP11-52N13)**
CP5	none	none
CP6	none	**5p15.1 (RP11-88L18)**
CP7	**19p13.2 (RP11-79F15)**	none
CP8	none	Chromosome3
CP9	none	none
CP10	none	none
CP11	none	none
CP12	**6q25.3–q26 (RP11-43B19)**	none
CP13	**8p23.1 (RP11-287P18), 17p13.3 (RP11-582C6)**	none
CP14	**19p13.2 (RP11-79F15)**	none
CP15	8p23.2 (RP11-113B7~RP11-89I12), **8p23.1 (RP11-287P18), *22q11.1–q11.2 and 9q34.13–qter***	none
CP16	**19p13.2 (RP11-79F15)**	**8q21.2 (RP11-90G23)**
CP17	none	none
CP18	**17p13.3 (RP11-582C6)**, 17p11.2–qter	**17q12(CTD-2019C10)** 17pter–p12
CP19	none	none
CP20	**19p13.2 (RP11-79F15)**	**5p15.1 (RP11-88L18)**
CP21	**8p23.1 (RP11-287P18), 15q22.31 (RP11-50N10), 22q13.32 (RP11-133P21)**	**1q25.1 (RP11-177M16)**
CP22	none	none
CP23	none	none
CP24	none	**5p15.1 (RP11-88L18, RP11-90B23), 8q21.3 (RP11-91K2), 9q32 (RP11-95J4)**
CP25	none	none

22q11.1–11.2 and ***9q34.13–qter*** corresponds to Philadelphia chromosome.
Gain of 17p11.2–qter together with loss of 17pter–p12 represents isochromosome 17q (i(17q)).
Copy number changes involving a single BAC are indicated in bold. Underlined are the regions (or BAC loci) whose copy number changes were confirmed by FISH.

cases, and Table 3 summarizes the number of cases showing each copy number alteration in different stages of CML. Array CGH successfully detected cryptic gains and losses that had been missed by conventional karyotyping analysis as well as large chromosomal changes that had been observed in prior conventional karyotyping analysis (Tables 2 and 3).

When analysis was confined to copy number alterations that involved at least two consecutive BAC clones, only 4 copy number alterations were detected in 25 patients in CP, whereas 38 copy number alterations were identified in 30 patients in AP/BC (Table 2). The frequency of DNA copy number alterations was significantly higher in AP/BC than in CP ($P < 0.005$).

Large and Small Cryptic Changes Detected by High-Resolution Array CGH

In the current analysis, the most frequent alteration was gain of extra Ph chromosomes (6 cases in AP/BC, 1 case in CP), which was inferred from gains of a distal part of 9q and a proximal part of 22q. Alterations of whole chromosomes, including gains of chromosomes 8 (4 cases in BC), 19 (2 cases in AP/BC), 13, 21, and 22 (1 case each in AP), and losses of chromosomes 3 (1 case in CP), 4, and 13 (1 case each in BC) were also observed (Tables 2 and 3). One CP patient (case CP18) displayed both gain of 17p11.2–qter and loss of 17pter–p12 material, suggesting the presence of an isochromosome 17q—i(17q)—which has repeatedly been reported in association with CML BC (Prigogina et al., 1978; Alimena et al., 1987; Fioretos et al., 1999; Melo et al., 2003), although the conventional karyotyping analysis had missed this abnormality.

Our array CGH analysis also uncovered cryptic changes that had not been reported in CML and therefore were novel regions implicated in the pathogenesis and progression of CML. Case BC3 was found to have a balanced t(9;22) translocation as the sole chromosomal abnormality in karyotyp-

TABLE 3. Summary of Copy Number Alterations Detected by Array CGH

	Stage	
	CP ($n = 25$)	AP + BC ($n = 30$)
Gains		
Unbalanced translocations or gains that were also detected by G-banding analysis		
Ph (22q11.1–q11.2 and 9q34.13–qter)	0	3
Chromosome 8	0	2
Chromosome 13	0	1
Chromosome 19	0	2
Chromosome 21	0	1
Chromosome 22	0	1
Gains in cases in which G-banding analysis was not done		
Chromosome 8	0	1
3q26.2–q29	0	1
7p15.2–p14.3	0	1
8p11.21–q24.3	0	1
Cryptic gains that were not detected by G-banding analysis (involving at least two consecutive BAC clones spotted on the array)		
Ph (22q11.1–q11.2 and 9q34.13–qter)	1	3
i(17q) (gain of 17p11.2–qter and loss of 17pter–p12)	1	0
Chromosome 8	0	1
6p22.3	0	1
8p12	0	1
8p21.3	0	1
8p23.2	1	0
8q24.13–q24.21	0	1
9p21.2–qter	0	1
9q	0	1
19p13.2–p12	0	1
22q13.1–q13.32	0	z1
Total number	3	26
Losses		
Losses in cases in which G-banding analysis was not done		
Chromosome 3	1	0
Chromosome 4	0	1
Chromosome 13	0	1
7p21.3–p11.2	0	1
22q13.1–q13.31	0	1
Cryptic losses that were not detected by G-banding analysis (involving at least two consecutive BAC clones spotted on the array)		
2q36.2–q37.3	0	1
5q23.1–q23.3	0	1
5q31.2–q32	0	1
7q31.1–q31.33	0	1
8pter–p12	0	1
8pter–p11.2	0	1
9p	0	1
18pter–q11.2	0	1
Total number	1	12

ing analysis (Tables 1 and 2). However, in array CGH, multiple copy number alterations, including gains in 8p12 and 9q, and an extra Ph chromosome, and losses in 5q23.1–q23.3, 5q31.2–q32, 7q31.1–q31.33, 8pter–p12, and 9p were reproducibly detected in duplicate experiments (Table 2, Fig. 2a). Case BC16 had a karyotype showing 48,XY, t(3;21;18)(q21;q22;p11),+8, t(9;22)(q34;q11), +12 (Table 1), whereas array CGH also detected an extra Ph chromosome as well as losses in 2q36.2–q37.3 and 18pter–q11.2 (Table 2, Fig. 2b). Also, in case BC22, CGH analysis disclosed cryptic copy number gains in three consecutive BACs within a small 6p22.3 region spanning 505 kb (Table 2, Fig. 2c).

These array CGH results were confirmed by FISH analysis using affected BAC clones as probes when Carnoy samples were available (Table 2, Fig. 2b and c). For example, the sample from patient BC16 showed, consistent with trisomies 8 and 12, three signals from clones RP11-150N13, on chromosome 8 (with an average $\log_2$ ratio of 0.449), and RP11-91I15, on chromosome 12 (with an average $\log_2$ ratio of 0.474), whereas clones RP11-116M19, on chromosome 2 (with an average $\log_2$ ratio of −0.538), and RP11-105C15, on chromosome 18 (with an average $\log_2$ ratio of −0.701), produced only one signal, confirming the presence of an allelic deletion in these regions (Fig. 2b). In patient BC22, clones RP11-228M24, at 6p22.3 (with an average $\log_2$ ratio of 1.158), showed multiple signals, in agreement with the copy number gain found in array CGH (Fig. 2c).

Copy number changes that involved only a single BAC locus (Table 4) were verified by FISH analysis for selected cases (Table 2 and Fig. 2d). In total, 75 single BAC copy number changes (SBCs) were identified in 24 BAC loci among 55 CML patients. Because 35 of the 75 SBCs, found in three BAC loci, were also identified in normal individuals (3 SBCs, at RP11-88L18, RP11-287P18, and RP11-586C6, in 10 healthy Japanese individuals; data not shown) and 37 SBCs in six BAC loci appeared as both copy number gains and losses depending on samples, suggesting that many of these are likely to represent polymorphisms known as large-scale copy number variations (LCVs; Iafrate et al., 2004; Sebat et al., 2004; Table 4). Indeed, 11 of the 24 BAC loci showing SBCs conformed to regions previously reported as LCVs (Table 4) (Iafrate et al., 2004; Sebat et al., 2004).

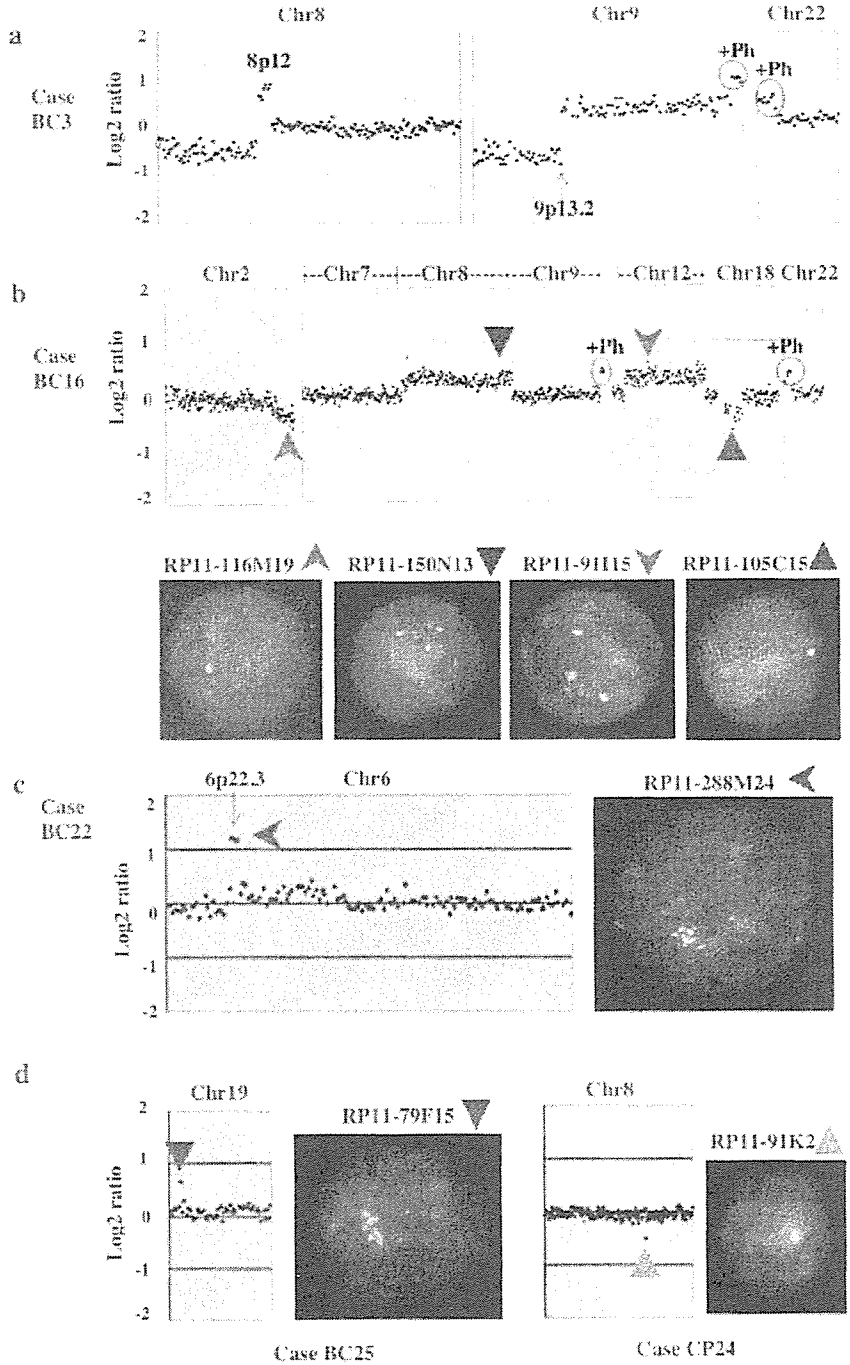

Figure 2. Gains and losses detected by array CGH and confirmed by FISH analysis. (a) Array CGH profile of case BC3, showing an extra Ph chromosome, gains in 8p12 and 9q and losses in 8pter–8p12 and 9p, not detected by karyotyping analysis; (b) Array CGH profile of case BC16, in which an extra Ph chromosome, gain in chromosome 8 with a higher-level of gain of the clone RP11-287P18, gain in chromosome 12, and losses in 2q36.2–2q37.3 and 18pter–18q11.2 were identified. Interphase FISH analysis of this case used the indicated biotin-labeled BAC clones as probes. Consistent with trisomies 8 and 12, clones RP11-150N13, on chromosome 8 (average $\log_2$ ratio of 0.449), and RP11-91H15, on chromosome 12 (average $\log_2$ ratio of 0.474), showed 3 signals, whereas clones RP11-116M19, on chromosome 2 (average $\log_2$ ratio of −0.538), and RP11-105C15, on chromosome 18 (average $\log_2$ ratio of −0.701), produced only one signal, confirming an allelic deletion in these regions. (c) Array CGH profile of case BC22, for which clone RP11-288M24, at chromosome 6p22.3 (average $\log_2$ ratio of 1.158), showed multiple signals, confirming copy number gains in this region. (d) Array CGH profiles of cases BC25 and CP24, in which single BAC copy number changes were observed. Copy number gain (RP11-79F15) and loss (RP11-91K2) were verified by FISH analysis.

Candidate Genes Implicated in Pathogenesis or Disease Progression of CML

The regions showing gain or loss in DNA copy number or breakpoint regions of unbalanced chromosomal translocations could harbor one or more genes implicated in the pathogenesis of CML or disease progression to BC. Supplementary Table 2 lists the representative genes within these regions identified in this study, not including the single BAC regions showing both gains and losses. Among previously reported cellular oncogenes or leukemia-related genes were *EVI1* (3q26), *FGFR1* (8p12), and *MYC* (8q24), which were included in the regions showing copy number gains in 3q26.2–q29, 8p12, and 8q24.13–q24.21, respectively (Supplementary Table 2). The 505-kb region showing

TABLE 4. Copy Number Alterations Involving a Single BAC Locus

	Stage	
	CP ($n = 25$)	AP + BC ($n = 30$)
Gains		
4p15.33 (RP11-143I20)[a]	0	1
5p15.1 (RP11-88L18)[a]	1	5
5p15.1 (RP11-90B23)	0	1
6q25.3–q26 (RP11-43B19)[a]	1	0
8p23.1 (RP11-287P18)[a]	4	6
8q21.2 (RP11-90G23)[a]	0	2
15q22.31 (RP11-50N10)	1	0
17p13.3 (RP11-582C6)[a]	4	5
17q12 (CTD-2019C10)	1	0
17q21.31 (RP11-52N13)	1	0
17q22 (RP11-143M4)	0	1
19p13.2 (RP11-79F15)[a]	4	4
22q11.21 (RP11-278E23)[a]	0	1
22q13.32 (RP11-133P21)	1	0
Losses		
1q25.1 (RP11-177M16)	4	1
1q25.3 (RP11-196B7)	0	1
1q25.3 (RP11-173E24)	1	1
1q25.3–q31.1 (RP11-162L13)	0	1
5p15.1 (RP11-88L18)[a]	5	4
5p15.1 (RP11-90B23)	1	0
7q11.21 (RP11-90C3)[a]	0	1
8p23.1 (RP11-287P18)[a]	0	1
8q21.2 (RP11-90G23)[a]	1	0
8q21.3 (RP11-91K2)[a]	1	0
9q32 (RP11-95J4)[a]	1	0
9q22.32 (RP11-223A21)	0	1
17q12 (CTD-2019C10)	1	0
17q21.31 (RP11-52N13)	1	2
17q25.2 (RP11-145C11)	1	0
21q22.12 (RP11-17O20)	0	1

Shaded areas point to the loci that showed both gains and losses in different samples.

[a]Regions previously reported to show large-scale copy number variations (LCVs).

copy number gain at the chromosome band 6p22.3 contained *OACT1* (*O*-acetyltransferase domain containing 1) and *E2F3* (E2F transcription factor 3), both known genes (Supplementary Table 2). It is not clear whether this region overlapped with the breakpoint region of the recurrent translocations t(6;19)(p22;q13) and t(6;9;22)(p22;q34;q11) in CML (Huret et al., 1989; Meza Espinoza et al., 2004; Yehuda et al., 1999), because the precise molecular breakpoints at 6p22 in these cases have not been characterized. The 346-kb region at 8p23.2 that showed copy number gain includes *CSMD1* (CUB and sushi multiple domains protein 1 precursor), the only transcriptome (Supplementary Table 2). Other abnormalities newly identified in this study involved mostly large regions of 2q26.2–q37.3 (16.8 Mb), 5q23.1–q23.3 (10.6 Mb), 5q31.2–q32 (6.50 Mb), 7p15.2–p14.3 (6.14 Mb), 7p21.3–p11.2 (41.7 Mb), 7q31.1-q31.33 (17.9 Mb), 8p21.3 (2.18 Mb), and 19p13.2–p12 (12.1 Mb), which made it difficult to pinpoint the candidate target genes.

DISCUSSION

In this article, we have shown genomewide detection of DNA copy number changes in a total of 55 CML patients at different stages using high-resolution array CGH. Using this technique, we delineated not only previously reported abnormalities, but also novel alterations involving narrow regions that may harbor only one or several candidate genes involved in the pathogenesis or disease progression of CML.

A number of cryptic copy number alterations that had been missed by karyotyping analysis were detected in array CGH analysis. Seven patients were found to have extra Ph chromosomes, which was the most frequent alteration in our series, although this alteration had not been detected by prior G-banding analysis in four of the seven patients (57%). In addition, more than 10 novel, cryptic copy number alterations were uncovered at a significantly higher frequency in patients in BC and AP, suggesting that these regions may contain genes relevant to the pathogenesis of CML, especially in progressive stages. Considering the wide variety of copy number alterations detected in AP/BC cases and that the majority of these abnormalities were observed in a single patient in our series, there might be a large heterogeneity in the molecular pathogenesis of CML AP/BC cases, and it may be possible that analysis of a larger number of patients could disclose novel recurrent molecular defects in CML. Alternatively, the genes included in the affected regions may also be deregulated by other mechanisms such as point mutations or epigenetic effects, which could not be detected by copy number analysis.

Many of the cryptic gains or losses affecting a single BAC locus are thought to represent copy number polymorphisms or LCVs rather than tumor-specific changes, and given their high frequency, it would be difficult to discriminate tumor-specific changes from LCVs. In our analysis, SBCs (or LCVs) seemed to be more frequently found in CML than in normal individuals using the same reference set (55 of 75 in CML vs. 3 of 10 in normal individuals, $P = 0.021$). Although