An Isoform of the Coactivator AIB1 That Increases Hormone and Growth Factor Sensitivity Is Overexpressed in Breast Cancer*

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The AIB1 (amplified in breast cancer 1) protein is a coactivator that potentiates the transcriptional activity of nuclear hormone receptors, and its gene is amplified in a subset of human breast cancers. Here we report a splice variant of AIB1 mRNA that lacks the exon 3 sequence. We determined that the AIB-Δ3 mRNA encoded a 130-kDa protein that lacks the NH₂-terminal basic helix-loop-helix and a portion of the PAS (Per-Arnt-Sim homology) dimerization domain. The 130-kDa protein was detected in MCF-7 breast cancer cells at levels that were 5–10% of the full-length protein, whereas in nontransformed mammary epithelium lines, the AIB-Δ3 protein was present at significantly lower levels compared with the full-length AIB1. Consistent with this finding, the abundance of AIB1-Δ3 mRNA was increased in human breast cancer specimens relative to that in normal breast tissue. To determine whether there were phenotypic changes associated with the overexpression of the AIB-Δ3 isoform, we performed functional reporter gene assays. These revealed that the ability of AIB1-Δ3 to promote transcription mediated by the estrogen or progesterone receptors was significantly greater than that of the full-length protein. Surprisingly, the AIB-Δ3 isoform was also more effective than AIB1 in promoting transcription induced by epidermal growth factor. Overexpression of AIB1-Δ3 may thus play an important role in sensitizing breast tumor cells to hormone or growth factor stimulation.

Ligands such as estrogen and progesterone that interact with nuclear receptors regulate gene expression predominantly at the transcriptional level. The ligand-bound receptors interact specifically with DNA and activate transcription by recruiting a preinitiation complex. Although such gene activation was originally thought to be mediated by interaction of the receptors with components of the basal transcriptional machinery (1–6), a variety of screening techniques has identified a family of receptor-interacting proteins known as nuclear receptor coactivators (7–11). A common characteristic of this superfamily of proteins is that, when overexpressed in the presence of nuclear receptors, they potentiate ligand induction of transcription (12, 13). Members of the related p160 group of coactivators, which include steroid receptor coactivator 1 (SRC-1), SRC-2, and SRC-3 (also known as AIB1, ACTR, RAC3, TRAM-1, and p(CIP) (14–20), possess several similar structural features including a receptor interaction domain, a bHLH (basic helix-loop-helix)-PAS (Per-Arnt-Sim homology) dimerization domain, and a CBP interaction domain (13). Coactivators are thought to function as bridges between nuclear receptors and either other coactivators or the basal transcriptional machinery (13). However, the discovery that coactivators possess a histone acetylase domain (15, 21–24) suggests that these proteins also might serve to regulate chromatin structure.

A portion of human chromosome 20q that is frequently amplified in breast cancer contains the gene for the nuclear coactivator AIB1 (amplified in breast cancer 1) (25). The AIB1 gene is amplified in 5–10% of breast cancers, and the abundance of the corresponding mRNA and protein is increased in some breast tumors and breast cancer cell lines (14, 25–27). It has recently been shown that AIB1 binds directly to the estrogen receptor (ER) (28) and that AIB1 is rate-limiting for estrogen-induced growth of MCF-7 cells (29). However, the overall role of AIB1 for breast tumorigenesis is not clear because AIB1 potentiates not only the action of estrogen (14, 16) and progesterone (16) receptors but also that of various other nuclear receptors (9, 15, 17–20) and transcription factors (30, 31). In addition, several splice variants of SRC family members have been described, although the functions of these variants remain unknown (13).

Here we report the identification of a splice variant of AIB1 that is overexpressed in breast cancer tissue and cell lines. The AIB-Δ3 transcript encodes an NH₂-terminal truncated version of AIB1 that lacks the bHLH and PAS A domains. In functional studies we have determined that the AIB-Δ3 protein is a significantly more effective coactivator of estrogen, progesterone, and EGF signaling than the wild type ER, suggesting a role for this AIB1 isoform in hormone and paracrine signaling in breast cancer.

EXPERIMENTAL PROCEDURES

Plasmids—We subcloned the full-length AIB1 cDNA from pCMX-ACTR (provided by R. Evans, Salk Institute, La Jolla, CA) into pcDNA3 (Invitrogen) with the use of the flanking KpnI and XhoI sites, thereby creating the expression vector pcDNA3-AIB1. We subcloned the smaller of the two RT-PCR products generated from MCF-7 cell total RNA with exon 1- and exon 9-specific primers (Fig. 1b) into pcRII (Invitrogen). The resulting plasmid was digested with BamHI and HpaI, recognition sequences that flank the splice sites of AIB1-Δ3 cDNA, and the released fragment was purified and used to replace the corresponding sequence of pcDNA3-AIB1, thereby creating pcDNA3-AIB1-Δ3. The pcDNA3-bHLH, basic helix-loop-helix; PAS, Per-Arnt-Sim homology; ER, estrogen receptor; ERE, estrogen response element; PCR, polymerase chain reaction; RT, reverse transcription; IMEM, Iacov's modified Eagle's medium; FBS, fetal bovine serum; EGF, epidermal growth factor; CHO, Chinese hamster ovary; FGF-BP, fibroblast growth factor-binding protein; TEF, transcription-enhancing factor.

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‡ The abbreviations used are: SRC, steroid receptor coactivator; TEF, transcription-enhancing factor.
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AIB1 and pcDNA3-AIB1-Δ3 vectors contain identical 5'- and 3'- untranslated regions, differing only in the loss of exon 3 in the latter. The inserts were verified by sequencing.

The expression vectors for human estrogen receptor α and progesterone receptor B were provided by P. Chamoun (CNRS, France). The firefly luciferase reporter plasmid containing the estrogen response element (ERE) from the Xenopus vitellogenin gene was provided by V. C. Jordan (Northwestern University, Chicago), and the plasmid containing the mouse mammary tumor virus promoter was provided by G. Hager (National Cancer Institute, Bethesda, MD). The luciferase reporter plasmid containing the human FGF-BP gene promoter has been described previously (32). The Renilla luciferase vector (pRL-CMV) was from Promega.

Cells and Tissue Samples—All cell lines were obtained from the tissue culture core facility of the Lombardi Cancer Center. MCF-7, ME-180, and COS-1 cells were cultured in Iscove’s modified Eagle’s medium (IMEM, Life Technologies, Inc.) supplemented with 10% fetal bovine serum (FBS). MCF-10A and A1N4 cells were grown in a 1:1 mixture of IMEM and Ham’s F-12 medium (Life Technologies, Inc.) that was supplemented with 5% horse serum, insulin (10 μg/ml), and hydrocortisone (500 ng/ml). CHO cells were maintained in F-12 nutrient mixture (Life Technologies, Inc.) supplemented with 10% FBS.

Frozen tissue samples were obtained from the Lombardi Cancer Center Histopathology and Tissue Shared Resource Core. The six normal samples were obtained from individuals undergoing reduction mammoplasty (mean age at time of surgery, 29 years; range, 19 to 54 years); the eight primary breast carcinoma specimens were obtained from women with a mean age at the time of surgery of 51 years (range, 29 to 64 years).

Immunoblot Analysis—Whole cell extracts were prepared as described previously (32), and equal portions (30 μg of protein) were resolved either on denaturing 4–20% polyacrylamide gradient gels or on 4% polyacrylamide gels containing Tris-glycine. The separated proteins were transferred to a nitrocellulose membrane and then subjected to immunoblot analysis with a 1:500 dilute of a mouse monoclonal antibody specific for amino acids 376–389 of human AIB1 (Transduction Laboratories), horseradish peroxidase-conjugated goat antibodies to mouse immunoglobulin (1:10,000 dilution; Amersham Pharmacia Biotech), and enhanced chemiluminescence reagents (Amersham Pharmacia Biotech).

RT-PCR—Isolation of total RNA and synthesis of cDNA by RT were performed as described previously (33). The amplification of AIB1 cDNA sequences was achieved by PCR according to the following protocol: incubation at 95°C for 5 min followed by 30 cycles of 95°C for 1 min, 60°C for 1 min, and 72°C for 90 s. The oligonucleotides used as primers for PCR or as probes for hybridization were as follows: exon 1, 5'-GACTGTTGATGACATGCTTCTCT-3'; exon 2, 5'-GCCATGATGTCATGCTGACTCCAC-3'; exon 3, 5'-CTGACTGATACATTCCGCAACT-3'; exon 4, 5'-ACCGCAGTATGATCTCAAGG-3'; exon 5, 5'-ATGTTTGCTGTCGATGTTGCAC-3'; exon 6, 5'-CCTGACACAGGATGACCAAC-3'; and exon 9, 5'-CTGACACAGGATGACCAAC-3'. The PCR products were separated by electrophoresis on a 1% agarose gel, transferred to a polyvinylidene difluoride membrane, which was then cut, and the lanes were subjected separately to hybridization with 32P-labeled oligonucleotides specific for exons 2, 3, or 8 of AIB1. The positions of PCR products corresponding to the full-length (AIB1) and truncated (AIB1-Δ3) transcripts are indicated. bp, base pairs. c, comparison of the structures of AIB1 and AIB1-Δ3 mRNAs. The alternative splicing event that results in the loss of exon 3 causes the open reading frame (ORF) to shift and terminate at a TAA codon in exon 4. A potential initiation site (AUG) for AIB1-Δ3 mRNA is present at nucleotide 778; the use of this site would be consistent with the AIB1-Δ3 protein lacking the NH2-terminal 26 kDa of full-length AIB1. The shaded regions indicate the open reading frame, and exons in the mRNAs are numbered. The positions of the splice junctions in AIB1-Δ3 mRNA and of the encoded protein domains are indicated. UTR, untranslated region.

PhosphorImager (Molecular Dynamics 445SI). Quantification of PCR products was performed with a

In Vitro Transcription-Translation—In vitro transcription-translation was performed with the TnT coupled reticulocyte lysate system (Promega). Plasmid DNA (1 μg) was combined with 25 μl of rabbit reticulocyte lysate, 2 μl of TnT reaction buffer, 1 μl of T7 RNA polymerase, 1 μl of amino acid mixture, 1 μl of RNasin (Ambion) ribonuclease inhibitor (40 units), and 1 μl of Transcend biotin-lysyl-TRNA (Roche) and the final volume was adjusted to 50 μl. The reaction was performed at 30 °C for 90 min, after which 5 μl of the reaction mixture was subjected to SDS-polyacrylamide gel electrophoresis and either to immunoblot analysis with antibodies to AIB1 or to direct detection with streptavidin-conjugated horseradish peroxidase (1:10,000 dilution in phosphate-buffered saline containing 0.05% Tween 20) and enhanced chemiluminescence.

RESULTS

Detection of the AIB1-Δ3 Isoform—In this study we determined whether there are naturally occurring splice variants of
AIB1 present in breast cancer cells that might encode proteins with altered function relevant to breast cancer progression. The exon-intron structure of AIB1 was assembled as shown in Fig. 1a by comparing the published sequence of the cDNA (14) with the contiguous genomic sequence available through the NCBI database. We arbitrarily designated the most 5′ exon of AIB1 as exon 1, with the result that the first codon is located in exon 2. Our initial strategy was to determine whether RNA from MCF-7 cells, which overexpress AIB1 (14), contained any splice variant forms of AIB1 RNA. To achieve this end, we performed reverse transcription and polymerase chain reaction (RT-PCR) analysis of total RNA from MCF-7 human breast cancer cells with primers amplifying the region between exons 1 and 9. This revealed two PCR products that differed in size by ~150 base pairs. These PCR products were then subjected to Southern blot analysis, and individual lanes from the membrane were probed separately with oligonucleotides specific for each exon from 2 to 8. Typical hybridizations with exons 2, 3, and 8 are shown in Fig. 1b. This analysis revealed that the smaller PCR product hybridized with all probes except the one specific for exon 3 (Fig. 1b), indicating that the lower band corresponds to an RNA splice variant (designated AIB1-Δ3) of AIB1 that lacks the exon 3 sequence. We subsequently subcloned and sequenced this PCR product, confirming that nucleotides 267–439 (exon 3) of the full-length AIB1 cDNA were missing (Fig. 1c).

**Translation of the AIB1-Δ3 mRNA in Vitro and in Vivo**—To determine whether an AIB1-related protein was encoded by the AIB1-Δ3 mRNA, we performed in vitro transcription and translation of AIB1-Δ3 cDNA. Western blot analysis with an AIB1-specific antibody of the proteins translated in vitro revealed the production of a 130-kDa protein (Fig. 2). Interestingly, we had also consistently detected a similar 130-kDa protein, in addition to the 156-kDa full-length AIB1, by immunoblot analysis of MCF-7 cell extracts with antibodies to AIB1 on 5–20% polyacrylamide gels (27). To determine whether the MCF-7 130-kDa species and the in vitro transcription translation product had identical electrophoretic properties, we performed high resolution electrophoresis on 4% polyacrylamide gels containing Tris-glycine followed by immunoblot analysis. This analysis demonstrated that the mobility of the 130-kDa protein detected in MCF-7 cell extracts was identical to that of the 130-kDa protein produced by in vitro transcription and translation of AIB1-Δ3 cDNA (Fig. 2). This observation suggested that the 130-kDa MCF-7 cell protein was translated from AIB1-Δ3 mRNA present in these cells.

To verify that the AIB1-Δ3 mRNA was translated in vivo we performed transient transfection of CHO cells (Fig. 2; see Fig. 4a) or COS-1 cells (see Fig. 5a) with the AIB1-Δ3 cDNA. Analysis of cell extracts demonstrated that this indeed resulted in the production of a 130-kDa protein, whereas transfection with the full-length AIB1 cDNA yielded only the 156-kDa full-length protein. This latter observation demonstrated that the 130-kDa protein was clearly not the product of proteolytic processing of the full-length protein. The electrophoretic mobility of the 130-kDa protein synthesized in cells transfected with the AIB1-Δ3 cDNA was identical to that of both the 130-kDa AIB1 species present in MCF-7 cell extracts and the product of in vitro transcription-translation of the AIB1-Δ3 cDNA (Fig. 2). Together these data indicated that the endogenous AIB1-Δ3 mRNA present in MCF-7 cells encodes a 130-kDa protein.

An examination of the sequence of AIB1-Δ3 mRNA indicated that the open reading frame of AIB1, which initiates at nucleotide 184 in the full-length mRNA would terminate after 90 amino acids in the splice variant (Fig. 1c). We did not detect this predicted low molecular mass product in vivo or in vitro.

The 130-kDa species is detected by an AIB1 antibody raised against amino acids 376–389 in the amino terminus of the protein. This suggests that the AIB1-Δ3 isoform most likely represents an NH2-terminally truncated form of AIB1, with synthesis being initiated at an internal translation start site downstream of the splice junction but prior to amino acid 376. Such internal translational initiation has been described for various mRNAs with extended 5′-untranslated regions (34–37). The difference in size between the 156-kDa full-length AIB1 protein and the 130-kDa species suggested that the latter lacks ~210 amino acids of the former, including all of the bHLH region (residues 16–88) and most of the PAS A domain (residues 116–171) (Fig. 1c). This would place the initiation codon of the 130-kDa protein most likely at the codon at position 778 (Fig. 1c). Interestingly, for cells transfected with equivalent amounts of cDNA, the intracellular concentration of AIB1-Δ3 protein was ~10% of that of full-length AIB1 (Fig. 2; see Figs. 4a and 5a), suggesting that translation initiation of the splice variant was inefficient, possibly because of the long 5′-untranslated region of the AIB1-Δ3 mRNA.

**AIB1-Δ3 mRNA is Overexpressed in Human Breast Cancer**—Given that we first detected the AIB1-Δ3 splice variant in a breast cancer cell line, we next examined whether its expression was restricted to tumor cells. MCF-7 cells are derived from a pleural effusion of metastatic breast cancer, whereas MCF-10A and AIN4 cells are not malignantly transformed and were derived from atypical human breast epithelial hyperplasia (38) and from human mammary epithelial cells treated with benzo[a]pyrene (39), respectively. RT-PCR followed by Southern blot analysis revealed that the amounts of AIB1-Δ3 mRNA in MCF-10A and AIN4 cells were lower than that of MCF-7 cells (Fig. 3a). By subsequent real-time PCR analysis, using primers specific for AIB1 or its isoform, we have assessed that the ratio of AIB1-Δ3 mRNA/full-length AIB1 is 5% in MCF-7 cells, whereas in MCF-10A and AIN4 cells the ratio is 0.5% (data not shown). We then compared the abundance of the AIB1-Δ3 mRNA in a series of eight human breast tumors with that in normal tissue obtained from six women undergoing breast reduction mammoplasty. The amount of the full-length AIB1 mRNA in tumor samples was slightly greater than that in the normal tissue samples, but this difference was not significant (Fig. 3b). In contrast, the abundance of the AIB1-Δ3 mRNA in the tumor specimens was significantly greater than that in the normal tissue samples, with all but one of the tumors showing an increased amount of this transcript compared with the normal range.

**Effect of the AIB1-Δ3 Isoform on Nuclear Receptor Function**—We next examined the effect of the deletion of the bHLH

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**Fig. 2. Immunoblot analysis of AIB1 isoforms in extracts of MCF-7 cells and transfected CHO cells.** Extracts of untransformed CHO cells or CHO cells transfected with plasmids encoding AIB1 or AIB1-Δ3, or MCF-7 cells were fractionated by electrophoresis on high resolution 4% polyacrylamide gels containing Tris-glycine, and the separated proteins were transferred to a nitrocellulose membrane and probed with a monoclonal antibody specific for amino acids 376–389 of human AIB1. The products of in vitro transcription-translation of AIB1-Δ3 cDNA were similarly analyzed.
The reaction products were resolved on a 1% agarose gel and then subjected to RT-PCR with primers specific for exons 2 and 5 of AIB1 mRNA in malignant and nonmalignant human breast tissue and cell lines. A total RNA isolated from MCF-7, MCF-10A, and A1N4 cells was subjected to RT-PCR with primers specific for exons 2 and 5 of AIB1. The reaction products were resolved on a 1% agarose gel and then subjected to Southern blot analysis with a ^32P-labeled oligonucleotide probe specific for exon 4 of AIB1. Total RNA isolated from six normal breast and eight breast cancer tissue samples was analyzed as in panel a. The amounts of PCR products corresponding to AIB1 and AIB1-Δ3 mRNAs were quantitated by densitometry, and the abundance of the latter was expressed as a percentage of that of the former. The signal of the full-length AIB1 transcript was compared between breast tumors and normal breast tissue with the use of an arbitrary scale; the signals in tumor and normal samples were 1.0 ± 0.46 and 0.7 ± 0.24 (means ± S.E.), respectively, and they did not differ significantly (p > 0.05; Student’s t test). The inset shows a typical blot of 5 of the 14 samples.

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and PAS A domains in AIB1-Δ3 on protein function. AIB1 acts as a coactivator for several nuclear receptors, including those for estrogen and progesterone, which are important in breast carcinogenesis. We therefore transfected CHO cells with expression vectors encoding full-length AIB1 or AIB1-Δ3, an expression vector for estrogen receptor α, and a luciferase reporter plasmid containing an ERE. Transfection of CHO cells with 3 μg of the AIB1 expression vector resulted in a 1.4-fold increase in estrogen-induced luciferase activity, whereas transfection with 3 μg of the vector for AIB1-Δ3 resulted in a 3.8-fold increase in the estrogen response (Fig. 4a). However, given that the abundance of recombinant AIB1 in the transfected cells was about 10 times that of AIB-Δ3, we also transfected CHO cells with 0.3 μg of the AIB1 vector, which yielded about the same amount of intracellular recombinant protein as did 3 μg of the AIB1-Δ3 vector (Fig. 4a). A comparison of transfected cells containing approximately equal amounts of recombinant protein thus revealed that AIB1 and AIB1-Δ3 potentiated the estrogen response by factors of 1.1 and 3.8, respectively. Similar transfection experiments with COS-1 cells (which express endogenous AIB1) also demonstrated a greater potentiation of the estrogen response by AIB1-Δ3 than by full-length AIB1 (Fig. 5a). The differences between full-length and the AIB1-Δ3 isoform were seen at different concentrations of estrogen (0.1–10 nM) and thus were not due to a change in the affinity of the hormone for its receptor but rather suggest enhanced efficacy of the signaling (data not shown). We also obtained similar results in COS-1 cells with an expression vector encoding progesterone receptor B; the transcriptional response to the progesterone analog R5020 was thus potentiated to a greater extent by AIB1-Δ3 than by AIB1 in both CHO and COS-1 cells (Figs. 4b and 5b). Of particular note is that small amounts of transfected AIB-Δ3 protein had significant effects on ER- and progesterone receptor-induced transcription even against a relatively high background of full-length AIB1 (Fig. 5, a and b).

**Effect of the AIB1-Δ3 Isoform on EGF Signaling**—The fact that members of the p160 SRC family act as coactivators in intracellular signaling pathways that activate transcription factors other than nuclear receptors (30, 31) prompted us to examine whether AIB1-Δ3 might be able to sensitize breast cancer cells to growth factor signaling. Overexpression of members of the families of epidermal growth factor (EGF) ligands or EGF receptors is important in the malignant progression of breast cancer (40). Such growth factors also contribute to the hormone-independent phenotype of breast tumors, and the HER-2 receptor is a target of current therapies (41). To determine whether AIB1 and AIB1-Δ3 affect EGF signaling, we transfected ME-180 human squamous cell carcinoma cells with the respective expression vectors and with a luciferase reporter plasmid containing the promoter of the fibroblast growth factor-binding protein (FGF-BP) gene. FGF-BP functions as an
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In this study, we have provided evidence for the presence of a splice variant of AIB1 that has exon 3 deleted. The AIB1-Δ3 mRNA is translated in vivo in breast cancer cells into an NH2-terminal truncated form of AIB1 that has several properties of interest. The first is that on a per molecule basis it is a more potent transcriptional coactivator of both the estrogen receptor and progesterone receptor B in COS-1 cells. Another is that the conformation of this isoform is more favorable than that of the full-length protein for interaction with nuclear receptors or for recruitment of other coactivators such as CBP/p300. An alternative possibility is suggested by the observation that the bHLH-PAS domain of SRC-1 interacts with and potentiates the activity of members of the TEF (transcription-enhancing factor) family of transcription factors (30). Thus, full-length AIB1 might be unavailable for interaction with nuclear receptors because it is sequestered or squelched by other intracellular factors. In contrast, AIB1-Δ3, which lacks an intact bHLH-PAS domain, would not bind to factors such as TEF and would be available for nuclear receptor coactivation. This model might explain why relatively small amounts of recombinant AIB1-Δ3 are able to induce significant potentiation of nuclear receptor activity in transfected COS-1 cells with a high background of endogenous full-length AIB1. This model also predicts that the relative coactivating effects of AIB1 and AIB1-Δ3 might be cell type-specific, depending on the endogenous expression of AIB1-sequestering molecules such as TEF. Interestingly, a recent report has described that the human MMS19 protein can interact with the PAS-HLH domain of AIB1 and can regulate ER-mediated transcriptional activity (43). It is possible that the lack of interaction of AIB1-Δ3 with this protein may explain some of its increased effectiveness in vivo. Whatever the reason for the increased activity of the AIB1-Δ3 isoform, our data suggest that its expression would sensitize cells to the effects of estrogen and progesterone.

The second interesting aspect of the function of the AIB1-Δ3 isoform was that it also potently increased EGF signaling in ME-180 squamous carcinoma cells. This could be through direct interactions with a nuclear receptor. However, our analysis of the fragment of the FGFBP gene promoter (nucleotides −118 to +62, relative to the transcription start site) used in this study did not reveal obvious consensus recognition sites for known nuclear receptors. In fact, EGF induction of this promoter is dependent on the factors AP-1 and c/EBPβ (32), either of which may interact directly or indirectly with AIB1. Alternatively, it may be that a common intermediary of both nuclear receptor and AP-1 signaling such as CBP/p300 (44, 45) may be the target of the superactivating effects of the AIB1-Δ3 isoform. Whatever the mechanism of the increased potentiation of growth factor signaling by the AIB1-Δ3 isoform, our data suggest that an increase in the abundance of the AIB1-Δ3 isoform in mammary epithelial cells may be an important step in tumor progression and in the development of a more aggressive, hormone-independent phenotype.

Finally, of major interest for breast cancer is our finding that the AIB1-Δ3 mRNA is overexpressed in breast cancer cell lines...
and in human breast tumors. Our analysis of tumor cell lines suggests that there is an overall increase in the AIB1-Δ3 mRNA relative to the full-length AIB1, although we do not know whether this is related to the gene amplification status of the endogenous gene. Alternatively, the increase in AIB1-Δ3 mRNA may be because of an increase in RNA splicing of exon 3 in breast cancer cells. It is also possible that the increase in expression in tumors may be due in part to dilution effects of surrounding stromal tissue, but this seems unlikely given that we also see lower AIB1-Δ3 mRNA expression in non-transformed versus malignant mammary epithelial cell lines. To date a number of laboratories, including ours, have reported measurement of increased levels of AIB1-Δ3 mRNA, and protein in breast tumor tissue, although the assessment of the portion of breast cancers overexpressing AIB1 varies widely between groups (14, 27, 46, 47). In addition, some groups have determined that AIB1 overexpression is correlated with ER and progesterone receptor status (26), whereas others have found an inverse relationship with steroid receptor expression but a positive correlation with HER-2 and p53 expression (47). However, all of these RT-PCR or immunohistochemical analyses of expression levels have not distinguished the AIB1-Δ3 isoform signal from that of the wild type. Our data indicates that the overexpression of relatively low levels of the AIB1-Δ3 isoform can sensitize cells to estrogen, progesterone, and growth factors. Therefore we believe that measurement of increased levels of AIB1-Δ3 levels might be a sensitive indicator of the progression of breast cancer to a more hormone-independent phenotype.

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REFERENCES

1. Blanco, J. C., Wang, I. M., Tsai, S. Y., Tsai, M. J., O’Malley, B. W., Jurutka, P. W., Haussler, M. R., and Ozato, K. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 1535–1539
2. Ing, N. H., Beekman, J. M., Tsai, S. Y., Tsai, M. J., and O’Malley, B. W. (1992) J. Biol. Chem. 267, 17617–17623
3. McEwan, I. J., and Gustafsson, J. A. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 8485–8490
4. Chiquet-Ehrismann, R., and Gubler, F. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 1535–1539
5. Ing, N. H., Beekman, J. M., Tsai, S. Y., Tsai, M. J., and O’Malley, B. W. (1992) J. Biol. Chem. 267, 17617–17623
6. Caudill, S. E., and Gustafsson, J. A. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 1535–1539
7. McEwan, I. J., and Gustafsson, J. A. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 8485–8490
8. Chiquet-Ehrismann, R., and Gubler, F. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 1535–1539
9. Ing, N. H., Beekman, J. M., Tsai, S. Y., Tsai, M. J., and O’Malley, B. W. (1992) J. Biol. Chem. 267, 17617–17623
10. Caudill, S. E., and Gustafsson, J. A. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 1535–1539
11. Voelkel, J. P., Chiquet, S., Gubler, F., and Gustafsson, J. A. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 1535–1539
12. Glass, C. K., Rose, D. P., and Rosenfeld, M. G. (1997) Curr. Opin. Cell Biol. 9, 222–228
13. McKenna, N. J., Lanza, R. B., and O’Malley, B. W. (1999) Endocr. Rev. 20, 321–344
14. Anzick, A. L., Kononen, J., Walker, R. L., Azorsa, D. O., Tannir, N. M., Gaining, D., Sauter, G., Kallioniemi, O. P., Trent, J. M., and Meltzer, P. S. (1997) Science 277, 965–968
15. Chen, H., Lin, R. J., Schiltz, R. L., Chakravarti, D., Nash, A., Nagy, L., Privalsky, M., L., Nakatani, Y., and Evans, R. M. (1997) Cell 90, 569–589
16. Suen, C. S., Berodkin, T. J., Mastroneti, R., Cheska, B. J., Littlye, C. R., and Frail, D. E. (1998) J. Biol. Chem. 273, 27645–27653
17. Li, H., Gomes, P. J., and Chen, J. D. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 7847–7849
18. Takashita, A., Cardona, G. R., Nishikawa, N., Suen, C.-S., and Chin, W. W. (1997) J. Biol. Chem. 272, 27629–27634
19. Yeh, S., Miyamoto, H., Shima, H., and Chang, C. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 5527–5532
20. Kamei, Y., Xu, L., Heinzel, T., Torchia, J., Kurokawa, R., Gloss, B., Lin, C. S., Heyman, R. A., Rose, D. W., Glass, C. K., and Rosenfeld, M. G. (1996) Cell 85, 403–414
21. Yang, X.-Y., Ogryzko, V. V., Nishikawa, J.-I., Howard, B. H., and Nakatani, Y. (1996) Nature 382, 319–324
22. Ogryzko, V. V., Schiltz, R. L., Russanov, V., Howard, B. H., and Nakatani, Y. (1996) Cell 87, 953–961
