Estrogen-Dependent and Estrogen-Independent Mechanisms Contribute to AIB1-Mediated Tumor Formation

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Abstract

We have previously reported the oncogenic properties of the gene amplified in breast cancer 1 (AIB1), a member of the p160 family of hormone receptor coactivators. In a transgenic mouse model, AIB1 overexpression resulted in a high incidence of tumors in various tissues, including mammary gland, uterus, lung, and pituitary. To determine whether the AIB1 oncogenicity in this model depended on its function as an estrogen receptor (ER) coactivator, we abolished ER signaling through two independent approaches, by performing ovariectomy on AIB1 transgenic (AIB1-tg) mice to prevent gonadal estrogen production and by crossing AIB1-tg mice with ERα-null mutant mice. Ovariectomized (ovx) mice, but not AIB1 × ERα−/− mice, still developed mammary gland hyperplasia and ductal carcinoma in situ. Both approaches, however, completely prevented the development of invasive mammary tumors, indicating that invasive mammary tumor formation is strictly estrogen dependent. Once developed, AIB1-induced mammary tumors can subsequently lose their dependence on estrogen: Injection of ERα(+) tumor cell lines derived from such tumors into ovx or untreated wild-type mice resulted in a similar rate of tumor growth in both groups. Surprisingly, however, ovx mice had an ~4-fold higher rate of metastasis formation, suggesting that estrogen provided some protection from metastasis formation. Lastly, our experiments identified oncogenic functions of AIB1 that are independent of its ER coactivation, as both approaches, ovariectomy and ER−/− crosses, still resulted in a high incidence of tumors in the lung and pituitary. We therefore conclude that AIB1 can exert its oncogenicity through tissue-specific estrogen-dependent and estrogen-independent functions.

Introduction

Breast cancer is the most prevalent form of cancer in women in the United States. The prognosis and response to therapy largely depends on tumor phenotype. Targeted therapies are available for estrogen receptor α (ERα)–positive tumors and for those tumors harboring HER2 amplifications. However, tumors lacking hormone receptor expression or HER2 amplification, so-called triple-negative breast cancers, lack defined targeted therapies and have a poor prognosis. Furthermore, both de novo and acquired resistance to both endocrine and HER2-targeted therapies remain a significant problem, and once patients develop metastatic disease, cures remain extremely rare.

We have previously developed a mouse model for breast cancer by overexpressing the human gene AIB1 in the mammary gland of FVB mice (1) to investigate mechanisms of breast cancer development, metastasis formation, and drug resistance. AIB1 was discovered as a gene frequently amplified or overexpressed in breast cancer (2). It belongs to the p160 family of hormone receptor coactivators, and consequently, one of its most studied functions is as an ERα coactivator (3). The AIB1 transgenic (AIB1-tg) mice in our model developed invasive mammary gland tumors with a 75% incidence, as well as frequent tumors in other organs including the uterus, pituitary, lung, and skin. In addition, some of the mice harboring mammary gland tumors developed metastasis in bone and brain. These results showed for the first time the ability of AIB1 to function as an oncogene.

The development of mammary and uterine tumors in AIB1-tg mice was consistent with its role as an ER coactivator. However, the formation of tumors in the lung, skin, and pituitary was surprising, as these tissues are not known to be estrogen dependent. Moreover, about 20% of the mammary tumors in AIB1-tg mice lacked expression of ERα. These observations raised the question whether AIB1 exerted its role as an oncogene through ERα coactivation alone or whether it had other functions as well.

In the present study, we used two strategies to address the role of estrogen and ERα in AIB1-mediated tumor formation. First, we surgically removed the ovaries of prepubertal AIB1-tg mice to block gonadal estrogen production, which is the main source of estrogen in female mice. Ovariectomized (ovx) AIB1-tg mice did not develop any invasive mammary...
gland tumors. In sharp contrast, the incidence of pituitary, lung, skin, and bone tumors was higher than in non-ovx AIB1-tg mice, indicating that only a subset of tumors were affected by the abolishment of gonadal estrogen production. Second, we crossed AIB1-tg mice with ERα-null mutant mice. Lacking ERα, these mice are unable to respond to estrogen through this receptor, whether the estrogen is produced in the ovaries or in other tissues via aromatization of adrenal androgens. Again, these mice showed no signs of mammary tumors but developed tumors in lung, skin, and pituitary with the same high incidence as their AIB1-tg counterparts expressing ERα.

Our results indicate that AIB1 can cause tumor formation by estrogen-dependent and estrogen-independent mechanisms, depending on the organ or tissue affected. These results further suggest that AIB1 exerts oncogenic activities in cell signaling and/or gene regulation that are independent of its function as an ER coactivator.

Materials and Methods

Mouse strains and treatments. Animal experiments were compliant with the guidelines of the Dana-Farber Cancer Institute. The AIB1-tg mouse line (FVB-MMTV-AIB1) has been previously described (1). FVB wild-type (wt) mice were purchased from Charles River Laboratories.

Ovaries from wt and AIB1-tg mice were surgically removed before onset of puberty. Mammary gland development was assessed after ovarioectomy by whole-mount analysis and H&E staining of cross sections (see below). Ovx animals were also aged and monitored for tumor formation. Mice were sacrificed when tumors developed or at 16 months of age, depending on tumor status and overall health conditions. Complete necropsies were then done. DNA, RNA, and protein were extracted from various tissues for further analysis.

Heterozygous ERα deletion mutant mice (+/-) of the C57BL/6 strain were kindly provided by Dr. Pierre Chambon (Institute of Genetics and Molecular and Cellular Biology, CNRS/INSERM/University of Strasbourg, Strasbourg, France; ref. 4). These mice were crossed with the AIB1-tg mouse line FVB-MMTV-AIB1 to obtain the double-mutant mouse line AIB1-tg × ERα−/− and then inbred to obtain the homozygous null mutant strain AIB1-tg × ERα−/−. Mammary gland development and tumor formation were assessed as described above.

Mammary gland whole mounts and immunostaining. Mammary glands were dissected, mounted on glass slides, and stained as described in http://mammary.nih.gov/tools/histological/Histology/index.htm1. Tissues were fixed overnight in 10% neutralized buffered formalin and embedded in paraffin by standard procedures. Sections of 4-μm thickness were stained with H&E or processed for immunohistochemistry analysis following antigen retrieval, using polyclonal ERα (Santa Cruz Biotechnology; diluted 1:3,000) or monoclonal anti-AIB1 antibody (BD Biosciences; diluted 1:200) and hematoxylin counterstaining.

RNA isolation and quantitative PCR. RNA isolation from tissue or tumors was done with Trizol (Invitrogen) according to the manufacturer’s protocol. Five micrograms of RNA were reverse transcribed, using the ABI High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). The resulting cDNAs were used for quantitative PCR analysis using an Applied Biosystem 7300 RT PCR device and ABI SYBR Green master mix. The following primers were used: AIB1-sense, 5′-GGCCGCGAGTTCCGATTTA-3′; antisense, 5′-GGATCACCAAGCAGGAGT-3′; actin-sense, 5′-GGATGGGAGAGAGCGC-3′; antisense, 5′-TCGTCCCGGAGAAGAC-3′; aromatase-sense, 5′-AGGGAAATAGCGCAAGATG-3′; antisense, 5′-CGGGACCATGATGGTGACA-3′; ERα-sense, 5′-CCTGCTGCTGGA-GATTTG-3′; antisense, 5′-GCTTCCGGGTTCC-3′.

Protein extraction and Western blot analysis. The Western blot analysis protocol has been previously described (5).

Mammary gland tumors. The AIB1-tg mouse line has been maintained as previously described (5). Monoclonal anti-AIB1 antibody (BD Biosciences) and polyclonal anti-aromatase (Novus) were used for Western blots. Polyclonal calnexin (Stressgen) or β-actin antibodies (Sigma) were used as control for protein loading.

Statistical analysis. Statistical analyses for PCR and tumor injection studies were done using unpaired two-tailed Student’s t tests. Statistical significance was considered to be present at levels greater than 95% (P < 0.05). Means and SDs have been estimated for continuous variables. Tumor-free survival was calculated using the product-limit method of Kaplan-Meier (7). Comparisons between subgroups of study mice and the significance of differences between survivals rates were ascertained using a two-sided log-rank test (8). Differences between the two populations were considered significant at confidence levels greater than 95% (P < 0.05). All statistical analyses were carried out using GraphPad Prism version 5.0b for Mac OS X (GraphPad Software).

Results

Tumors formed in AIB1-tg mice lack a correlation between AIB1 and ER expression levels. We have previously reported that AIB1-tg mice display a high incidence of mammary tumors. Whereas many of these mammary tumors are ERα(+) or ERα(−), a significant proportion of them do not express detectable levels of ERα (1). This finding was somewhat surprising as ERα coactivation is thought to be the primary function of AIB1. This finding raised the question of whether AIB1-induced formation of ERα(−) mammary tumors is ER independent. Alternatively, formation of ERα(−) tumors
initially requires AIB1 coactivation of ER, but the tumors later become ER independent and lose ER expression.

Before we determined whether ERα and estrogen were necessary for AIB1-mediated tumorigenesis, we evaluated the correlation between AIB1 and ERα expression in mammary gland tumors derived from AIB1-tg mice. We compared AIB1 and ERα mRNA and protein levels in a large number of tumor samples, using quantitative PCR (Fig. 1A) and Western blot (Supplementary Fig. S1). Notably, several tumors with high AIB1 expression levels had undetectable or only barely detectable levels of ERα. A statistical analysis of the expression levels revealed the lack of a correlation between AIB1 and ERα mRNA levels (Fig. 1B).

**Ovariectomy impairs mammary gland development in AIB1-tg mice.** To address the causal relationship between ER function, AIB1 expression, and mammary tumor formation, we abolished ovarian estrogen production in 25 AIB1-tg FVB mice and 25 wt FVB mice by performing ovariectomy before onset of puberty. First, we analyzed mammary gland development in these mice and compared it to that in untreated AIB1-tg and wt mice. Whole-mount analysis of 20-week-old ovx mice revealed severe impairment of mammary gland development, consistent with previous reports showing the requirement of estrogen for normal mammary gland development (9–11). Whereas mammary glands of AIB1 and wt mice with intact ovaries showed complete ductal

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**Figure 1.** Lack of correlation of ERα and AIB1 expression in mammary tumor samples from AIB1-tg mice. A, relative mRNA expression levels of AIB1 and ERα were determined by quantitative PCR in the tumor samples indicated. B, for each tumor sample, the relative expression levels of AIB1 and ERα were plotted against each other. Linear regression analysis was done using GraphPad Prism version 5.0b for Mac OS X.
Figure 2. Effect of ovariectomy on mammary gland development in AIB1-tg mice. Wt and AIB1-tg mice were ovariectomized at 3 wk of age. At 20 wk, controls and ovx mice were sacrificed and mammary gland whole mounts or cross sections were prepared. A, whole mounts, top: left, untreated wt; right, untreated AIB1; bottom: left, ovx wt; right, ovx AIB1 mouse (×20 magnification). Arrow, example of branching that is absent in ovx wt mice. B, H&E-stained cross sections of mammary glands (×100). Ovariectomy results in impaired mammary gland development in wt and AIB1-tg mice. AIB1-tg mice display hyperplasia and disorganized mammary duct architecture, even after ovariectomy. Inset, detail of a hyperplastic mammary duct. Right, example of a DCIS observed in some of the ovx AIB1-tg mice. C, effect of ovariectomy on ERα expression. Mammary gland cross sections of untreated and ovx wt and AIB1-tg mice were probed with ERα antibody. All mice showed comparable ERα staining. D, ERα expression of DCIS.
elongation that fully branched out and penetrated the mammary fat pad (Fig. 2A), duct formation after ovariectomy was only rudimentary and ductal elongation and side branching were limited or absent in both AIB1-tg and wt mice (Fig. 2A).

We, however, observed a difference in overall mammary gland development between AIB1-tg and wt mice after ovariectomy. Whereas there was no detectable ductal branching in wt mice following ovariectomy, mammary glands of AIB1-tg mice showed limited but clearly present ductal elongation and branching (Fig. 2A, arrow). These results confirm that ovarian estrogen production is required for full ductal development. AIB1 overexpression alone cannot compensate for the lack of gonadal estrogen during mammary gland development, although it increases ductal elongation and branching to some extent. Serum estrogen levels were undetectable in the ovx mice that developed uterine atrophy and increased in body weight (not shown), in agreement with previous studies (9, 12–14).

Despite impaired mammary gland development, ovx AIB1-tg mice display mammary gland hyperplasia and ductal carcinoma in situ. We have previously reported that AIB1-tg mice develop mammary epithelial hyperplasia, as evidenced by increased cell numbers and a disorganized pattern of cells surrounding the ducts (1). We now find that despite ovariectomy, the mammary glands of AIB1-tg mice also show an increase in cell number and disorganized structures as compared with wt mice (Fig. 2B). Thus, despite impaired mammary gland development, ovx AIB1-tg mice develop hyperplasia and, in some cases, develop ductal carcinoma in situ (DCIS; Fig. 2B, right). These results indicate that the apparent proliferative effects of the AIB1 gene on mammary gland epithelial cells are exerted even in the absence of gonadal estrogen.

To determine the effect of estrogen removal on ERα expression levels, we performed immunohistochemistry. The pattern of ERα expression in luminal epithelial cells was not significantly different with or without ovariectomy, notwithstanding the smaller number of ducts and branches (Fig. 2C), indicating that estrogen deficiency does not influence ER expression levels or distribution. The DCIS lesions were also ERα(+)) (Fig. 2D).

Ovx AIB1-tg mice display a reduced incidence of tumors in hormone-dependent tissues but an increased incidence of tumors in hormone-independent tissues. Twenty-five AIB1-tg mice treated by ovariectomy and 27 control AIB1-tg mice were aged and monitored for tumor formation. When animals presented evidence of disease or, at the latest, at the end of the study, they were sacrificed and a full pathologic examination was conducted. Data are presented as Kaplan-Meier disease-free survival curves. Three categories of diseases were scored: invasive mammary gland tumors; noninvasive DCIS (both shown in Fig. 3A); and nonmammary gland tumors, such as lung, skin, bone, or pituitary tumors (Fig. 3B). DCIS does not lead to visible disease and, consequently, only was diagnosed when the animals suffered from other symptoms or at the end point of the study at 16 months. Most strikingly, none of the 25 ovx AIB1 mice developed invasive mammary gland tumors (Fig. 3A) or uterine tumors (not shown). Thus, the incidence of invasive tumors in these hormone-dependent tissues was significantly reduced as compared with non-ovx AIB1 mice. In sharp contrast, 13 of 25 (52%) ovx AIB1 mice developed tumors in hormone-independent tissues, including lung, skin, pituitary, and bone. The incidence of these tumors was even significantly higher than in untreated AIB1 mice, where 6 of 27 (22%) of mice developed such tumors (Fig. 3B). These data show that AIB1 overexpression can give rise to two distinct groups of tumors: those that are prevented by ovariectomy and thus are estrogen dependent (Fig. 3A) and those that remain unaffected or even increase in frequency on ovariectomy and thus are independent of gonadal estrogen production (Fig. 3B). Representative sections of tumors developing in ovx AIB1-tg mice are shown in Supplementary Fig. S2. They include bone sarcoma, skin papilloma, lung carcinoma, and pituitary adenoma.

Lack of ovarian production of estrogen leads to increased aromatase expression in the mammary gland. An essential step of estrogen biosynthesis is catalyzed by the enzyme aromatase. Aromatase is responsible not only for the estrogen produced in the ovaries but also for extragonadal estrogen production, for example, in adipose tissue...
and skin (15–17). It has been suggested that aromatase expression is increased on reduction of estrogen levels in an attempt by the organism to increase aromatase production to compensate for reduced estrogen signaling (18, 19). To determine whether abolishment of gonadal estrogen production leads to increased aromatase production in our system, we analyzed aromatase mRNA expression levels by quantitative PCR in whole mammary glands from mature ovx and non-ovx AIB1-tg and wt mice. Ovariectomy led to a 3- to 4-fold increase in aromatase levels in both AIB1-tg and wt mice (Supplementary Fig. S3). Interestingly, AIB1-tg mice had about 2- to 3-fold lower aromatase expression as compared with wt mice. This difference was apparent in treated and untreated AIB1 mice, indicating that there might also be a negative feedback loop by which increased ER activity (due to increased AIB1 coactivation) down-regulates estrogen production by aromatase.

**Estrogen Dependence of AIB1-Induced Tumors**

We next investigated whether extragonadal estrogen was responsible for mediating the phenotype of AIB1 mice after ovariectomy by abolishing all estrogen signaling through removal of ERα. We did this by generating crosses of AIB1-tg mice with ERα-null mutant mice. This approach should allow us to unequivocally dissect ERα-dependent and ERα-independent functions of AIB1-mediated tumorigenesis. Double-mutant mice of the AIB1-tg × ERα−/− genotype were aged and monitored for tumor formation. When tumors were detected or at the end point of the study, mice were sacrificed and dissected for pathologic evaluation. As expected, all 16 of the double-mutant mice displayed impaired mammary gland development. Specifically, mammary glands did not show signs of ductal elongation or side branching (Fig. 4), nor did they develop terminal end buds (not shown). A similar mammary gland phenotype was detected in control ERα−/− mice, in agreement with previous reports showing that ERα−/− mice only develop a truncated ductal rudiment, demonstrating that normal mammary gland development requires ERα signaling (20–24). As expected, no mammary or uterine tumors were found during 16 months of aging. However, 7 of the 16 mice displayed lung or pituitary tumors, a comparable incidence to the 12 of 25 AIB1-tg mice showing the same kinds of tumors after ovariectomy. The lung and pituitary tumors were of similar type and showed similar onsets than the tumors developing in the single-mutant AIB1-tg mice (not shown). These results show that lung and pituitary tumors are indeed arising in an ERα- and estrogen-independent manner.

**Lack of estrogen dependence of tumor growth in normal mice after injection with an ERα(+) AIB1 tumor cell line.**

Thus far, we have shown that the formation of mammary tumors in AIB1-tg mice is strictly estrogen dependent. However, that does not necessarily mean that once mammary tumors have formed, they continue to depend on estrogen. In fact, when we derived ERα(+) cell lines from AIB1-tg mammary tumors and injected those cell lines into normal mice, they only showed a limited response to tamoxifen, as we previously reported (5). On injection into normal, syngeneic mice, these mammary tumor cell lines have been shown to be particularly aggressive, leading to uniform tumor formation in 100% of injected animals within 2 weeks (5).

We wanted to determine whether tumor growth on injection of these ERα(+) AIB1 tumor cell lines still required the presence of gonadal estrogen. Therefore, groups of five female wt FVB mice were ovariectomized before onset of puberty. At 20 weeks of age, mice were injected with 1,000 cells of the ER(+) AIB1-tg tumor cell line. As control, five age-matched, intact female wt mice were injected, and five wt FVB male mice were treated with the same tumor cell line as the females. After injection, the volume of developing tumors was determined at regular intervals. Mice were sacrificed after 24 days or when tumor volumes reached 2 cm³. Tumors developed in 100% of the animals injected, regardless of gender or prior treatment with ovariectomy. The group of mice injected with the tumor cell line showed no difference in tumor growth rate (Fig. 5A). No tumors were obtained when we injected the nontumorigenic mammary
epithelial cell line SCP2 (6) into ovx or untreated female mice or into male mice (Fig. 5A). These results indicate that an ER (+) tumor cell line derived from an AIB1-induced mammary tumor can form subsequent tumors, in vivo, in the absence of circulating gonadal estrogen.

In addition to tumor formation and growth, we also assessed metastasis formation by dissection and full pathologic analysis, as we have previously shown that this cell line can give rise to metastasis in various organs (5). As expected from those studies, we observed a considerable number of metastasis in the injected animals. Surprisingly, however, ovx mice showed a dramatically larger proportion of metastasis (80–90% versus 20–30% for non-ovx mice; Fig. 5B). Furthermore, the rate of metastasis in male mice was also increased over female untreated mice (40–50% versus 20–30%), suggesting that estrogen signaling may play a role in the prevention of metastasis.

Discussion

We have examined the dependency of AIB1-mediated tumor formation on estrogen in a mouse model to differentiate the AIB1 function as an ER coactivator from other reported functions (25). We followed a dual strategy: In one set of experiments, we removed the capacity for gonadal estrogen production by performing ovariectomies. In a second set of experiments, we removed the capacity for ERα signaling by creating AIB1-tg × ERα−/− double-mutant mice. A comparison of the results from these two approaches allowed us to distinguish the effects of gonadal estrogen and ERα on normal and premalignant mammary gland phenotype, mammary tumorigenesis, and tumor formation in other hormone-dependent and hormone-independent tissues.

Consistent with previous reports (9–11, 20–22), ovariectomy and deletion of the ERα gene both resulted in severely impaired mammary gland development. However, wt and AIB1-tg mice revealed different degrees of impairment after ovariectomy, as mammary glands of AIB1-tg mice showed limited but clearly present ductal elongation and branching. These features were absent in AIB1 × ERα−/− mice, indicating that they are strictly dependent on ERα signaling.

Unexpectedly, despite a general impairment of mammary gland development, ovariectomy did not prevent the formation of AIB1-mediated mammary gland abnormalities, including disorganized mammary gland architecture, the development of epithelial cell hyperplasia, and the formation of ductal carcinoma in situ, indicative of a premalignant phenotype (26, 27). This observation raised the question whether these abnormalities were caused by AIB1 through an ERα-dependent mechanism. We were able to establish the requirement for ERα by creating AIB1 × ERα−/− mice and examining their phenotype. As these mice did not show any evidence of the above AIB1-mediated mammary gland abnormalities, we conclude that these features are strictly ER dependent. Thus, the premalignant mammary phenotype still present after ovariectomy must be the result of extragonadal estrogen production or ligand-independent activation of ERα, or both. We expect extragonadal estrogen levels to be somewhat increased after ovariectomy due to the increased levels of aromatase mRNA and protein, which is consistent with previous reports of increased aromatase levels after ovariectomy in mice or rats (18, 19). However, we conclude that the levels of extragonadal estrogen were not sufficient either for normal mammary gland development or for the generation of AIB1-induced mammary tumors.

Our findings establish that the development of mammary gland tumors and uterine tumors in the AIB1 model is strictly dependent on the production of gonadal estrogen, as ovx mice showed a 0% incidence of these tumors, as compared with a 75% incidence of mammary tumors.
(and 20% incidence of uterine tumors) for untreated AIB1-tg mice. These findings argue that AIB1 exerts its oncogenicity in the mammary gland and uterus by acting as a coactivator for estrogen-bound ERα, leading to the expression of genes involved in proliferation and survival. Unexpectedly, we found that ovariectomy suppressed not only ER(+) but also ER(−) mammary gland tumors, which developed in untreated AIB1-tg mice with an ∼15% incidence. This finding suggests that the formation of ER(−) tumors in AIB1-tg mice requires estrogen at some stage during tumorigenesis, but that this requirement is lost at a later stage of tumor progression.

This hypothesis is further supported by the results from an ER(+) mammary tumor cell line derived from an AIB1 mammary tumor that seems to represent an intermediate step on the path to estrogen independence. As shown in Fig. 5A, injection of this tumor cell line can give rise to solid tumors in ovx female mice or in male mice. Thus, this particular tumor cell line is no longer dependent on gonadal estrogen for proliferation and survival, in vivo. It remains possible that this cell line uses extragonadal sources of estrogen or possibly produces estrogen in an autocrine manner. However, we consider this possibility unlikely, as the cell line has also ceased

Figure 6. Models of estrogen-dependent and estrogen-independent AIB1 actions. A, role of estrogen and ERα in mammary gland development and the formation of a premalignant phenotype and invasive tumors. B, estrogen dependence of tumor progression and metastasis formation.
to respond to estrogen in vitro and is not affected by treatment with the ER antagonist ICI 182780 (data not shown). Therefore, this cell line seems indeed to be estrogen independent despite continued expression of ER (also see Fig. 6A).

A totally unexpected finding from these injection experiments was that estrogen seems to have a protective effect from the development of metastasis, as ovx mice have a significantly increased rate of metastasis following injection of the ER(+) tumor cell line. The increase in metastasis formation after ovariectomy seems indeed to be due to reduced estrogen levels rather than to the effect of a prior surgery, as male mice (not treated by any surgery) showed a similar increase in metastasis over female untreated mice. Thus, although this cell line does not seem to require ER signaling for proliferation and survival, ER must still provide some other signals that result in the protection from metastasis, most likely by regulating transcription of genes involved in migration or homing (see in Fig. 6B). These findings indicate that loss of ER may present additional, thus far unanticipated risks in the progression of breast cancer.

In sharp contrast to mammary tumors and uterine tumors, which were completely abolished by ovariectomy or deletion of the Efra gene, tumors in other organs, such as lung, pituitary, and skin, still persisted following these treatments. This indicates that AIB1 does not function as an ER coactivator in the formation of these latter tumors and thus must have functions other than ER coactivation that can result in oncogenesis. AIB1 has been reported to serve as a coactivator for several other transcription factors, besides the ER, including other hormone receptors such as progesterone receptor, thyroid hormone receptor, glucocorticoid receptor, and retinoic acid receptor, when overexpressed in the presence of ligand (28–30). AIB1 has also been shown to function in a hormone receptor-independent fashion to coactivate other p300/CBP-associated transcription factors, such as STAT and AP-1 (31). Furthermore, we and others have implicated AIB1 in the regulation of several signaling pathways, including the phosphoinositide 3-kinase/Akt/mammalian target of rapamycin pathway and the NF-κB pathway (1, 32). At this point, we do not know whether any of these functions, an entirely new function, or a combination of these is responsible for tumorigenesis in lung, pituitary, and skin mediated by AIB1.

It is interesting to note that in the absence of estrogen or ER, the incidence of nonmammary gland tumors increases rather than remaining unchanged (see Fig. 3B). This indicates that there may be a connection between the estrogen-dependent and estrogen-independent functions of AIB1. For example, various pathways or molecules (including ER) might compete for AIB1, such that AIB1 might be the limiting factor in deciding which pathway is being activated. This result thus highlights a tight regulation of the various functions of AIB1.

Our findings that AIB1 can mediate its oncogenicity in the lung in an estrogen-independent manner have some immediate medical relevance, as AIB1 overexpression and amplification have recently been shown to correlate with poor prognosis in non–small-cell lung cancer in humans (33). However, such correlation studies cannot address cause and effect, and therefore, it remains unclear whether in humans AIB1 overexpression is causal to lung cancer formation and whether AIB1 functions as an ERα coactivator in the lung. The data from our mouse model clearly show that AIB1 overexpression by itself is sufficient to cause lung cancer and does so in an estrogen-independent manner. Our findings may thus have important implications in the search for drugs and drug targets in lung cancer treatment. Taken together, our present study shows that AIB1 can exert its oncogenicity through at least two distinct, tissue-dependent mechanisms, one that is ER dependent and one that is ER independent.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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