Cancer Research

# EF Hand Protein IBA2 Promotes Cell Proliferation in Breast Cancers via Transcriptional Control of Cyclin D1

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## Abstract

EF hand (EFh) domain–containing proteins have been implicated in malignant progression, but their precise functional contributions are uncertain. Here, we report evidence that the EFh protein IBA2 promotes the proliferation of breast cancer cells by facilitating their transit through the $G_1$–S cell-cycle transition. Mechanistic investigations revealed that IBA2 acted at the transcriptional level to promote the expression of the critical cell-cycle regulator cyclin D1. Clinically, we found that levels of IBA2 were significantly upregulated in breast cancer specimens, where its expression correlated positively with histologic grade. Our results suggest a key role for IBA2 in mammary tumorigenesis. *Cancer Res; 76(15); 4535–45. ©2016 AACR.*

## Introduction

EF hand (EFh) domain–containing proteins are involved in various disease states including chronic inflammation, tumor progression, cardiomyopathy, and other clinical disorders. They are associated with multiple targets that promote cell growth and differentiation, cell-cycle regulation, and transcription activities (1, 2). EFh proteins have remarkable sequence homology and structural similarity, yet they function in a wide range of biological processes. The activities of these proteins are tailored in part by a distinct pattern of subcellular localization and tissue-specific expression (3).

Ionized calcium–binding adapter molecule 1 (IBA1) is an EFh containing protein that binds and crosslinks filamentous actin (F-actin) and localizes to membrane ruffles and phagocytic cups. Daintain (also known as IBA1 or AIF-1) is closely associated with cardiac allograft vasculopathy (4–6), rheumatoid arthritis (7), inflammatory skin disorders (8), systemic sclerosis (9, 10), and central nervous system injury (11–13). Furthermore, IBA1 plays important roles in the initiation and progression of cancers, including breast cancer (14–19).

Ionized calcium–binding adapter molecule 2 (IBA2) is a homolog of IBA1 (20, 21) and share similar overall structure and molecular function with IBA1 (22). However, the distinct expression patterns of the two proteins in various tissues of the body indicate different functions between IBA1 and IBA2 (23). To date, the potential roles in carcinogenesis and the cellular mechanisms of IBA2 have not been well characterized.

In this study, we demonstrate that IBA2 promotes the proliferation of breast cancer cells by facilitating the $G_1$–S transition through upregulation of cyclin D1. We found that IBA2 was frequently overexpressed in breast carcinomas, and that IBA2 expression levels were positively correlated with tumor grades in breast carcinoma samples.

## Materials and Methods

### Bioinformatics

The mRNA length, open reading frame, conserved domains, and chromosome location of IBA2 were predicted using NCBI databases (www.ncbi.nlm.nih.gov). The theoretical molecular weight and isoelectric point of IBA2 were predicted using ExPASy (www.expasy.ch/tools). Sequence alignments were performed with ClustalW (version 1.60; ref. 24), and phylogenetic analysis was performed using the Jotun Hein method (25).

### Tissue specimens and cell lines

Breast carcinoma tissues from human patients were obtained from Beijing 301 Military General Hospital (Beijing, China). Samples were frozen in liquid nitrogen immediately after surgical removal and maintained at −80°C until use. All human tissues were collected following protocols approved by the Ethics Committee of the Peking University Health Science Center. Normal human breast epithelia cell and human breast cancer cell lines were obtained from ATCC.

### Chromatin immunoprecipitation assays

ChIP assays were performed essentially as described previously (26). The following primers were used to amplify the *cyclin D1* promoter: forward, 5′-tgccgggctttgatcttt-3′; reverse,

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5′-cggtcgttgaggaggttgg-3′. The following primers were used to amplify a negative control sequence: forward, 5′-ttttggattttctttc-3′; reverse, 5′-gcattttgattttatt-3′.

### Tumor xenografts

Tumor xenografts were performed as described previously (27). MCF-7 breast cancer cells were plated and infected *in vitro* with mock (control) lentivirus encoding IBA2, or lentivirus encoding IBA2 shRNA. After 48 hours, $5 \times 10^6$ viable MCF-7 cells (in 200-mL PBS) were injected into the mammary fat pads of 6- to 8-week-old female BALB/c mice (Charles River Laboratories). Six animals per group were used in each experiment. Seventeen β-estradiol pellets (0.72 mg/pellet, 60-day release; Innovative Research of America) were implanted 1 day before the injection of MCF-7 cells. Tumor volume and weight were measured. All studies were approved by the Animal Care Committee of Peking University Health Science Center (Beijing, China).

## Results

### Cloning and characterization of IBA2

We cloned the *IBA2* gene in GenBank (ID NM_031426) from a mammary cDNA library. The cDNA of IBA2 is 3,455 bp in length and contains an open reading frame of 453 bp, which includes one potential phosphorylation site and one potential acetylation site (Fig. 1A and B). The predicted molecular mass and isoelectric point of IBA2 are approximately 17.0 kDa and 6.63, respectively. The *IBA2* gene is located on chromosome 9q34.13 and consists of five exons and four introns. IBA2 is predicted to have an EFh domain, thus belonging to the EFh proteins (Fig. 1A). These

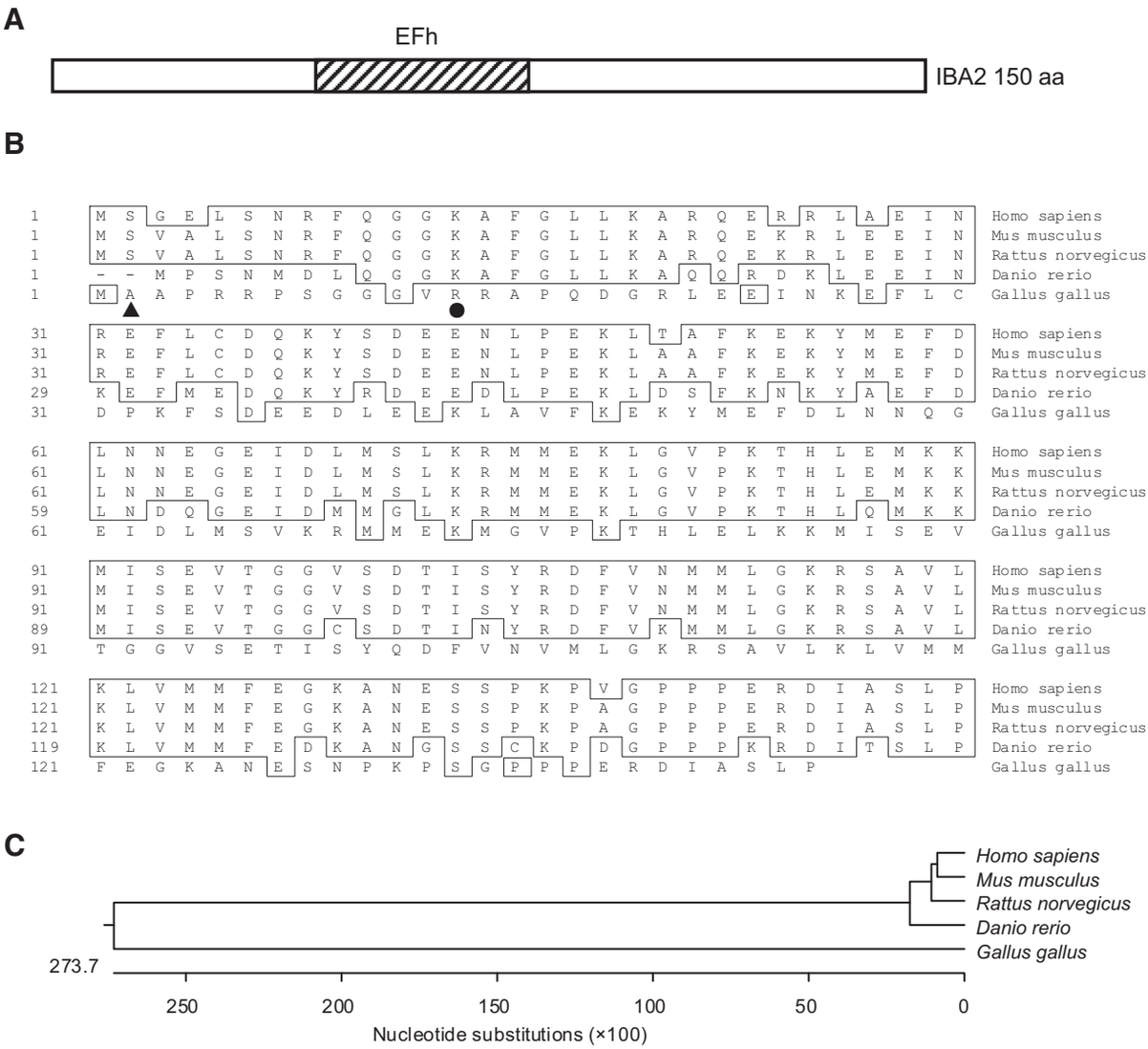



**Figure 1.**

Cloning and sequence analysis of IBA2. **A,** schematic diagram of IBA2 protein sequence. The conserved EFh domain is shown. **B,** sequence analysis of IBA2 proteins from different species. Boxed residues represent conserved regions. One potential phosphorylation site (residue 2) is indicated with ▲, and one potential acetylation site (residue 33) is labeled with ●. **C,** phylogenetic analysis of the evolutionary relationship among IBA2 family proteins in different species.

proteins have remarkable sequence homology and structural similarity, yet they function in a wide range of biological processes. On the basis of protein sequence, human IBA2 shares 82% identity with the mouse (*Mus musculus*) and rat (*Rattus norvegicus*) homologs, 67% with the zebrafish (*Danio rerio*) homolog, and 75% with the chicken (*Gallus gallus*) homolog (Fig. 1B). Phylogenetic analysis also indicated that IBA2 is an evolutionarily well-conserved gene (Fig. 1C).

To determine the expression profile of IBA2, we analyzed the expression of IBA2 mRNA in several mouse tissues (Fig. 2A). IBA2 mRNA expression was highest in the kidney, relatively high in the heart, and low in the brain, spleen, lung, skeletal muscle, and testis. IBA2 mRNA was not detected in the liver (Fig. 2A). We first examined the expression of IBA2 protein by transfecting a FLAG-tagged IBA2 expression construct into MCF-7 cells. After 48 hours, the total protein lysate was analyzed by Western blotting using an anti-FLAG mAb. As shown in Fig. 2B (left), IBA2 was expressed as an approximately 17 kDa protein, confirming its predicted molecular weight. We also generated polyclonal antibodies to IBA2 using recombinant IBA2. Western blotting analysis of endogenous and overexpressed IBA2 protein using the anti-IBA2 polyclonal antibody confirmed that endogenous IBA2 is also approximately 17 kDa (Fig. 2B, right). In addition, IBA2 mRNA and protein expression are higher in breast cancer cells than in normal human breast epithelial cells (MCF-10A; Fig. 2C). To gain insight into the biological function of IBA2, we examined its subcellular localization. Overexpressed EGFP-IBA2 and FLAG-IBA2 localized to the cytoplasm and nucleus in HeLa cells (Fig. 2D).

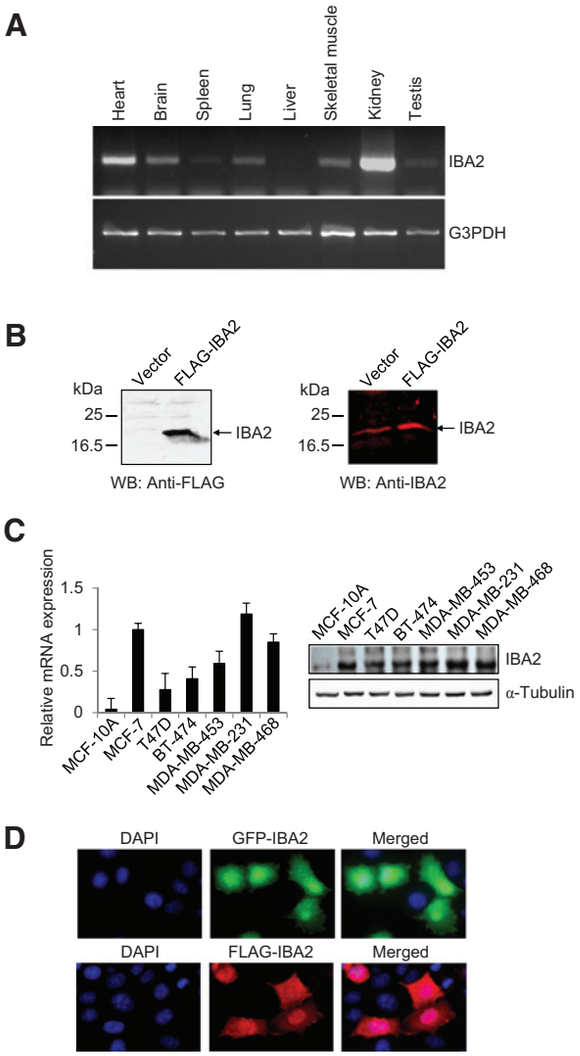
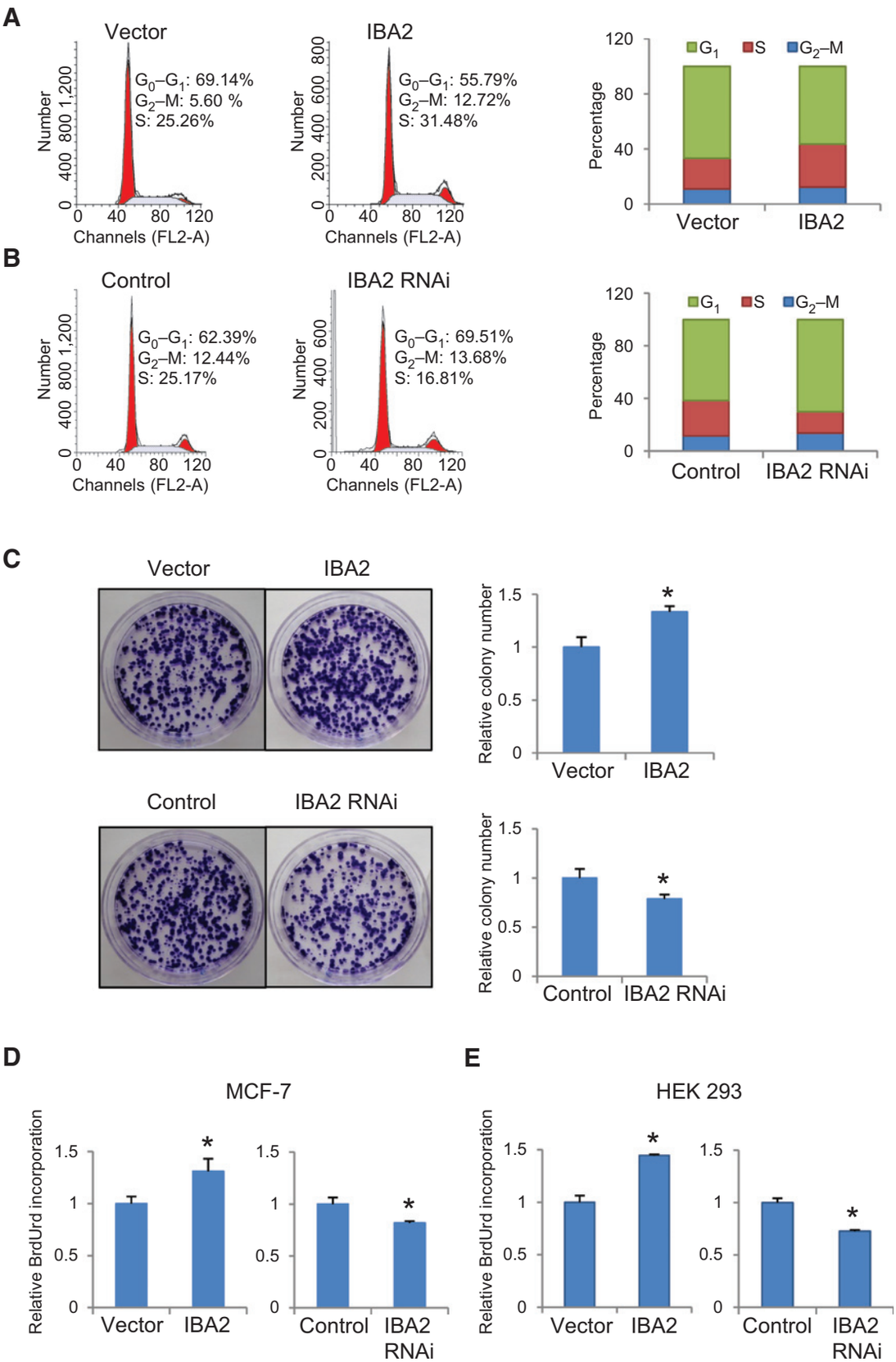



**Figure 2.**
The expression profile of IBA2 in mouse tissues and cell lines. **A,** real-time PCR analysis of the tissue-specific IBA2 mRNA expression. **B,** Western blotting analysis of IBA2 protein. MCF-7 cells were transfected with an empty vector or FLAG-IBA2. Cellular proteins were prepared and Western blotting was performed with anti-FLAG (left) or anti-IBA2 (right). **C,** real-time PCR (left) and Western blotting (right) analysis show that IBA2 is expressed at higher levels in breast cancer cells than in normal human breast epithelial cells (MCF-10A). GAPDH and α-tubulin were used as internal controls. **D,** subcellular localization of IBA2 protein. HeLa cells were transfected with EGFP-IBA2 (top) or FLAG-IBA2 (bottom). After 24 hours, EGFP fluorescence or immunofluorescence (rhodamine) of the FLAG tag was visualized by fluorescence microscopy. DAPI staining was included to visualize the cell nucleus.

### IBA2 promotes proliferation and tumorigenesis of breast cancer cells

Because of the crucial role of IBA1 in cell proliferation and tumorigenesis and its close homology to IBA2, we hypothesized that the misregulation of IBA2 expression in breast carcinomas and breast cancer cell lines may have pathologic relevance. Thus, we investigated whether IBA2 expression influenced breast cancer cell proliferation and tumorigenesis. First, we examined the effect of IBA2 overexpression or knockdown on cell-cycle regulation in mammary carcinoma cells. In these experiments, MCF-7 cells were synchronized at the $G_0$–$G_1$ phase by serum starvation (28–30). Cell-cycle profiling by FACS indicated that IBA2 overexpression was associated with an increase in the transition of cells to the S+$G_2$–M phases from the $G_0$–$G_1$ phase (Fig. 3A). Consistently, knockdown of IBA2 with specific siRNA resulted in an accumulation of cells in the $G_0$–$G_1$ phase (Fig. 3B). Collectively, these results indicated that IBA2 promotes the proliferation of breast cancer cells by facilitating the $G_1$–S transition.

To investigate the role of IBA2 in breast tumorigenesis, we overexpressed and knocked down IBA2 by infecting MCF-7 cells using lentiviruses encoding the *IBA2* gene or shRNA against IBA2, respectively. A mock lentivirus (carrying an empty vector) and a lentivirus encoding a nonsilencing control shRNA were used as controls for overexpression and knockdown, respectively. Colony formation assays demonstrated that overexpressing IBA2 increased the number of colonies formed, whereas knocking down IBA2 reduced the number of colonies formed (Fig. 3C). To further demonstrate the importance of IBA2 in cell proliferation, we performed BrdUrd incorporation assays, the indication of cell proliferation by which is more sensitive. The number of BrdUrd-positive MCF-7 and human embryonic kidney 293 (HEK 293) cells increased when IBA2 was overexpressed and decreased when IBA2 was downregulated (Fig. 3D and E). To demonstrate whether or not the oncogenic properties of IBA2 may also include an ability to promote migration, IBA2 overexpression and knockdown cells were used in cell invasion assays. We found that neither IBA2 overexpression nor knockdown affect cell invasion (Supplementary Fig. S1A and S1B). Western blotting also showed the epithelial markers E-cadherin and α-catenin and the


A
Vector
Number
Channels (FL2-A)
G0–G1: 69.14%
G2–M: 5.60 %
S: 25.26%
IBA2
G0–G1: 55.79%
G2–M: 12.72%
S: 31.48%
G1
S
G2–M
Percentage
Vector
IBA2
B
Control
G0–G1: 62.39%
G2–M: 12.44%
S: 25.17%
IBA2 RNAi
G0–G1: 69.51%
G2–M: 13.68%
S: 16.81%
Control
C
Vector
IBA2
Relative colony number
Control
IBA2 RNAi
D
MCF-7
Relative BrdUrd incorporation
E
HEK 293

mesenchymal markers vimentin and S100A are expressed to similar extents in both IBA2 overexpression and knockdown cells (Supplementary Fig. S1C). These results suggest that the oncogenic properties of IBA2 reside in its ability to promote proliferation rather than migration.

To assess the importance of IBA2 on breast tumorigenesis *in vivo*, equal numbers of IBA2-overexpressing and IBA2 shRNA–expressing MCF-7 cells and their respective controls were implanted into the mammary fat pads of athymic BALB/c mice, and the growth of the implanted tumors ($n = 6$ for each group) was measured over a period of 8 weeks. Overexpression of IBA2 was associated with significant tumor growth, and knockdown of IBA2 expression resulted in a dramatic reduction in tumor volume and weight (Fig. 4A–C).

### Identification of cyclin D1 as a downstream target for IBA2

To further dissect the signaling pathway underlying IBA2-mediated cell proliferation and tumorigenesis, the expression of cell-cycle–regulated genes was monitored by Western blotting and real-time PCR (Fig. 5A and B). As shown in Fig. 5A, while the overexpression or knockdown of IBA2 had little effect on the protein expression of some cell-cycle–regulated genes, including *cyclin E1*, *cyclin D3*, *cyclin B1*, *CDK2*, *CDK4*, *CDK6*, and *p-RB*, the protein and RNA expression of cyclin D1 was elevated in cells overexpressing IBA2 and reduced in cells when IBA2 was downregulated. We also detected the senescence markers P53 and P21 and found that the overexpression or knockdown of IBA2 had little effect on the expression level of both proteins (Fig. 5A). These results suggest that the *cyclin D1* gene is a downstream target of IBA2 in breast cancer cells.

We further tested the role of cyclin D1 in promoting IBA2-dependent proliferation of breast cancer cells by overexpressing cyclin D1 when IBA2 was knocked down to determine whether cyclin D1 could alleviate the effect of $G_0$–$G_1$ accumulation induced by IBA2 knockdown. MCF-7 cells were cotransfected with either IBA2 siRNA and a cyclin D1 expression construct, or IBA2 siRNA and an empty expression vector as a negative control. FACS analysis revealed that cyclin D1 expression in IBA2-downregulated cells resulted in a significant decrease in the percentage of cells in the $G_0$–$G_1$ phase (Fig. 5C). These results strongly suggest that cyclin D1 is a critical downstream mediator of IBA2 in promoting the $G_1$–S transition and cell proliferation. To examine whether the EFh domain is critical for the role of IBA2 in regulating cell proliferation, we generated an IBA2 mutant in which the EFh domain was deleted (Fig. 5E, left). A FLAG tag was added to the N terminus of the deletion construct to monitor its expression by Western blotting using an anti-FLAG antibody (Fig. 5E, right). Deletion of the EFh domain reduced the effect of IBA2 in promoting cell-cycle progression (Fig. 5D), indicating that the EFh domain is essential for this function.

### Transcriptional regulation of cyclin D1 by IBA2

Although cyclin D1 is overexpressed in approximately 50% of human breast cancers, *cyclin D1* gene amplification accounts for only 10% of these cases (31). Therefore, other mechanisms, such as increased gene transcription, must be responsible for the overexpression of this cell-cycle regulator. To determine whether IBA2 regulates cyclin D1 expression in the transcription level, we examined the effect of IBA2 on *cyclin D1* promoter using luciferase reporter assays. As shown in Fig. 6A, IBA2 induced expression of the *cyclin D1* gene promoter in a dose-dependent manner in both MCF-7 cells and HEK 293 cells. These results indicate that IBA2 upregulates *cyclin D1* gene transcription.

We mapped *cis*-element(s) within the *cyclin D1* promoter that is/are required for IBA2-dependent gene transcription. MCF-7 cells were transfected with a series of 5′-truncated *cyclin D1* promoter luciferase constructs (32) and with or without IBA2 expression constructs, and luciferase activity was measured (Fig. 6B). Deletion of the sequence spanning −186 bp to −106 bp resulted in decrease in IBA2-dependent gene transcription. Further deletion up to −53 bp led to a complete loss of IBA2-dependent gene transcription. These results reveal that the *cyclin D1* promoter region spanning nucleotides −186 to −53 harbors essential element(s) responsible for mediating IBA2-dependent gene transcription.

To determine whether IBA2 directly regulates *cyclin D1* gene transcription, we performed ChIP assays to determine whether IBA2 is directly recruited to the *cyclin D1* promoter. As shown in Fig. 6C, IBA2 was recruited to the proximal region of the *cyclin D1* promoter (−86 to −234 bp). The recruitment of IBA2 was sequence-specific as negligible binding was detected either with the genomic sequence 2.5 kb upstream of the *cyclin D1* gene transcription start site or with a control antiserum. This result demonstrates that IBA2 physically associates with the *cyclin D1* promoter.

To determine which domain of IBA2 is required for *cyclin D1* gene transcription, we tested the EFh domain deletion mutant of IBA2. Deletion of the EFh domain was associated with a diminished IBA2-dependent *cyclin D1* gene transcription (Fig. 6D), indicating that the EFh domain of IBA2 is important for *cyclin D1* gene transcription.

### IBA2 is overexpressed in human mammary tumors and its level is positively correlated with tumor grade

Using IHC, we screened paraffin-embedded mammary tissue sections from 24 normal and 30 breast cancer patients for the

**Figure 3.**

IBA2 promotes proliferation of breast cancer cells. **A,** the effect of IBA2 overexpression on cell-cycle progression. MCF-7 cells were transfected with an empty vector or FLAG-IBA2 for 24 hours and then switched to conditioned medium without serum for 24 hours. The cells were then cultured in medium containing 10% FBS for 24 hours and collected for cell-cycle analysis by flow cytometry. The data shown in the right panel represent data from three independent experiments. Representative profiles are shown in the left panel. **B,** the effect of IBA2 knockdown on cell-cycle progression. After MCF-7 cells were transfected with control siRNA or IBA2 siRNA for 24 hours, cells were switched to conditioned medium without serum for 24 hours. The cells were then cultured in medium containing 10% FBS for 24 hours and collected for cell-cycle analysis by flow cytometry. The data shown in the right panel represent data from three independent experiments. Representative profiles are shown in the left panel. **C,** colony formation assay. MCF-7 cells stably expressing the indicated plasmids were maintained in culture media for 14 days and stained with crystal violet. The number of colonies in each condition was counted and expressed as the mean ± SD from triplicate experiments. *, $P < 0.05$ (two-tailed unpaired *t* test). **D** and **E,** effect of IBA2 on cell proliferation. MCF-7 cells (**D**) and HEK 293 cells (**E**) were transfected with the indicated constructs, and cell proliferation was measured by BrdUrd incorporation. Data are means ± SD from triplicate experiments. *, $P < 0.05$ (two-tailed unpaired *t* test).

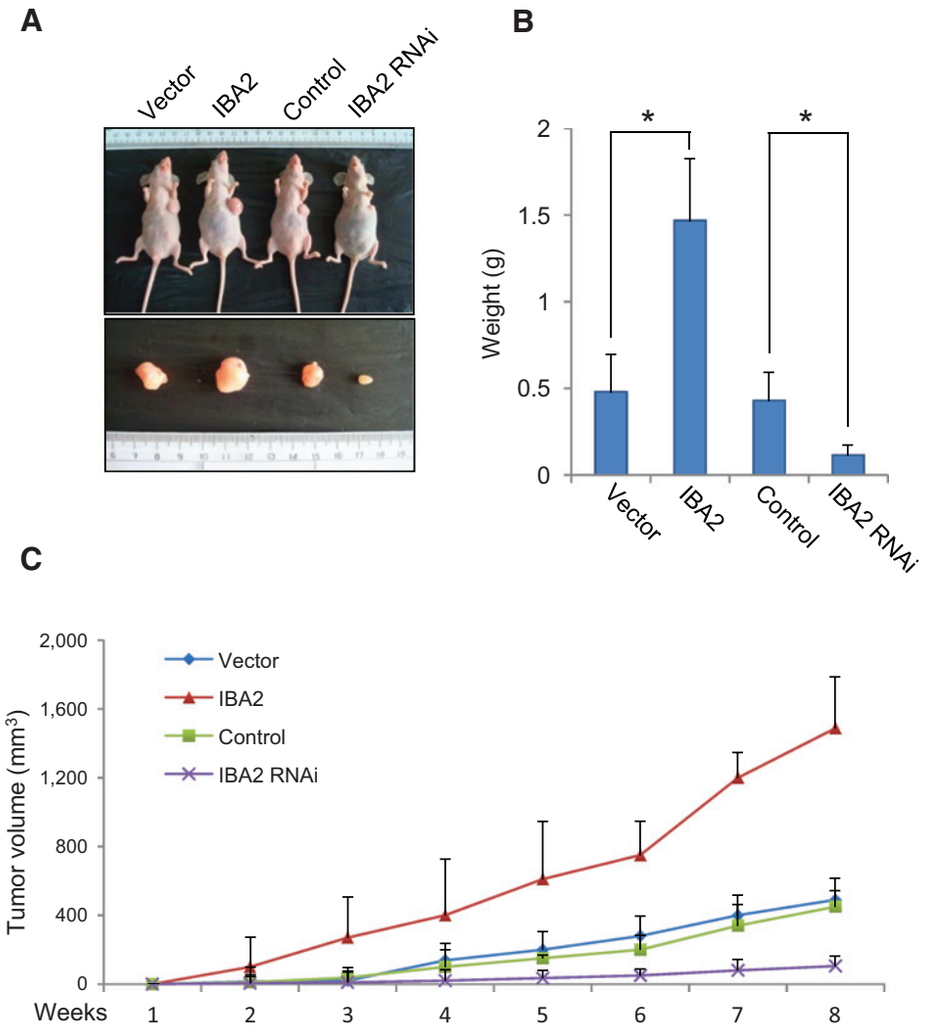



**Figure 4.**
IBA2 promotes tumorigenesis of breast cancer cells. **A–C,** IBA2 promotes breast tumorigenesis. IBA2-overexpressing or IBA2 shRNA–expressing MCF-7 cells were transplanted into ovariectomized athymic mice. Tumors were measured weekly using a Vernier caliper and the volume was calculated according to the formula: $\pi/6 \times \text{length} \times \text{width}^2$. Representative images of tumor-bearing mice (**A**), the average tumor mass of each group (**B**), and the growth curves of tumors (**C**) are shown. Each point represents the mean ± SD. *, $P < 0.05$ (two-tailed unpaired *t* test).

expression of IBA2. While normal mammary epithelial cells displayed no or weak IBA2 staining (Fig. 7A, left), breast carcinoma cells displayed strong IBA2 staining in both the cytoplasm and nucleus (Fig. 7A, right).

To determine whether there is a correlation between the expression level of IBA2 and the development and progression of breast tumors, we measured IBA2 mRNA expression levels in primary tumors using quantitative real-time PCR. We collected normal mammary tissue from 24 normal patients and breast carcinoma tissue from 30 breast cancer patients. IBA2 was significantly upregulated in tumors and its expression correlated positively with histologic grades (Fig. 7B). Increased IBA2 mRNA expression was also evident in the majority of breast carcinomas compared with adjacent normal tissues (Fig. 7C). Cyclin D1 mRNA levels in carcinoma samples were also analyzed and were plotted against the levels of IBA2 mRNA (Fig. 7D). Statistical analysis found a Pearson correlation coefficient of 0.5679 ($P < 0.0001$), indicating a strong positive correlation between the expression of these two genes in breast carcinomas. These data further support a role for IBA2 in cyclin D1 regulation and in breast carcinogenesis. Collectively, these data suggest that overexpression of IBA2 may be a frequent event in human breast cancer and promotes the initiation and progression of breast cancer.

## Discussion

EFh domain–containing transcription factors play important roles in carcinogenesis. They have structural similarity and affect a wide range of biological processes. The activities of these proteins are determined in part by a distinct pattern of subcellular localization and tissue-specific expression. IBA1, an EF protein and homolog of IBA2, may contribute to the progression of epithelial–mesenchymal transition (EMT; refs. 33, 34) and is overexpressed in human mammary tumors and cervical cancer tissues (18, 35). Overexpression of IBA1 promotes the proliferation of human breast cancer cells and facilitates tumor growth in female nude mice (10, 18). These results indicate that IBA1 may function as an oncogene. IBA2 has similar overall structure to, but distinct expression patterns from IBA1, which indicates that IBA2 may have other important roles in carcinogenesis. However, the potential role in carcinogenesis and the cellular mechanism of IBA2 have not been well characterized. In the current study, we showed that

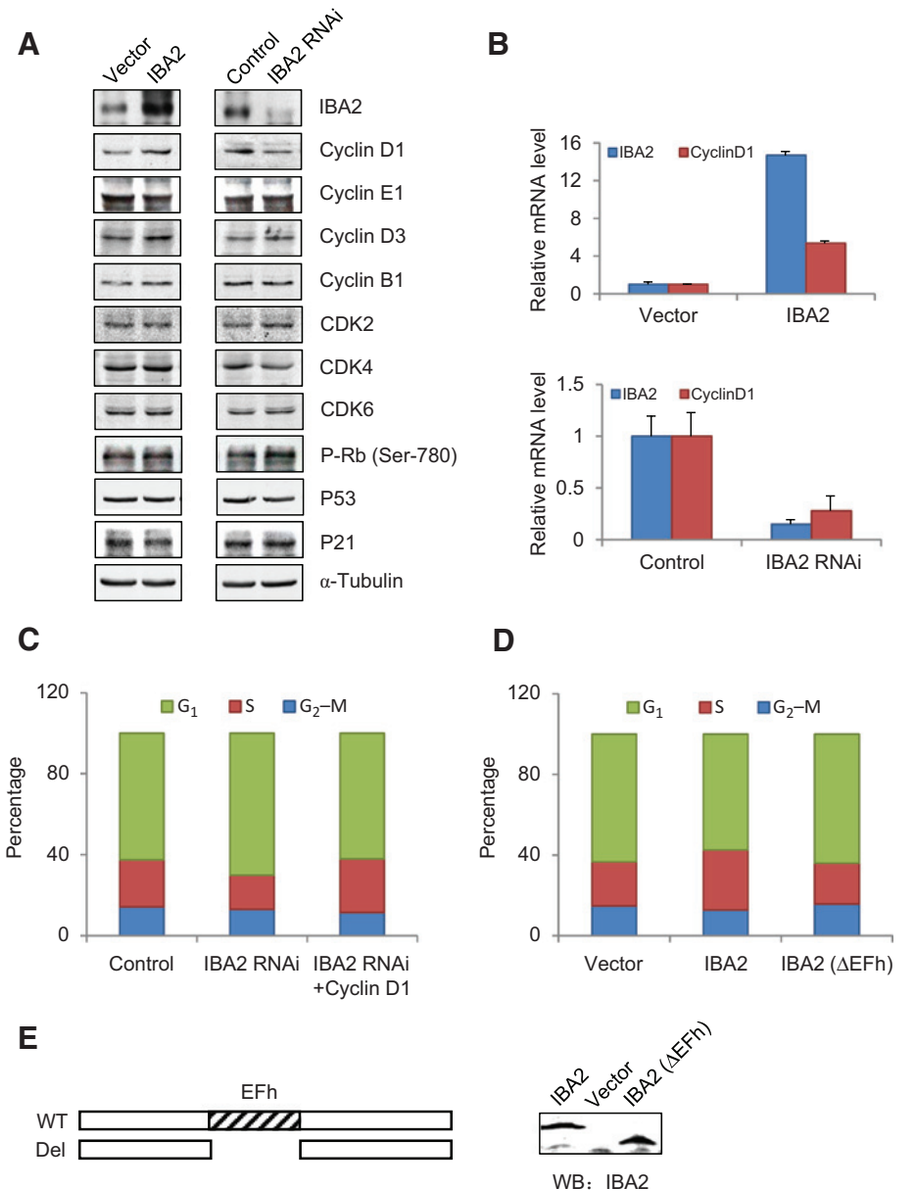



**Figure 5.**
Identification of cyclin D1 as a downstream target of IBA2. **A,** Western blotting analysis of the main proteins involved in the $G_1$–S transition in IBA2-overexpressing or IBA2 knockdown cells. **B,** real-time PCR analysis of the expression of IBA2 and cyclin D1 in IBA2-overexpressing or IBA2 knockdown cells. **C,** the effect of cyclin D1 and IBA2 knockdown on cell proliferation. **D,** the effect of IBA2-ΔEFh on cell proliferation. **E,** functional domain of IBA2 implicated in the cell proliferative effect.

IBA2 promotes cell proliferation and tumorigenesis in breast cancer cells via regulating cyclin D1.

EFh proteins regulate the expression of downstream target genes and play a role in carcinogenesis; therefore, target identification is essential to understand the oncogenic potential of these proteins. In this study, we identified the *cyclin D1* gene as a downstream target of IBA2 in breast cancer cells. Cyclin D1 is a critical regulator of the $G_0$–$G_1$ to S transition in the cell cycle; thus, its identification as the downstream target of IBA2 is consistent with the effect of IBA2 in promoting the $G_0$–$G_1$ to S transition. On the other hand, cyclin D1 expression or transcriptional activities can be regulated by oncogenes, growth factors, ion channels, and G-protein–coupled receptors (36). We show here IBA2 can regulate cyclin D1 expression and transcriptional activities.

Cyclin D1 amplification and/or overexpression is one of the most prevalent alterations in breast carcinomas, occurring in approximately 50% of cases (31, 37–39). The oncogenic activity of cyclin D1 in mammary tissue was demonstrated in mouse models wherein cyclin D1 overexpression led to the development of mammary carcinoma (40), whereas *cyclin D1* ablation resulted in mice resistant to cancer induced by several oncogenes (41). In addition, *cyclin D1*–null mice exhibited defective postnatal mammary development as revealed by a lack of proliferation of alveolar epithelial cells in response to the sex steroid milieu of pregnancy (42, 43). In fact, cyclin D1 is a classic oncogene that is also amplified and/or overexpressed in a substantial proportion of other human cancers including parathyroid adenoma, colon cancer, lymphoma, melanoma, and prostate cancer (44).

Another approach to understanding the oncogenic potential of IBA2 is to decipher its molecular actions, that is, the molecular details involved in its regulation of transcription. IBA1 promotes human vascular smooth muscle cell proliferation (45, 46) and this ability is lost by mutations in the EFh region (47). IBA2 binds

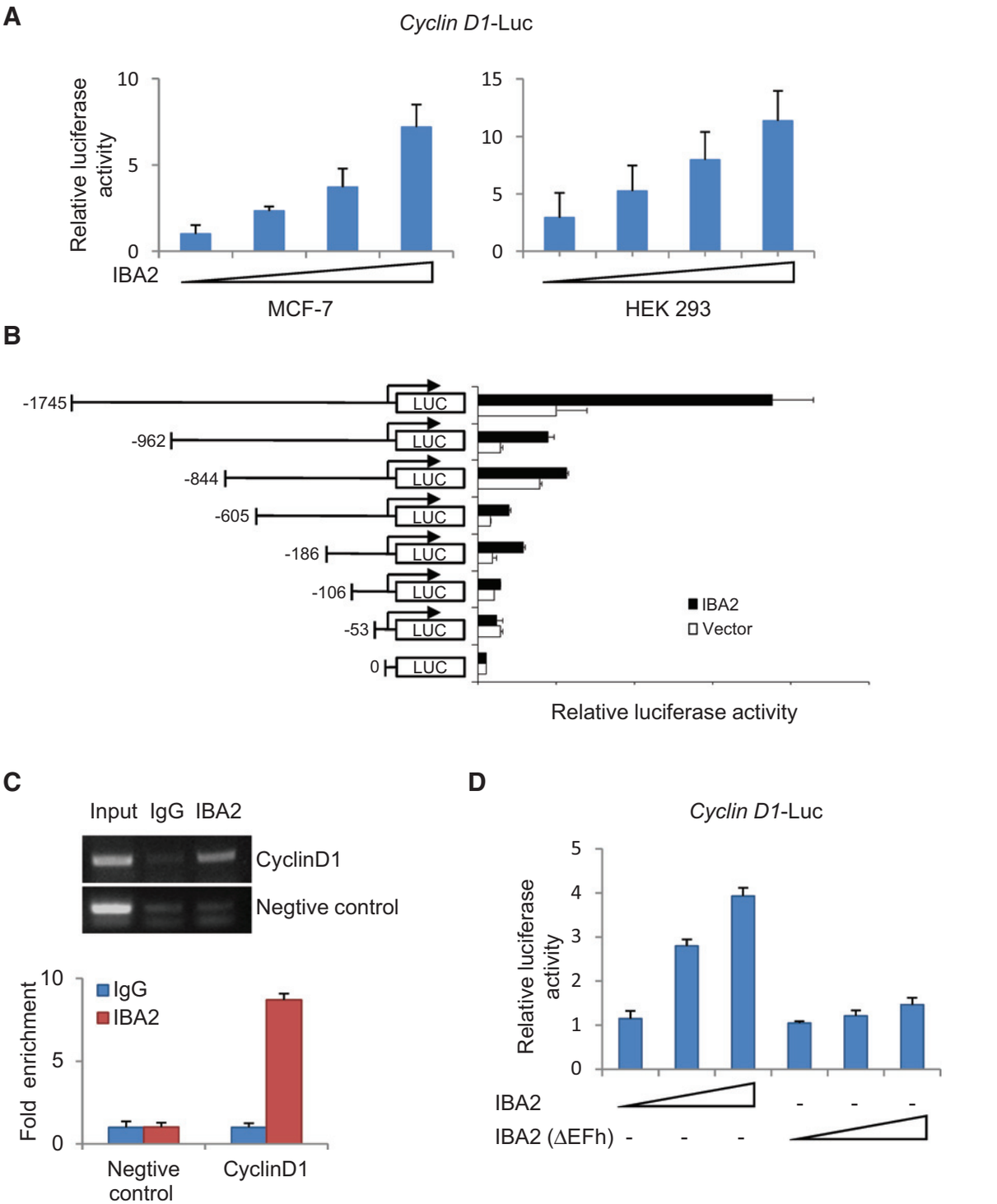



**Figure 6.**

Transcriptional regulation of cyclin D1 by IBA2. **A,** IBA2 transactivates *cyclin D1* promoter expression. MCF-7 (left) and HEK 293 cells (right) were cotransfected with a *cyclin D1*-Luc reporter construct, a *Renilla* construct, and different amounts of the IBA2 expression construct (0.05, 0.2, and 0.8 μg/well). After 24 hours, cells were harvested and assayed for luciferase activity. **B,** mapping of *cis*-acting elements in the *cyclin D1* promoter that are responsible for IBA2 transactivation. Shown is a schematic representation of the 5′-truncated *cyclin D1* promoter luciferase constructs and their activities in MCF-7 cells. Cells were transiently transfected with the indicated reporter plasmids with or without cotransfection of an IBA2 expression construct (0.8 μg/well). **C,** the recruitment of IBA2 to the *cyclin D1* promoter. Soluble chromatin from MCF-7 cells was immunoprecipitated with anti-IBA2 or a control rabbit normal IgG. The final DNA extractions were amplified by PCR and real-time PCR using primers that cover the proximal promoter region of the *cyclin D1* gene or the upstream control region. **D,** functional domain of IBA2 implicated in its transactivation activity of cyclin D1. Shown are the transactivation activities of the IBA2 deletion mutant on the *cyclin D1* promoter. Data are means ± SD from triplicate experiments.

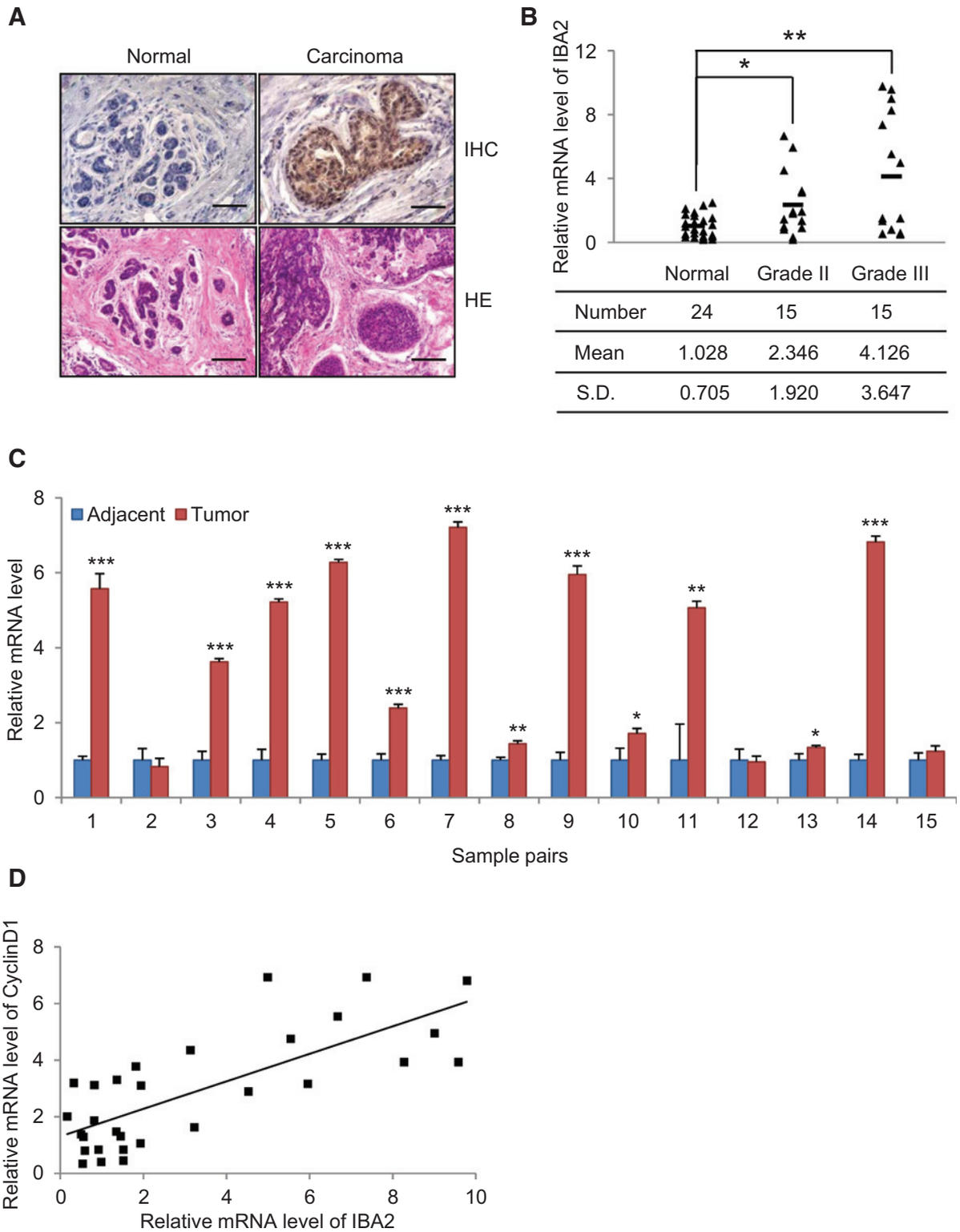



**Figure 7.**

IBA2 is overexpressed in human mammary tumors and its expression level correlates with tumor grade. **A,** representative IHC of IBA2 protein in paraffin-embedded human primary breast cancer (right) and adjacent normal tissue (left). Hematoxylin and eosin staining shows standard morphology. **B,** IBA2 expression levels in normal and carcinoma breast samples. Normalized IBA2 mRNA expression was measured by quantitative real-time PCR using GAPDH mRNA as the internal control. **C,** IBA2 mRNA levels in paired samples of breast carcinomas compared with adjacent normal mammary tissues. **D,** the relative level of IBA2 expression was plotted against the relative level of cyclin D1 expression.

specifically to DNA sequences through its EFh domain. EFh proteins activate multiple targets that are involved in cell growth, cell-cycle regulation, transcription, and other cellular activities (1, 2, 48, 49). As a result, IBA2 requires formation of stable protein–DNA complexes to promote cell proliferation and tumorigenesis by regulating cyclin D1.

In summary, we report here that IBA2 is overexpressed in breast carcinomas. We have demonstrated that IBA2 is able to promote cell proliferation and tumorigenesis of breast cancer, possibly by facilitating the $G_1$–S transition in the cell cycle. We identified the *cyclin D1* gene as a downstream target of IBA2 in breast cancer cells. These results will further our understanding of the oncogenic potential of IBA2, which will be useful for the prognosis and therapy of breast cancer.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

## Authors' Contributions

**Conception and design:** Y. Zhang, L. Li
**Development of methodology:** Y. Zhang
**Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.):** Y. Zhang, S. Wang
**Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis):** Y. Zhang, L. Li
**Writing, review, and/or revision of the manuscript:** Y. Zhang, L. Li
**Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases):** Y. Zhang, S. Wang
**Study supervision:** Y. Zhang, L. Li
**Other (provided research funding for this study):** Y. Zhang, L. Li

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