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In Breast Cancer, Amplification of the Steroid Receptor Coactivator Gene *AIB1* Is Correlated with Estrogen and Progesterone Receptor Positivity[1]

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Abstract

The *AIB1* gene was isolated upon microdissection of the homogeneously staining regions observed in breast cancer cell lines. It was subsequently shown to map at a region at 20q12 that is frequently amplified in breast tumors. In a screen of breast tumor cell lines, of all the genes mapping to the region, *AIB1* appeared to be the most consistently amplified and overexpressed. *AIB1* shares homology with the SRC-1 family of nuclear receptor coactivators. It was found to interact in a ligand-dependent manner with the estrogen receptor (ER) and to result in increased levels of estrogen-dependent transcription. These properties could be of important biological significance in breast and ovarian cancerigenesis, and we were, therefore, interested in determining whether the amplification of the *AIB1* gene was associated with a particular phenotype or subgroup in these tumors. We tested a population of 1157 breast and 122 ovarian tumors in which DNA amplification had been determined previously at 15 chromosomal locations. Amplification of the *AIB1* gene was observed in 4.8% of breast cancers and 7.4% of ovarian cancers. In breast tumors, *AIB1* was correlated with ER and progesterone receptor positivity, as well as with tumor size. Correlation was also observed with the amplification of *MDM2* and *FGFR1* genes, but interestingly, no correlation was found with the amplification of *CCND1*, which is known to be strongly associated with ER. Furthermore, analyzing at 20q12–q13 range, we show the existence of three amplification cores, represented by *AIB3/AIB4*, *AIB1*, and *RMC20C001*. *AIB1* and *CCND1* amplifications may, thus, represent two different subsets of ER-positive breast tumors.

Introduction

In human breast tumors, DNA amplification is a prevalent mode of expression of genetic instability (1). Comparative genomic hybridization studies showed that 28 chromosomal arms developed DNA copy gains in this tumor type, of which a subset of 7 regions is most frequently involved (2–4). The long arm of chromosome 20 is, along with 1q, 8q, and 17q, one of the most recurrent sites of DNA copy gains in breast cancer: gains are observed on this chromosomal arm in up to 18% of primary tumors and 40% of cell lines (2, 5). Amplification at 20q has been shown to range from 20q11 to 20q13, and three independently amplified regions have been described (6). Three genes, *AIB1*, *AIB3*, and *AIB4*, mapping at 20q12 (*AIB1*) and 20q11 (*AIB3* and *AIB4*), have been isolated upon chromosomal microdissection of homogeneously staining regions (7). All three genes have been shown to be amplified and overexpressed in breast cancer cell lines, although *AIB1* appeared to be the most frequently involved. *AIB1* belongs to the SRC-1 family of nuclear receptor coactivators and was shown to interact at the protein level with ERs[3] in a ligand-dependent manner (8). Furthermore, in transient transfection assays, its overexpression resulted in increased levels of estrogen-dependent transcription. Given the steroid hormone responsiveness of a large fraction of breast and ovarian cancer, the overexpression of the *AIB1* gene, secondary to DNA amplification, could be of biological importance in these tumors.

A number of genetic alterations affecting primary cancers have been shown to bear diagnostic and/or prognostic significance. This suggests that gathering a set of alterations that is extensive as possible could open the way to a classification of tumors according to their genetic profiles. As part of such an approach, we studied, in a recent work, DNA amplification using 26 probes to genes or genetic markers mapping at 15 chromosomal locations in a cohort of 1875 breast tumors. Genes or genetic markers tested were included in the study because they were either known to be amplified or suspected to be in breast cancer. We showed that amplification patterns could be associated with cancer phenotype, thus allowing the definition of phenotypic subgroups (9). We were, thus, interested in testing

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[3] The abbreviations used are: ER, estrogen receptor; PR, progesterone receptor.

Table 1 Incidence and levels of DNA amplification in the tested breast and ovarian tumors

	Breast tumors		Ovarian tumors	
Amplified locus	No./total (%)	Amplification level (fold)	No./total (%)	Amplification level (fold)
AIB1	56/1157 (4.8)	2–8	9/122 (7.4)	2–10
AIB3	16/379 (4.2)	2–5	ND[a]	ND
AIB4	16/379 (4.2)	2–5	ND	ND
RMC20C001	66/1097 (6.0)	3–10	3/120 (2.5)	2–5

[a] ND, not determined.

for the amplification of the *AIB1* gene in this set of tumors, the amplification patterns of which had been characterized. Our aim was to relate *AIB1* amplification to previously determined amplifications, as well as to clinicopathological parameters. Here, we have analyzed 1157 breast tumor DNAs by Southern blotting with a genomic probe to *AIB1*, and our data indicate that this gene shows increased copy number in 4.8% of the tumors. Although the amplification of *AIB1* frequently occurs concomitantly with that of the *RMC20C001* locus, which maps at 20q13, both events correspond to different subsets of breast tumors. *AIB1* amplification clearly correlated with ER and/or PR positivity, whereas *RMC20C001* did not. Furthermore, we have analyzed *AIB1* amplification in a series of 122 ovarian cancers. We observed that 7.4% of ovarian tumors showed *AIB1* gene amplification, and interestingly, levels of amplification were, on average, higher than those observed in breast tumors.

Materials and Methods

Tumor Samples and Clinical Material. Collection and handling of tumor material and processing of clinical data were as described previously (9). The breast tumor series was composed of 72% invasive ductal carcinoma, 12% invasive lobular carcinoma, 14.5% untyped or other invasive adenocarcinomas, and 1.5% ductal carcinoma *in situ*. Nodal invasion stratified as follows: N^-, 52%; and N^+, 48%. Scarff and Bloom grading was: grade 1, 7%; grade 2, 49%; and grade 3, 44%. Steroid receptor status was: ER^+ (>10 fmol/mg protein), 68%; ER^-, 32%; PR^+ (>10 fmol/mg protein), 56.5%; and PR^-, 43.5%. Sixty-seven patients (5%) had received presurgical treatment. Histological types were determined according to WHO guidelines. The series of ovarian tumors was composed of 122 primary ovarian carcinomas, including 84 serous, 11 mucinous, 9 clear cell, 16 endometrioid, and 59 untyped tumors. Tumor stages were: stage 1, 40; stage 2, 10; stage 3, 72; and stage 4, 26.

Preparation of Southern Blots and Hybridization. Southern blots preparation and hybridization were as described previously (9).

DNA Probes. The probe to *AIB1* was a 1.087-kb genomic fragment spanning nucleotides 1376–2276 in the *AIB1* cDNA sequence subcloned from a bacterial artificial chromosome clone containing *AIB1* (8). This probe hybridizes to an ~9-kb *Eco*RI fragment in genomic DNA. Probe to *AIB3* corresponded to a 3-kb *Eco*RI-*Kpn*I fragment isolated from the R8-7 cDNA clone (7). It hybridized to 2-kb *Eco*RI fragment. The *AIB4* probe corresponded to a 1.5-kb cDNA clone, revealing a 14-kb *Eco*RI signal (7). The *RMC20C001* and reference probes were as described previously (10).

DNA Amplification Analysis and Quantification of Hybridization Signals. DNA amplification analysis and quantification of hybridization signals were performed essentially as described previously (9).

Statistics and Data Analysis. Clinical and molecular data associated with each patient were pooled in a computer-assisted database (Paradox for DOS; Borland Software). Statistical analyses were performed with the EpiInfo Version 3.0 software package from the Centers for Disease Control (Atlanta, GA) for classical χ^2 tests. Tests for data stratification were performed with the Knowledge Seeker Version 3.0 from Angoss Software (11).

Results

Incidence of AIB1 Amplification in Breast and Ovarian Tumors. We analyzed, as described previously (9), a series of 1157 breast tumor and 122 ovarian DNAs digested with *Eco*RI and immobilized on Southern blotting membranes with a genomic probe to the *AIB1* locus. As shown in Table 1, amplification was observed in 56 of 1157 (4.8%) breast tumors. On average, copy number increases ranged from 2- to 8-fold. These numbers were in keeping with those found with the RMC20C001 (6% of amplified tumors and amplification levels ranging from 2- to 10-fold). Interestingly, ovarian tumors showed a somewhat different picture. Whereas *AIB1* was found amplified in 7.4%, RMC20C001 amplification occurred less frequently (2.5%). Correspondingly, amplification levels of *AIB1* exceeded those of RMC20C001 (Table 1).

Patterns of Amplification at 20q in Breast Tumors. To obtain a picture of DNA amplification patterns at 20q, we analyzed, in combination to *AIB1* and *RMC20C001*, a subset of 379 breast tumor DNAs with probes to *AIB3* and *AIB4*, two genes that are amplified in breast cancer cell lines and that map to 20q11 (7). These 379 tumors correspond to subset of reference DNAs that we use for exploratory studies. If genes or genetic markers showed correlations or trends of associations in this set of tumors, the analysis was extended to a larger cohort. Patterns of amplification are presented in Fig. 1. Our data show that *AIB1* and *RMC20C001* were amplified alone in 16 and 13 tumors, respectively, whereas 5 cases showed coamplification of both these genes. *AIB3* and *AIB4*, which map in close proximity to each other, were systematically coamplified in 11 tumors and concomitantly with *AIB1* in 3 further cases. We noted that amplification levels for *AIB3*/*AIB4* were inferior to those observed with either *AIB1* or *RMC20C001*. Altogether, 50 of 379 tumors (13.2%) presented amplification of at least one of the tested loci. In addition to these tumors tested by Southern

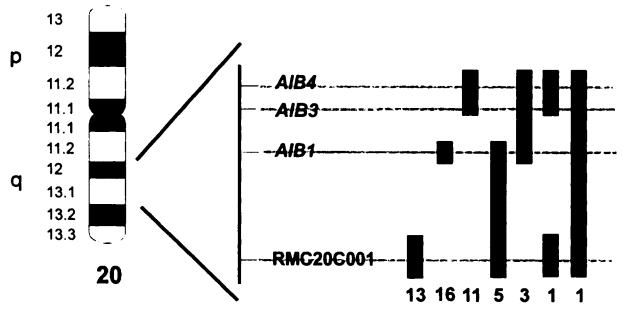

Fig. 1 Patterns of DNA amplification observed at 20q in breast cancer. A subset of 379 tumor DNAs was analyzed with probes to *AIB1*, *AIB3*, *AIB4*, and *RMC20C001*, and their amplifications were assessed. ■, DNA amplification for the corresponding marker or group of markers. Number of tumors presenting the respective amplification patterns are shown (*bottom*). Overall, 50 of 379 tumors presented amplification at 20q.

blotting, we analyzed 20 tumors presenting gains at 20q upon comparative genomic hybridization analysis by fluorescence *in situ* hybridization with probes to *AIB1* and *RMC20C001*. Of these 20 tumors, 10 were amplified at either locus. Eight tumors showed elevated copy numbers with the *AIB1* probe (range, 6–10-fold), 4 with *RMC20C001* (similar amplification levels), and 2 tumors were amplified concomitantly at both loci.

Clinicopathological Correlations. Correlations between *AIB1* amplification and clinicopathological parameters were analyzed, and results are presented in Table 2. *AIB1* amplification correlated positively with either ER or PR. Results showed that the *AIB1* gene was amplified 2-fold more frequently in ER^+ or PR^+ tumors than it was in ER^- or PR^- tumors. It was interesting to see that, although *AIB1* amplification occurred in 2% of ER^-/PR^- tumors, it was observed in 6.2% of ER^+/PR^+, 5.0% of ER^+/PR^-, and 5.9% of ER^-/PR^+ tumors. Apart from steroid receptor status, *AIB1* amplification correlated only with large tumor size (>2 cm). Neither *AIB3* nor *AIB4* correlated to any clinicopathological marker tested.

Correlations with Other Amplifications. We tested for correlations with the amplifications of the genes or loci most frequently amplified in breast tumors and consistently showing clinicopathological correlations in our tumor cohort: *i.e.*, *FGFR1* (8p12), *MYC* (8q24), *CCND1-EMS1-GARP* (11q13), *MDM2-SAS* (12q13), *ERBB2* (17q12), and *RMC20C001* (20q13). *AIB1* amplification expectedly correlated to that of the *RMC20COO1* locus, and the level of correlation between these two loci colocalized on 20q was used as an internal standard (Table 3). Correlation was also observed with *MDM2* and *FGFR1* amplifications, with *MDM2* showing the strongest association as testified by the χ^2 value (Table 3). No correlation was found with *CCND1-EMS1-GARP*, *ERBB2*, or *MYC*.

Discussion

We and others have suggested that breast cancer heterogeneity may find its source in its genetic variability and, hence, that phenotypic subsets could be defined according to patterns of genetic alterations carried by the tumors. The tumors presently analyzed for the amplification of the *AIB1* gene had been characterized previously for that of 26 genes or genetic markers mapping at 15 chromosomal locations. Of the 26 genes or loci tested, 11 showed elevated levels of amplification in a substantial fraction of tumors and 9 of 11 consistently presented clinicopathological correlations allowing to define subsets of breast tumors according to DNA amplification patterns (9). *AIB1*, together with two others genes *AIB3* and *AIB4*, maps to the long arm of chromosome 20, in a region known to be frequently amplified in breast cancer (8). These three genes have been recently cloned as part of a microdissection based effort to characterize homogeneously staining regions in breast cancer cell lines (12). Screening breast cancer cell lines, *AIB1* was found to be most consistently amplified and overexpressed (7). Furthermore, *AIB1* presents distinct homology at the amino acid level with members of the SRC-1 family of nuclear receptor coactivators and was shown, in transient transfection assays, to activate ER expression at the transcriptional level (8). These properties made this gene particularly interesting in primary breast cancer and motivated us to test for the incidence of its amplification and verify whether this anomaly was characteristic of a specific subset of tumors.

In our dataset, *AIB1* amplification was observed in 4.8% of the tumors. Amplification levels were moderate (2–8-fold), in keeping with those observed for *RMC20C001*, another locus amplified at 20q13 (10). Searching for clinicopathological correlations, we found that *AIB1* amplification was significantly associated with ER and/or PR positivity. Interestingly, when both ER and PR were considered in combination, a significant difference was observed when ER^-/PR^- tumors were compared with ER^+/PR^+, ER^+/PR^-, or ER^-/PR^+cancers. These results

Table 2 Clinicopathological correlations observed in breast tumors with *AIB1* amplification

Clinicopathological parameter	*AIB1* amplification	
	No./total (%)	P (χ^2)[a]
Scarff and Bloom grade		
1	0/57 (0.0)	
2	22/398 (5.5)	
3	21/353 (5.9)	NS (3.51)
Lymph node status[b]		
N^-	18/429 (4.2)	
N^+	27/407 (6.6)	NS (2.44)
Hormonal receptors[c]		
ER^-	10/364 (2.7)	
ER^+	45/769 (5.9)	0.02 (5.1)
PR^-	16/494 (3.2)	
PR^+	39/636 (6.1)	0.02 (5.0)
Clinical size (cm)		
≤2	5/216 (2.3)	
>2	25/369 (6.8)	0.02 (5.5)
Age (yr)		
<45	8/158 (5.1)	
45–55	12/261 (4.6)	
>50	30/617 (4.9)	NS (0.04)

[a] Statistical significance was considered at $P < 0.05$ (χ^2 shown in parentheses). NS, not significant.

[b] Axillary lymph node status was divided into two subclasses: absence of metastatic node (N^-) and one or more than one invaded node (N^+).

[c] Tumors were considered ER or PR when measured levels exceeded 10 fmol/mg of proteins.

Table 3 Amplifications correlated with *AIB1*

Gene or locus analyzed	Status	*AIB1* amplification		
		No./total	%	P (χ^2)[a]
RMC20C001	Amplified	18/66	27.3	10^{-5} (75)
	Nonamplified	36/1031	3.5	
CCND1	Amplified	6/120	5.0	NS (0.15)
	Nonamplified	35/827	4.2	
FGFR1	Amplified	9/103	8.7	0.02 (5.4)
	Nonamplified	32/845	3.8	
MDM2	Amplified	10/63	15.9	0.0001 (17.5)
	Nonamplified	46/1093	4.2	

[a] Statistical significance was considered at $P < 0.05$ (χ^2 shown in parentheses). NS, not significant.

suggest that *AIB1* amplification may, in breast cancer, be related not only to ER activation but also to PR activation. Nevertheless, the positive correlation observed with ER^+ tumors is in agreement with the biological activity of the *AIB1* gene. The fact that *AIB1* amplification was also found to correlate with increased tumor size could suggest a role in the growth of hormone-dependent breast tumors. To check whether *AIB1* amplification was characteristic of a distinct subset of breast tumors, we searched for correlations with amplifications affecting other loci. It was, indeed, interesting to verify whether the *AIB1* gene was preferentially coamplified with genes or loci, such as *CCND1* (or other loci colocalized at 11q13), which were known to be strongly associated with ER positivity (9). Correlation was tested against nine other loci, of which three presented a significant association with the amplification of *AIB1*. As expected, the strongest correlation was found with the *RMC20C001* locus, localized distally from *AIB1* at 20q13. The next strongest, according to χ^2 levels, were the amplifications of *MDM2* (12q13) and, finally, of *FGFR1* (8p12). These correlations suggest possible cooperative pathways of oncogenic activation. Indeed, the p95-mdm2 protein is the main repressor of the p53 protein; thus, amplification and overexpression of the *MDM2* gene in a tumor may be functionally equivalent to a *p53* gene inactivation. The *FGFR1* gene codes for a class IV tyrosine kinase receptor that is preferentially activated by FGF1 and FGF2. Its role in human cancer is clearly substantiated by its amplification and overexpression in 10–15% of breast tumors and its activation by balanced chromosomal rearrangements involving the short arm of chromosome 8 in myeloproliferative disorders (13, 14). Noticeably, neither *CCND1* nor any of the 11q13 loci were preferentially coamplified with *AIB1*. These data could, thus, mean that *AIB1* and *CCND1* amplification correspond to distinct subsets of ER-positive breast tumors. This may be further supported by the fact that, although the incidence of *AIB1* amplification was clearly different in ER^- and ER^+ tumors, no variation was observed according to the level of ER expression (measured by scatchard in protein extracts). This was not the case for *CCND1* amplification, the frequency of which showed a gradual increase according to levels of ER expression. It was, indeed, lowest in tumors expressing 0–10 fmol/mg of ER (5.4% of *CCND1* amplification), intermediate in the 10–70 fmol/mg of ER range (12%), and highest above 70 fmol/mg of ER (20%). Furthermore, *CCND1* was found to correlate with tumor type, showing preferential amplification in lobular breast cancer, whereas no such relation was observed with *AIB1*. It is also noteworthy that the *MDM2* gene was also preferentially amplified in ER^+ or PR^+ tumors (9, 10), thus suggesting the existence of two groups of hormone-responsive breast cancers; one clustering around the amplification of *CCND1* and the second around that *AIB1* and/or *MDM2*. Moreover, it is interesting to see that, although colocalized on 20q, amplifications of *AIB1* and *RMC20C001* present very different trends of association with clinicopathological parameters; the latter did not show any correlation with the hormonal status but instead correlated with nodal invasion (9, 10). These results suggest that these two amplification events, despite their colocalization at 20q, define different subsets of tumors.

Data presented here are further evidence of the existence of complex amplification patterns in human tumors and, particularly, in breast cancer. There have been a number of reports showing that amplification can encompass large portions of the genome. Best characterized were the amplifications at 11q13 and 12q13 (9, 15–17), but similar observations were also made at 17q12 with the coamplification of *ERBB2* with four other genes: *MLN50*, *MLN 51*, *MLN 62*, and *MLN 64* (18). Our data suggest that amplifications at 20q could correspond to at least three distinct core regions of amplification represented by *RMC20C001*, *AIB1*, and *AIB3/AIB4*, respectively, supporting previous observations reported by Tanner and coworkers (19). We cannot exclude that amplification at 20q involves further genes or markers, and a full picture will require the use of a larger number of markers based on a physical map of the region.

As shown by Anzick and coworkers (8), *AIB1* amplification is also observed in ovarian tumors. Results presented herein show that, whereas in breast tumors *AIB1* and *RMC20C001* are amplified in equivalent proportions, this is not the case in ovarian cancer. Indeed, *AIB1* amplification is 3 times more frequent than that of *RMC20C001*. These results reinforce previous observations showing that breast and ovarian cancers present different patterns of amplification (20).

Finally, it should be noted that recent biochemical studies of the murine orthologue of *AIB1*, p/CIP, suggest that the role of this protein extends to other signaling pathways beyond those involving nuclear receptors (21). This is a consequence of the interaction of AIB1/p/CIP with the transcriptional integrator CBP/p300, which itself interacts with multiple transcription factors. Indeed, the presence of p/CIP in CBP/p300 complexes appears to be important for cAMP and IFN-γ signaling, as well as nuclear receptor function. Therefore, although it is tempting to speculate that tumor clones bearing *AIB1* amplification emerge as a result of increased ER/PR signaling. It will be important to examine additional signal transduction pathways in *AIB1*-amplified tumors. The use of high-density cDNA microarray technology (22) may prove useful in determining the overall consequences of *AIB1* overexpression on gene expression.

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In breast cancer, amplification of the steroid receptor coactivator gene AIB1 is correlated with estrogen and progesterone receptor positivity.

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