Somatic Gene Alteration of AIB1 Gene in Patients with Breast Cancer

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Abstract. AIB1 (amplified in breast cancer 1) is a coactivator which stimulates the transcriptional activity of the liganded-estrogen receptor (ER). It has been reported that AIB1 gene is amplified and overexpressed in some breast cancer cell lines. AIB1 contains a stretch of homopolymeric glutamines (poly-Q). We reported that the poly-Q shows polymorphism, which provides an opportunity to study somatic gene alteration such as loss of heterozygosity (LOH). In the present study, we aimed to investigate the frequency of somatic gene alteration in Japanese patients with breast cancer. Amplification of AIB1 gene was detected only in 1 (2.6%) of 39 breast cancer tissues by DNA dot blot analysis. On the other hand, LOH was found in 2 (8%) breast cancer tissues out of 25 patients showing heterozygosity in peripheral blood mononuclear cells (PBMC). Taken together, both LOH and amplification of AIB1 gene were identified in breast cancer patients, raising the possibility that AIB1 have oncogenic as well as antioncogenic potential for the pathogenesis of breast cancer.

Key words: AIB1, LOH, Polymorphism, Amplification, Breast cancer

ESTROGEN contributes to the tumorigenesis and progression of breast cancer [1, 2]. AIB1 is a member of the SRC-1 (Steroid Receptor Coactivator-1) family and enhances ligand-dependent transactivation interacting with ligand-bound nuclear receptors, including estrogen receptors (ERs) [3]. Anzick et al. reported that AIB1 gene is amplified in approximately 10% of 105 unselected specimens of primary breast cancers [3]. Bautista et al. reported that amplification of the AIB1 gene was observed in 4.8% out of 1157 breast cancer samples, and that the amplification of AIB1 gene was related to the estrogen receptor status and size of breast tumor [4]. Gene amplification frequently occurs in oncogenes and is correlated with the clinical behavior and histological characteristics of the cancer cells [5-8]. Taken together, it is suggested that AIB1 has an oncogenic potential for breast cancer. However, the frequency of AIB1 gene amplification in Japanese patients with breast cancers is not known.

AIB1 gene is located at 20q 12 and its cDNA encodes 1420 amino acids-protein which contains a stretch of homopolymeric glutamine residues (poly-Q) between amino acid positions from 1244 to 1272 [3]. The poly-Q residues are encoded by the repeat of CAG triplet, which is often polymorphic among populations. Our group as well as others have previously reported that a stretch of poly-Q in AIB1 shows polymorphism in healthy subjects [9, 10]. The CAG repeats in several genes harbor instability, and expanded triplet repeats in several genes are known to cause neurodegenerative disorders [11]. In
addition, poly-Q itself can activate transcription when fused to the DNA binding domain of GAL4 factor and its activity was affected by the length of poly-Q [12], suggesting that the length of poly-Q might modify the function of proteins carrying the residue. Indeed, the length of poly-Q in human androgen receptor correlates with a decrease in ligand-dependent transactivation [13]. Yet the possible relation between the polymorphism of poly-Q in AIB1 gene and breast cancer has not been studied. The polymorphism also provides an opportunity to study loss of heterozygosity (LOH), which is reported in tumor suppressor gene such as p53, RB1 and BRCA1 [14].

In the present study, we investigated possible somatic gene alteration, such as gene amplification and LOH, in AIB1 gene in breast cancer patients by analyzing the polymorphism in poly-Q. We also compared the polymorphism in the Japanese patients with breast cancer to that of control subjects to evaluate whether the length of poly-Q in AIB1 gene affects the pathogenesis of breast cancer.

**Materials and Methods**

**DNA samples and clinical data**

Genomic DNA was extracted from peripheral blood mononuclear cells (PBMC) of 86 consecutive series of patients who were diagnosed to have breast cancer in the Second Department of Surgery, Nagoya University, during the period between April 1993 and December 1997. Informed consent was obtained from each subject before blood sampling. Genomic DNA was also extracted from breast cancer-free patients who consented to the study and were used as control controls. Mean age was 52.1 ± 11.7 and 44.9 ± 13.8 for breast cancer patients (86 females) and controls (10 males and 48 females), respectively. Genomic DNA was also extracted from 51 breast cancer specimens obtained at surgery in the Second Department of Surgery, Nagoya University. Data on maximum diameter of tumor, ER status and lymph node metastasis were available in 77, 75 and 79 out of 86 patients with breast cancer, respectively. Details of these data are presented in Table 1.

**DNA dot blots and hybridization**

Five μg of DNA samples from 39 breast cancer tissues were spotted onto GeneScreen Plus membrane

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<td><strong>AIB1 Genotype</strong></td>
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Breast cancer patients as well as controls are divided into groups by AIB1 genotype. For breast cancer patients, mean ± SD of age, tumor size, estrogen receptor status (ER(+/−)) and the presence or absence of lymph node metastasis (N(+/−)) are shown. The number in parentheses for tumor size indicates the number of patients. The asterisks were placed to indicate that the numbers are smaller than that of each group because information was not applicable or missing.
LOH OF AIB1 GENE IN BREAST CANCER

(NEN Life Science) according to manufacturer protocol. The AIB1 cDNA probe which spans nucleotides 2318–3700 was labeled with [α-32P]dCTP (3000 Ci/mmol; New England Nuclear) using a random primed labeling kit (Boehringer Mannheim). The membrane was hybridized as previously described [15]. After hybridization, the membrane was washed and radioactivities for each dot were quantified by BAS 2000 bioimage analyzing system (Fuji Film Co., Tokyo, Japan). To control the amount of DNA, the membrane was rehybridized with Alu sequence, which presents as multiple copies in human genome, as a probe to normalize the dosage of AIB1 gene.

**PCR analysis and statistical analysis.**

The region encoding poly-Q in AIB1 gene was amplified by PCR and the length of the amplified fragments were analyzed as previously described [9, 16]. Difference of the length of poly-Q between groups was compared using the Mann-Whitney test. LOH was estimated by comparing the amplified fragments from PBMC and tumor tissue.

**Results**

First, the frequency of AIB1 gene amplification was evaluated using 39 breast cancer tissues, from which more than 5 µg of DNA could be obtained for dot blot analysis. DNA dot blot revealed AIB1 gene amplification in only 1 (2.6%) sample (Fig. 1). The patient was operated on age 55. The tumor was 60 mm in diameter and estrogen receptor positive. Axillary lymph node metastasis was present.

Next, to investigate the polymorphism of AIB1 gene in 58 control subjects, 86 patients with breast cancer and 51 tissue samples, poly-Q coding region in AIB1 gene was amplified by PCR. The fragment length of PCR products was analyzed and the number of glutamines in each allele was determined (Fig. 2). Allele type of each subject was shown at the bottom of the Figure. To examine the somatic gene alteration on AIB1 locus, the polymorphism of AIB1 gene was compared between PBMC and tissue samples of breast cancer patients. In 2 (8%) out of 25 patients showing heterozygosity in PBMC, only one allele could be amplified by PCR from breast cancer tissues indicating LOH (Fig. 3). These patients, who had 28Qs and 29Qs allele in PBMC, had only 29Qs allele in breast cancer tissues. The other patients showed no difference in allele pattern between PBMC and tumor samples. The two patients were operated on age 58 and 45, respectively. Both tumors were 30 mm in diameter. One was ER positive and showed axillary lymph node metastasis, while the other was ER negative with no lymph node metastasis.

Finally, the polymorphism of AIB1 gene was compared between control subjects and patients with breast cancer to investigate whether polymorphism of AIB1 gene affects the susceptibility to breast cancer. The number of glutamines in the poly-Q region ranged from 26 to 32 in control subjects with heter-
ozygosity of 48% and 22 to 29 in patients with breast cancer with heterozygosity of 39%. Genotype of AIB1, age of the patients, clinicopathological features are summarized in Table 1. Difference in distribution of alleles in control subjects and patients with breast cancer was evaluated using allele frequency. The most frequent allele was 29 in both groups. Although no allele longer than 29Qs was found in the patients with breast cancer, no statistically significant difference in distribution between the controls and the patients with breast cancer was found (Fig. 4). Considering that expanded poly-Q has an apoptotic potential [17], it is noteworthy that

Fig. 2. Fragment length analysis of PCR products.
A representative result for PCR fragment length analysis of breast cancer patients is shown. Length of the amplified fragment is also indicated as numbers of glutamine in the poly-Q stretch. Allele type of each subject is shown at the bottom of the figure. Q: glutamine

Fig. 3. LOH on AIB1 gene.
A representative result of PCR analysis is shown. Note that two alleles are present in PBMC while only one allele could be detected in breast cancer tissue. The faint 28Qs-band present in tissue may be derived from contamination of PBMC in tumor sample.

Fig. 4. Distribution of alleles in control subjects and patients with breast cancer.
Distribution of alleles in each group is shown as frequency (%). BC: patients with breast cancer, C: control subjects, NS, not significant.
an extremely short allele 22Qs was found in a patient with breast cancer. It was also evaluated whether the length of poly-Q in the patients with breast cancer correlates with clinical features such as maximum diameter of tumor, ER status and lymph node metastasis. No significant difference in distribution was found between the two groups, respectively (data not shown).

Discussion

Somatic gene alterations such as gene amplification and LOH play a role in the pathogenesis of breast cancer [14]. Amplification of oncogene and growth factor receptor is common in breast cancer. Up to 40% of breast cancer may have amplification in at least one of oncogenes including ERBB2, MYC and INT2 [14]. Amplification of ERBB-2 is found in 25-30% of primary breast cancers and is associated with poor clinical outcome [18]. Anzick et al. reported that evaluation of 105 unselected specimens of primary breast cancer revealed AIB1 amplification in approximately 10% [3]. Moreover, Bautista et al. reported that amplification of the AIB1 gene was observed in 4.8% out of 1157 breast cancer samples [4]. Our present study revealed that amplification of AIB1 gene was found in 2.6% of breast cancer tissues of Japanese. Since the amount of DNA required for dot blot analysis is considerably high, the number of the tissues analyzed in the present study was much less compared to that in Bautista’s study. At the same time, these results raise the question whether that amplification of AIB1 gene is playing a major role in the tumorigenesis of breast cancer. Since the frequency of AIB1 gene amplification studied by Bautista et al. as well as the present report is much less compared to that of ERBB-2 reported by Szollosi et al. [4,18], we believe that the contribution of AIB1 amplification for development of breast cancer is, at least, smaller compared to that of ERBB2, MYC and INT2 gene.

On the other hand, LOH is frequently identified in the locus close to tumor suppressor genes. Two tumor suppressor genes involved in pathogenesis of breast cancer, BRCA1 and BRCA2, have been identified by LOH analysis [19]. In sporadic breast cancer, the frequency of LOH in BRCA1 region using microsatellite markers are 4 to 21% for each marker [20-22]. In the present study, we reported for the first time that LOH of AIB1 gene was found in 2 (8%) out of 25 breast cancer tissues examined. This result suggest that the region coding poly-Q could be a genetic marker for tumor suppressor gene, which may be the AIB1 gene itself or a gene located close to AIB1 locus.

In the present study, both LOH and amplification of AIB1 gene were identified in Japanese patients with breast cancer. This result suggests that AIB1 can be oncogenic in some cases while it can be anti-oncogenic in other cases. Similar findings were reported in 53 gene. When it was overexpressed in various transformed cells, it was considered to be an oncogene, but when p53 was shown to suppress transformation, it turned out to be an antioncogene [23]. To clarify the role of AIB1 in pathogenesis of breast cancer, the frequency of LOH should be examined in breast cancer patients of different ethnic backgrounds. Although the length of poly-Q was not associated with susceptibility of breast cancer in the present study, further studies with larger numbers of patients should provide insights into the involvement of AIB1 in tumorigenesis.

Acknowledgements

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