Overexpression of the Steroid Receptor Coactivator AIB1 in Breast Cancer Correlates with the Absence of Estrogen and Progesterone Receptors and Positivity for p53 and HER2/neu

Toula Bouras, Melissa C. Southey, and Deon J. Venter

Abstract

The gene for the steroid receptor coactivator ampliﬁed in breast cancer 1 (AIB1), located on chromosome 20q12, is overexpressed at the mRNA level in up to 60% of primary breast carcinomas; however, only 5% of these tumors show DNA ampliﬁcation. The transcription factors and signaling pathways relevant to breast cancer, which in the absence of DNA ampliﬁcation are responsible for and targeted by elevated levels of AIB1 mRNA, are unknown. In the present study, in situ hybridization was used to examine AIB1 mRNA expression in 93 breast carcinomas of varying histological grade and immunohistochemical proﬁle. AIB1 mRNA was overexpressed relative to normal breast tissue in 26 of 83 (31%) invasive tumors. This was found to associate with high tumor grade (P = 0.0006), lack of immunohistochemical staining for the steroid receptors estrogen receptor (P = 0.002) and progesterone receptor (P = 0.002), and strong protein staining for p53 (P = 0.01) and HER2/neu (P = 0.002). These ﬁndings suggest that AIB1 overexpression may impact on breast cancer by a mechanism not wholly dependent on steroid receptor coexpression and which may involve other oncogenic events, such as p53 protein stabilization and HER2/neu overexpression.

Introduction

Steroid hormones, acting primarily through ligand-activated transcription factors and their interacting coactivator proteins, play a signiﬁcant role in mammary gland development and tumorigenesis. Recent attention has focused on the role of the SRC family, represented by SRC-1 (1), transcription intermediary factor 2 (also known as SRC-2; Ref. 2), and AIB1 (also termed SRC-3; Ref. 3). In transient overexpression experiments, coactivators interact with and enhance the transcriptional activity of steroid receptors. Their coactivation function has been linked to intrinsic histone acetyl transferase activity and to multiple interactions with basal transcriptional factors and chromatin remodeling proteins (4). The contribution of SRCs to hormonally regulated processes in the mammary gland has been highlighted by various in vivo observations. AIB1 (SRC-3) has been shown to be amplified in a small proportion (5%) of human breast cancers (3). In addition, mice null for the SRC-1 gene display less mammary gland alveolar development during pregnancy and respond to estrogen and progesterone with only partial duct differentiation (5).

Materials and Methods

Tumor Specimens and Tissue Arrays. Archived, formalin-ﬁxed, parafﬁn-embedded specimens of primary breast carcinoma were retrieved from ﬁles at the Department of Pathology (Peter MacCallum Cancer Institute). All specimens were obtained surgically from patients and ﬁxed in 10% buffered formalin within 1 h after surgery for 24 h before being embedded in parafﬁn wax in routine manner. Storage of parafﬁn blocks was at room temperature. A total of 93 breast tumors were used to construct tissue arrays, representing matched normal breast tissue, DCIS, and metastases. H&E-stained slides from each block were used as a guide to identify areas representing different stages of progression from each case, which were then sampled with a punch biopsy tool to remove a 2-mm-diameter cylinder of tissue. At least two punches of normal and two of tumor were taken for each case. These cylinders were then re-embedded in a predetermined position in a tissue array. Of the 93 cases, 83
contained regions of invasive cancer, and the remainder consisted of DCIS only. Of the 83 invasive tumors, 44 had only the invasive component scored, 18 were also sampled for DCIS, 18 had the accompanying metastases scored, and 3 had accompanying DCIS and metastases sampled. Of the 83 invasive tumors, 78 had a Scarff and Bloom grade on the pathology report as follows: 15 of 78 (19%) grade 1; 39 of 78 (50%) grade 2; and 24 of 78 (31%) grade 3. Axillary lymph nodes were examined for the presence of metastatic breast tumor in 75 patients, with 41 of 75 (55%) showing no evidence of metastatic tumor and 34 of 75 (45%) being positive for metastatic tumor. The Institutional Ethics Committee of the Peter MacCallum Cancer Institute approved the use of archival tumor tissue.

Immunohistochemistry. Staining was performed on 3-μm sections of formalin-fixed, paraffin wax-embedded tissue rehydrated through graded alcohols. Antigen retrieval was used for the ER, PR, AR, HER2/new, and p53 antibodies, which consists of 2 min of heating under pressure in a pressure cooker in 10 mM sodium citrate (pH 6.0). Sections were stained using a DAKO Autostainer (Dako Corp., Carpinteria, CA). All of the wash steps used 50 mM Tris-Cl (pH 7.6) and 0.05% Tween 20. Endogenous peroxidase activity was blocked by incubating sections with 3% hydrogen peroxide for 10 min. A 5-min blocking step (Protein Blocking Agent; Immunon, Pittsburgh, PA) was used for PSA and pS2. The primary antibody was applied at the following dilutions for 30 min at room temperature: ER (1:200); Dako, PR (1:800); Dako, AR (1:50); Dako, HER2/new (1:1600); Dako, p53 (DO7) (1:200); Novocastra Laboratories, Newcastle-Upon-Tyne, United Kingdom), pS2 (1:100; Novocastra Laboratories), and PSA (1:400; Dako). Biotinylated secondary antibodies were detected with streptavidin peroxidase by using the LSAB 1 kit (HER2/new, p53, pS2, and PSA) or the LSAB+ kit (PR, AR, and ER; Dako). The final color reaction was carried out using aminoethylcarbazole as a chromogen and counterstained with hematoxylin. Crystal mount (Biomeda, Foster City, CA) was applied to sections and dried on a 60°C hotplate. The sections were coverslipped with DPX mountant.

For all of the antibodies, the intensity of staining and proportion of positive cells were determined for the normal breast epithelium, DCIS, invasive carcinoma, and metastatic components for each case, according to a method described by Armes et al. (12). Briefly, a semiquantitative estimate of expression levels of the antigen was based on the combined score for the proportion of staining cells and the intensity of staining. The proportion score represented the estimated percentage of positive cells (0, <10%; 1, 11–25%; 2, 26–50%; 3, 51–75%; 4, 76–90%; and 5, >91%). The intensity score represented the average staining intensity for positive cells (0, none; 1, weak; 2, moderate; and 3, strong). Levels of staining were derived as follows: samples with an intensity score of 0 or having <10% of cells staining were designated negative; and samples with intensity score of 1 in >10% of cells were designated weak. For intensity levels 2 and 3, combined scores of 2–3 were designated as weak, 4–6 as moderate, and 7 or 8 as strong expression. For each antibody, only the cellular compartment described previously as expressing the antigen of interest was scored.

Preparation of Riboprobes. AIB1 mRNA in situ expression was determined by two nonoverlapping cDNA fragments used in combination described previously by Anzick et al. (3). CDNA sequences spanning nucleotides 1733–2579 and 3072–3580 of the AIB1 gene (GenBank accession number AF012108) were subcloned into the CIaI and SacI sites of BlueScript vector with T3 and T7 RNA polymerase at either end of the insert. A 335-bp region of the β2-microglobulin was used to assess RNA integrity (GenBank accession number AB021288) spanning nucleotides 1–335, which was subcloned in the Smal site of pBluescript vector. The identity and orientation of the inserts were validated by sequencing using the Amplicycle sequencing kit (Perkin-Elmer, Branchenlog, NJ).

Subclones were linearized at one end of the insert with the appropriate restriction enzyme. For each subclone, both sense and antisense probes were synthesized. Approximately 1 μg of linearized template DNA was used for in vitro transcription using [32P]-labeled UTP (New England Nuclear, Boston, Massachusetts) according to the manufacturer’s protocol (Stratagene, La Jolla, CA).

Hybridization and Washes. Sections of formalin-fixed, paraffin-embedded tissue were cut at 5 μm on aminopropyltriethoxysilane-coated slides and baked at 60°C for 1 h. Sections were dewaxed in histolene and rehydrated through graded ethanol. Prior to hybridization, sections were pretreated with 20 μg/ml of proteinase K for 10 min at room temperature in digestion buffer, 50 mM Tris-HCl (pH 7.5), and 5 mM EDTA (pH 8.0). The reaction was stopped in 0.2% glycerine in PBS buffer for 1 min. Sections were then fixed with 4% paraformaldehyde for 10 min at room temperature. The sections were dehydrated in increasing concentrations of ethanol and stored in 100% ethanol at −20°C until hybridization.

Hybridization was carried out on pretreated tissue sections at 60°C for 48 h in hybridization buffer [0.3 M NaCl, 10 mM Na2HPO4 (pH 6.8), 10 mM Tris-Cl (pH 7.5), 5 mM EDTA (pH 6.8), 50% formamide, 5% dextran sulfate, 100 mg/ml tRNA, and 100 mg/ml single-stranded DNA]. Hybridization buffer (50–100 μl) containing 100–200 ng/ml of each of the two probes was applied to each section. The sections were coverslipped and incubated in a humidified chamber at 60°C for 36–48 h. Posthybridization washes were performed twice at high stringency in hybridization solution [0.3 M NaCl, 10 mM Na2HPO4 (pH 6.8), 10 mM Tris-Cl (pH 7.5), 5 mM EDTA (pH 6.8), and 50% formamide] at 60°C for 20 min each. Nonspecifically bound probe was digested with RNase A. The slides were washed twice in 2× SSC at 65°C, dehydrated in graded ethanol, and air-dried. The slides were then dipped in Kodak NTB-2 emulsion (Eastman Kodak, Rochester, NY), air-dried, and exposed in light proof boxes at 4°C for 4–6 weeks before developing in undiluted Kodak D19 developer according to the manufacturer’s instructions and counterstaining in hematoxylin.

Hybridization Experiments and Analysis. Each tumor array section was hybridized with the two antisense probes for AIB1 used in combination. An adjacent section was hybridized with the sense probes as a negative control. Sections of archival lymph node were used as positive tissue control to assess reproducibility and technique performance because AIB1 was found to be highly expressed in germinal centers. Tumor-infiltrating lymphocytes also served as an internal positive control. To confirm the presence of intact RNA, adjacent tissue sections were hybridized with a probe to the housekeeping gene β2-microglobulin. A strong hybridization signal was present in all sections studied. A 3-point scoring system was used to assess AIB1 expression levels in each tumor relative to matched normal breast tissue. Low-level AIB1 mRNA expression was observed in all histologically normal breast tissues accompanying the tumors; at high magnification (×400), this consisted of a few irregularly distributed silver grains over the breast epithelial cells (averaging 1–4 grains/cell). Accordingly, tumors were designated to have low AIB1 expression if the same pattern of irregularly distributed silver grains was present over the tumor cells and the tumor architecture could not be easily discerned by darkfield examination at low power. Moderate to high levels were defined as the distinct clustering of silver grains present over the majority of cells in the tumor sample, which displayed an easily visible darkfield pattern consistent with the tumor architecture under low power. At higher magnification (×400), moderate levels were estimated as a 2–5-fold increase in the number of localized silver grains/cell relative to matched normal and high estimated to be a >5-fold increase. For each tumor array, two independent hybridization experiments were performed. Tumors were only deemed to overexpress AIB1 mRNA if their score in the two independent hybridization experiments was either moderate or high. AIB1 overexpression was confirmed by independent hybridization experiments on the original tumor biopsy blocks used to construct the arrays. Results obtained from whole biopsies were consistent with those observed on the tissue array. For analysis purposes, tumors have been subdivided into two groups: those with low AIB1 expression; and those deemed to overexpress AIB1 as having high levels.

Statistics. Statistical comparisons between groups were assessed by two-tailed Fisher’s exact test or the χ2 test for independence. Nominal Ps are given without adjustment for multiple comparisons. Raw data are provided together with the Ps.
to high levels of AIB1 mRNA occurred in 11 of 31 (35%) of ductal carcinomas in situ, 26 of 83 (31%) invasive cancers, and 8 of 21 (38%) metastases. Examples of low and high levels of AIB1 mRNA expression are shown in Fig. 1. Invasive tumors found to have high levels of AIB1 expression also had high levels of expression present in the corresponding DCIS and metastatic lesions.

**Clinicopathological Correlation.** AIB1 mRNA levels were correlated with various clinical and pathological parameters, as shown in Table 1. AIB1 overexpression was significantly associated with high tumor grade, with the majority of tumors expressing high AIB1 mRNA levels being poorly differentiated grade 3 lesions ($P = 0.0006$). Tumors with high AIB1 levels tended to occur in patients with an earlier onset of the disease ($\leq 50$ years of age at the time of diagnosis), suggesting a possible link to menopausal status ($P = 0.05$). No significant correlation was observed for the presence or absence of lymph node metastases (Table 1).

**Immunohistochemical Correlation.** To address the question of whether AIB1 overexpression correlates with expression of genes involved in steroid versus nonsteroid receptor pathways relevant to breast cancer, immunohistochemistry was performed for steroid receptors known to interact with AIB1 (ER, PR, and AR), their selected target genes (pS2 and PSA), as well as p53 and HER2/neu. This analysis included only the invasive tumor component of the study set (Table 2). High levels of AIB1 mRNA expression correlated with the absence of immunohistochemically detectable ER and PR. AIB1 mRNA was highly expressed in 15 of 26 (58%) ER-negative tumors compared with only 11 of 55 (20%) ER-positive tumors ($P = 0.002$). Similarly for PR, high levels of AIB1 mRNA expression occurred more frequently in PR-negative tumors 18 of 35 (51%) than in PR-positive tumors 8 of 48 (17%; $P = 0.002$). For both HER2/neu and p53, AIB1 up-regulation was correlated with moderate to strong staining for these antigens. High levels of AIB1 mRNA were observed in 19 of 38 (50%) tumors with moderate to strong staining of HER2/neu compared with 7 of 44 (16%) tumors with weak or negative immunohistochemical expression of the HER2/neu antigen ($P = 0.002$). This difference is further highlighted when the intensity and extent of staining was considered. For the invasive carcinomas with low levels of AIB1, only 1 of 56 (2%) expressed HER2/neu strongly, whereas of the tumors with high AIB1 levels, 10 of 26 (38%) showed strong HER2/neu staining ($P < 0.0001$). When this analysis was extended to the DCIS subset, the same trend was observed (data not shown), with 58% of DCIS (7 of 12) overexpressing AIB1 also showing strong HER2/neu staining. In contrast, only 1 of 18 (6%) DCIS cases with low AIB1 had strong HER2/neu staining ($P = 0.003$). Of note, 4 of the 7 DCIS cases with both high HER2/neu and AIB1 were subclassified as high-grade comedo DCIS. This type of DCIS is characterized histologically by prominent necrosis and by markers of high-grade malignancy, such as ER/PR negativity and overexpression of HER2/neu and p53 (13). This is consistent with the features observed in invasive tumors with high AIB1 mRNA; an example of such an invasive cancer is given in Fig. 2. For p53, AIB1 was highly expressed in 9 of 15 (60%) tumors with moderate to strong accumulation of p53 compared with 16 of 66 (24%) tumors with low p53 staining ($P = 0.01$). No significant difference was observed for immunohistochemical staining of AR, PSA, and pS2 in tumors expressing low levels compared with high levels of AIB1.

<table>
<thead>
<tr>
<th>Clinicopathological parameter</th>
<th>High AIB1 No./Total (%)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade$^a$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3/15 (20)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>7/59 (12)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>15/24 (63)</td>
<td>0.0006</td>
</tr>
<tr>
<td>Lymph node status$^b$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N+</td>
<td>10/34 (29)</td>
<td></td>
</tr>
<tr>
<td>N−</td>
<td>13/41 (32)</td>
<td>NS$^c$</td>
</tr>
<tr>
<td>Age (yr)$^d$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;45</td>
<td>8/17 (47)</td>
<td></td>
</tr>
<tr>
<td>45–50</td>
<td>5/12 (42)</td>
<td></td>
</tr>
<tr>
<td>&gt;50</td>
<td>12/52 (23)</td>
<td>NS</td>
</tr>
</tbody>
</table>

$^a$ Seventy-eight tumors had reliable grading information.

$^b$ Seventy-five patients had an axillary node clearance. Lymph node status was stratified into two classes: absence of metastatic tumor in axillary lymph node (N−); and one or more axillary lymph nodes invaded with metastatic tumor (N+).

$^c$ Not significant ($P > 0.05$).

$^d$ The $P$ for comparison of patients $\leq 50$ years to those $>50$ did reach significance, $P = 0.05$.
Discussion

Cellular signaling by the steroid receptors is a complex process involving the interaction of multiple cofactors. Efficient activation of target gene expression depends on several coactivators that associate with the ligand-activated receptor and act as bridging factors to the basal transcriptional apparatus. Altered expression or modification of these cofactors may result in changes in gene expression and lead to disturbances in cellular growth control. Indication that this may be of importance has come from the identification of the SRC AIB1 as a gene amplified on chromosome 20q12 in breast cancer (3). Buasista et al. (14) using Southern analysis reported recently that AIB1 gene amplification occurs in 4.8% of breast tumors and correlates with ER and PR positivity. At the transcript level, however, initial reports indicate that AIB1 RNA is overexpressed in 60% of breast tumors, occurring in both the ER+ and ER− subtypes (3). Because mechanisms other than DNA amplification appear to predominate, the effect of AIB1 overexpression in the presence of normal gene copy number in breast cancer remains poorly understood.

To first evaluate whether AIB1 overexpression contributes to breast cancer by overstimulating steroid receptor-mediated transactivation, the relationship between AIB1 mRNA expression and immunohistochemical staining for steroid receptors (ER, PR, and AR) and target genes (p52 and PSA; Ref. 15 and 16) was examined. Of particular interest is our significant observation of an inverse relationship between AIB1 mRNA expression and protein staining for ER and PR. The inverse relationship observed in tumors is of interest in the light of immunohistochemical studies showing that the structurally related coactivator SRC-1 in the rat mammary gland is not colocalized with ER but expressed in a subpopulation of epithelial cells distinct from those expressing ER and PR (17). Taken together, these results suggest that AIB1-mediated pathways not dependent on coexpression of ER and PR protein (at the levels usually detected by immunohistochemistry) may be predominately active in the mammary gland and in a significant subset of breast cancers.

To further examine the relationship between AIB1 mRNA up-regulation and other factors that may influence the efficacy of antiestrogen therapy or potentially offer a point for signaling cross-talk, we investigated the same tumor cases for coexpression of HER2/neu, a receptor tyrosine kinase that is overexpressed in 20–30% of breast cancers (18). Overexpression of HER2/neu in breast cancer is inversely correlated with ER levels and predicts clinical resistance to the antiestrogen tamoxifen (19). We detected a strong correlation between AIB1 mRNA and HER2/neu protein overexpression, which is consistent with the inverse correlation observed for ER and PR. This relationship may simply reflect an association with high tumor grade or, alternatively, may be an indicator of some form of cooperative cross-talk between AIB1 and HER2/neu. The latter possibility offers interesting insight. Newman et al. (20), using the ER-positive breast cancer cell line ZR75-1, demonstrated that in antiestrogenic media the structurally related coactivator SRC-1 activates the HER2/neu enhancer. This coactivator competition study suggests that AIB1 may contribute to the transcriptional up-regulation of HER2/neu. Alternatively, cross-talk may occur at the protein level. The precise molecular mechanisms are still not clear; however, evidence supports the notion that steroid receptor phosphorylation (through MAPK), a downstream target of HER2/neu, promotes coactivator recruitment (8). Tremblay et al. (9) demonstrated that MAPK induced phosphorylation of specific serine residues within the NH−terminal AF1 transactivation domain of ERβ enhances coactivator recruitment and estrogen-independent transactivation. Other studies have extended these findings to demonstrate in prostate cancer models the enhancement of AR-mediated transactivation in the presence of increased HER2/neu/MAPK activity, again possibly because of enhanced AR-coactivator interaction (21, 22). In addition to nuclear receptors, MAPK phosphorylation sites have also been mapped in coactivators, specifically SRC-1 and AIB1 (10, 11). MAPK phosphorylation of AIB1 has been shown to stimulate its intrinsic histone acetyl transferase activity and the recruitment of the transcriptional coactivator p300 (11). These exper-

<table>
<thead>
<tr>
<th>Antigen</th>
<th>AIB1 RNA status</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>ERb</td>
<td>44/55 (80)</td>
<td>44/55 (80)</td>
</tr>
<tr>
<td>PRb</td>
<td>40/57 (70)</td>
<td>8/26 (31)</td>
</tr>
<tr>
<td>ARb</td>
<td>40/55 (73)</td>
<td>14/26 (54)</td>
</tr>
<tr>
<td>p53c</td>
<td>6/56 (11)</td>
<td>9/25 (36)</td>
</tr>
<tr>
<td>HER2/neuc</td>
<td>19/56 (34)</td>
<td>12/26 (73)</td>
</tr>
<tr>
<td>p52d</td>
<td>28/56 (50)</td>
<td>12/26 (46)</td>
</tr>
<tr>
<td>PSAe</td>
<td>13/56 (23)</td>
<td>4/26 (15)</td>
</tr>
</tbody>
</table>

Table 2. Immunohistochemical characteristics observed in breast tumors by AIB1 mRNA status.

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Low (%)</th>
<th>High (%)</th>
<th>Low vs. High</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERb</td>
<td>44/55</td>
<td>11/26</td>
<td>0.002</td>
</tr>
<tr>
<td>PRb</td>
<td>40/57</td>
<td>8/26</td>
<td>0.002</td>
</tr>
<tr>
<td>ARb</td>
<td>40/55</td>
<td>14/26</td>
<td>0.1</td>
</tr>
<tr>
<td>p53c</td>
<td>6/56</td>
<td>9/25</td>
<td>0.01</td>
</tr>
<tr>
<td>HER2/neuc</td>
<td>19/56</td>
<td>12/26</td>
<td>0.002</td>
</tr>
<tr>
<td>p52d</td>
<td>28/56</td>
<td>12/26</td>
<td>0.8</td>
</tr>
<tr>
<td>PSAe</td>
<td>13/56</td>
<td>4/26</td>
<td>0.6</td>
</tr>
</tbody>
</table>

* Not all cases stained for each antibody because of availability of tissue.
* Proportion showing >10% of cells positive at an intensity of weak and above.
* Proportion showing >10% of cells positive at an intensity of moderate and strong.

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Fig. 2. Immunohistochemical expression pattern of an invasive breast carcinoma with high-level AIB1 mRNA expression. A, low power darkfield section after in situ hybridization with the AIB1 antisense probe, showing high levels of AIB1 mRNA expression in invasive carcinoma. D, dark-field section after hybridization with the AIB1 sense probe. Immunohistochemical staining of the same tumor reveals strong staining for HER2/neu (B) and p53 antigen (C) compared with lack of staining for ER (E) and PR (F). All panels, ×100.
iments, taken together with our findings of a strong correlation be-
tween AIB1 and HER2/neu in breast cancer, suggest that HER2/neu
is acting upstream of MAPK phosphorylation of AIB1 and may
promote its coactivator activity and interaction with specific steroid
receptors or other yet to be defined transcription factors. This may
potentially lead to autonomous (ligand independent) and/or growth
factor-modulated transactivation of target genes, accounting for es-
trogen-independent tumor growth. Genes involved in these processes
could be developed as markers to assess disease progression.

The view that AIB1 dysregulation cooperates with other oncogenic
events during breast cancer progression has also been put forward by
Bautista et al. (14). Unlike our study, which focuses on AIB1 mRNA
expression, these workers concentrated on AIB1 gene amplification
(which occurs in only 5% of breast tumors) and found a strong
correlation between amplification of AIB1 and MDM2. The MDM2
gene negatively regulates p53, and its amplification in tumors is
thought to be equivalent to p53 inactivation (23). This is consistent
with our findings of a correlation between high AIB1 and strong
protein staining for the tumor suppressor gene p53 (which is thought
to accompany p53 inactivation and protein stabilization). The tran-
scriptional cointegrators p300/CBP, with which AIB1 forms a com-
plex, regulate the transactivation function and protein abundance of
p53 (24, 25), further emphasizing the potential interaction between the
AIB1 and p53/MDM2 pathways. Whether AIB1 in complex with
CBP/p300 contributes to these functions remains to be elucidated but
may have important implications for more precisely understanding
AIB1 action in breast cancer.

In conclusion, our study reports up-regulation of AIB1 gene tran-
scripts in 30% of nonselected human breast carcinomas. The new
findings outlined here indicate that AIB1 overexpression occurs in a
minority of steroid receptor-expressing tumors and suggest a possible
convergence of AIB1 with other pathways involving HER2/neu over-
expression and p53 inactivation, which may be involved in estrogen-
indepedent growth. Further studies to examine the role of AIB1
overexpression in patients with known response to endocrine therapy
and the identification of transcription factors specifically affected by
AIB1 dysregulation are now necessary to further define how this gene
impacts on the clinical phenotype and biology of breast cancer.

Acknowledgments

We thank Dr. Andrew Holloway and Wee Kheng Soo for critical reading of
the manuscript and helpful comments. We much appreciate the expert assis-
tance of Ryan Van Laar with the preparation of the figures.

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