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The Mitotic Kinase Aurora-A Induces Mammary Cell Migration and Breast Cancer Metastasis by Activating the Cofilin-F-actin Pathway

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Abstract

The mitotic kinase Aurora-A (Aur-A) is required to form the bipolar spindle and ensure accurate chromosome segregation before cell division. Aur-A dysregulation represents an oncogenic event that promotes tumor formation. Here, we report that Aur-A promotes breast cancer metastasis. Aur-A overexpression enhanced mammary cell migration by dephosphorylation and activation of cofilin, which facilitates actin reorganization and polymerization. Cofilin knockdown impaired Aur-A–driven cell migration and protrusion of the cell membrane. Conversely, overexpression of activated cofilin abrogated the effects of Aur-A knockdown on cell migration. Moreover, Aur-A overexpression increased the expression of the cofilin phosphatase Slingshot-1 (SSH1), contributing to cofilin activation and cell migration. We found that phosphatidylinositol 3-kinase (PI3K) inhibition blocked Aur-A–induced cofilin dephosphorylation, actin reorganization, and cell migration, suggesting crosstalk with PI3K signaling and a potential benefit of PI3K inhibition in tumors with deregulated Aur-A. Additionally, we found an association between Aur-A overexpression and cofilin activity in breast cancer tissues. Our findings indicate that activation of the cofilin-F-actin pathway contributes to tumor cell migration and metastasis enhanced by Aur-A, revealing a novel function for mitotic Aur-A kinase in tumor progression. *Cancer Res; 70(22); 9118–28. ©2010 AACR.*

Introduction

The serine/threonine Aurora kinase family, including Aurora-A, Aurora-B, and Aurora-C, play an important role in ensuring genetic stability in cell division (1). Activation of Aurora A (Aur-A) requires a variety of cofactors, such as TPX2, Ajuba, PAK1, HEF1, and hBora, and it is inhibited by protein phosphatase-1–dependent dephosphorylation (2). We and others have shown that Aur-A is essential in accurate timing of mitosis and maintenance of bipolar spindles (3, 4). Indeed, Aur-A is physically associated with hBora, which in turn promotes activation of Polo-like kinase 1, causing the activation of cyclin-dependent kinase 1 (CDK1) and mitotic entry (5). Overexpression of Aur-A leads to centrosome amplification and subsequent formation of multipolar spindle structures, causing genetic instability or aneuploidy (4). Mouse NIH3T3 cells transfected with Aur-A give rise to tumors when injected into nude mice (6). The expression of Aur-A increases in certain types of cancer including breast, bladder, ovarian, colon, and pancreas (7). Recently, our data showed that Aur-A increases laryngeal cancer cell migration (8). However, the precise role of Aur-A in cancer invasion and metastasis remains largely unknown.

In tumor cells, acquisition of migratory ability is a characteristic that contributes to the spread of metastatic tumor cells to distant organs (9). Cell migration is physically mediated by actin cytoskeleton and is initiated by the protrusion of the cell membrane (10). Cofilin has emerged as an essential player for the localized formation of the barbed ends, which act as sites for new local actin polymerization, thus determining the direction of cell protrusion and movement (11). Expression of the wild-type or the nonphosphorylatable form of the cofilin mutant S3A increases melanoma cell migration and invasion (12), whereas depletion of cofilin impairs cell motility (13). Indeed, the phosphorylation site of cofilin localizes in the actin-binding domain and inhibits its binding to filamentous actin (F-actin), completely inhibiting cofilin ability to promote filament disassembly (14–17). Once dephosphorylated, cofilin binds actin filaments to promote actin turnover (18). Therefore, cofilin activity regulated

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by its phosphorylation status determines the cytoskeleton dynamics and cell motility.

The activity of cofilin is tightly regulated by phosphorylation and the binding of phosphatidylinositol-(4,5)-bisphosphate (19, 20). The serine/threonine kinases LIMK and TESK phosphorylate cofilin on the Ser3 residue, rendering it inactive (14, 21, 22). Suppression of cofilin activity by LIMK overexpression abolishes lamellipodium formation and polarized cell migration (23). Phosphatase Slingshot-1 (SSH1), which may be regulated by phosphatidylinositol 3-kinase (PI3K), dephosphorylates and activates cofilin, thereby stimulating the severing and depolymerization of actin (18, 24). Insulin-stimulated MCF-7 cells exhibit SSH1 activation and cofilin dephosphorylation that can be abrogated by PI3K inhibition (25). Additionally, SSH1 activity is directly stimulated by F-actin binding, indicating that existing actin filaments may promote actin reorganization through SSH1-mediated cofilin activation (26).

Our present data showed that overexpression of Aur-A enhanced cell motility and promoted the lung metastasis of breast cancer. Overexpression of Aur-A increased the nonphosphorylated, active form of cofilin by enhancing SSH1 expression, which in turn promoted actin reorganization. Importantly, we found a significant correlation between Aur-A expression and cofilin dephosphorylation in the immunohistochemical analysis of clinical breast cancer specimens, supporting a novel signaling mechanism by which Aur-A induced cofilin dephosphorylation and actin reorganization, thus promoting mammary cell movement and breast cancer metastasis.

Materials and Methods

Cell lines and cell culture conditions

Human breast epithelial cell lines (MCF-10A, MCF-7, SK-BR-3, and MDA-MB-435) and a cervix epithelial cell line (HeLa) were obtained from the American Type Culture Collection and used within 2 months after resuscitation of frozen aliquots. Cell lines were authenticated based on viability, recovery, growth, morphology, and isoenzymology by the supplier. Culture conditions are described in Supplementary Data.

Experimental lung metastasis model

Female BALB/c nude mice (5–7 weeks old) were purchased from SLRC Laboratory Animal and maintained and treated under specific pathogen-free conditions. Cells (2×10^6 per mouse) were injected i.v. via the tail vein. After 4 weeks, lungs were removed and examined macroscopically or detected in paraffin-embedded sections stained with H&E.

Magnetic resonance imaging

Magnetic resonance imaging (MRI) examinations were conducted on a GE (Signal Twin Speed Excite II) 1.5-T scanner with a 77 mT/m (150 mT/m ms) gradient system at room temperature.

Plasmid construction and stable cell line generation

Cofilin S3A or S3E was subcloned into pBabe vector. Aur-A (Flag tagged) was subcloned into pcDNA6B vector. MCF-10A cells stably expressing Aur-A were constructed as previously described (8). MDA-MB-435 cells stably expressing Aur-A (Flag tagged) were constructed with a lentivirus vector (pLVPT).

Western blot analysis

Western blot was done with antibodies against glyceraldehyde-3-phosphate dehydrogenase (Ambion), Flag (Sigma), SSH1 (Abcam), Aur-A (Upstate), phosphorylated Aur-A (Thr288; Cell Signaling), cofilin (Cell Signaling), phosphorylated cofilin (Ser3; Cell Signaling), Akt1 (Cell Signaling), and phosphorylated Akt1 (Ser473; Cell Signaling).

Transwell migration assay

Transwell assay was performed as described previously (8). Results were shown as average from at least three independent experiments. Error bars represented the SD.

Wound healing assay

Cells were grown to confluence on 24-well plates. The monolayers were wounded with a P10 micropipette tip. Photographs of wound healing were taken at the indicated time points.

Cellular F-actin/G-actin assay

F-actin and globular actin (G-actin) fractions were obtained using an F-actin/G-actin assay kit (BK 037, Cytoskeleton).

Phalloidin staining

Whole cell phalloidin staining was performed according to the manufacturer's protocol (Sigma P5282). Nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI) and viewed with Olympus IX71.

Analysis of Triton-soluble and insoluble actin

To measure Triton-soluble actin, cytoskeletal proteins were extracted and subjected to Western blot analysis with the indicated antibodies as previously described (27).

Immunofluorescence staining

Cells were fixed and permeabilized. Slides were incubated with the indicated antibodies for 60 minutes. The immune complexes were stained with secondary antibody conjugated to Alexa-488 or Alexa-680 (Molecular Probes). Nuclei were stained with DAPI and viewed with Olympus IX71.

Small interfering RNA transfection

Cells were transfected as previously described (8). RNA oligonucleotide duplexes targeted to Aur-A, cofilin, and SSH1 as well as negative control were purchased from Shanghai

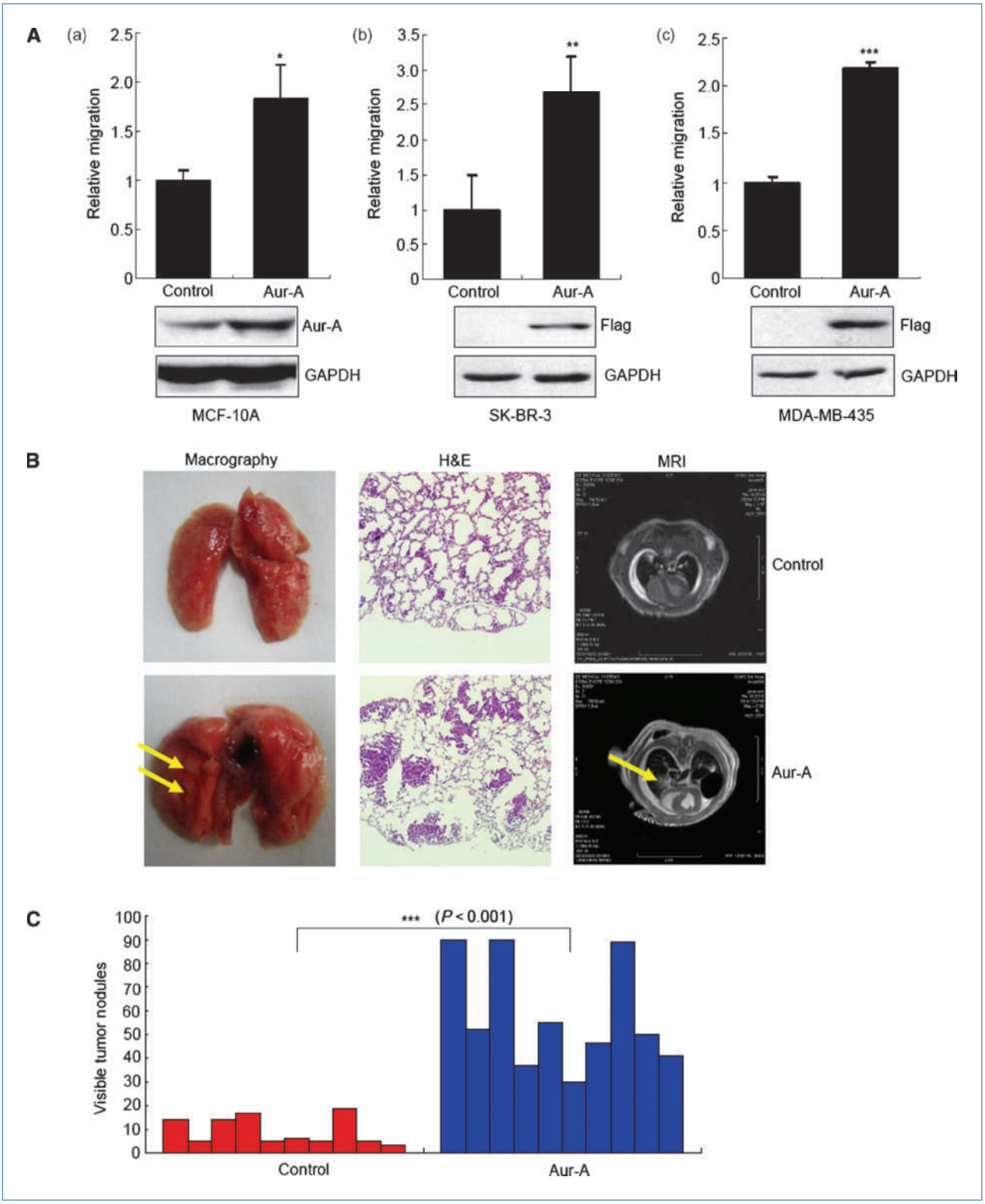

Figure 1. Overexpression of Aur-A significantly enhances cell migration and breast cancer metastasis. A, the migratory properties of control and Aur-A–overexpressing cells were analyzed by transwell assay (top). Data were summarized from three independent experiments. Aur-A expression level was detected by Western blot analysis (bottom). B, left, representative photograph of a lung specimen. Lung tissues from mice injected with MDA-MB-435-control and MDA-MB-435-Aur-A cells were analyzed by H&E staining (middle; ×100) and MRI (right). Arrows, metastatic nodules. C, the number of lung metastasis was counted in paraffin-embedded sections stained with H&E.

GenePharma Co., Ltd. The RNA sequences are provided in Supplementary Data.

Polyadenylate tail length assay

The polyadenylate tail length (PAT) assay was carried out as described previously (28). The sequences of the primers are provided in Supplementary Data.

Cell synchronization and cell cycle analysis

Cell synchronization and cell cycle analysis were performed as previously described (29).

Patients and clinical tissue specimens

A total of 99 breast tissue specimens were selected for analysis. Specimens were fixed in formalin and embedded in paraffin in the diagnostic histopathology laboratory at Cancer Center, Sun Yat-sen University.

Immunohistochemical staining and statistical analysis

Immunohistochemical staining was performed as described previously (8). The staining intensity and extent of Aur-A and phosphorylated cofilin (Ser3) were graded as described previously (30, 31). Due to the small sampling size, Fisher's exact test was used to analyze the significance of differences.

Statistics

The χ^2 test and Student t test were used to make statistical comparisons between groups. All P values were two sided. $P < 0.05$ was considered statistically significant. We performed each study at least three times under identical conditions.

Results

Overexpression of Aur-A significantly enhances cell migration and breast cancer metastasis

A noncancerous breast epithelial cell line, MCF-10A, and two breast cancer cell lines, SK-BR-3 and MDA-MB-435, were transiently transfected with Aur-A. Cell migration assay was performed, and we found that Aur-A enhanced cell migration significantly in all three cell lines (Fig. 1A). Wound healing assay showed that MCF-10A-Aur-A cells migrated into the wound area faster than MCF-10A-control cells (Supplementary Fig. S1). We compared cell growth rates between cells and found that overexpression of Aur-A did not produce a significant increase in cell number up to 2 days (Supplementary Figs. S2A and S3A). Cell migration assay was performed in less than 1 day, indicating that the higher rate of migration caused by Aur-A overexpression was not contributive to a more rapid division. To clarify whether Aur-A–enhanced cell

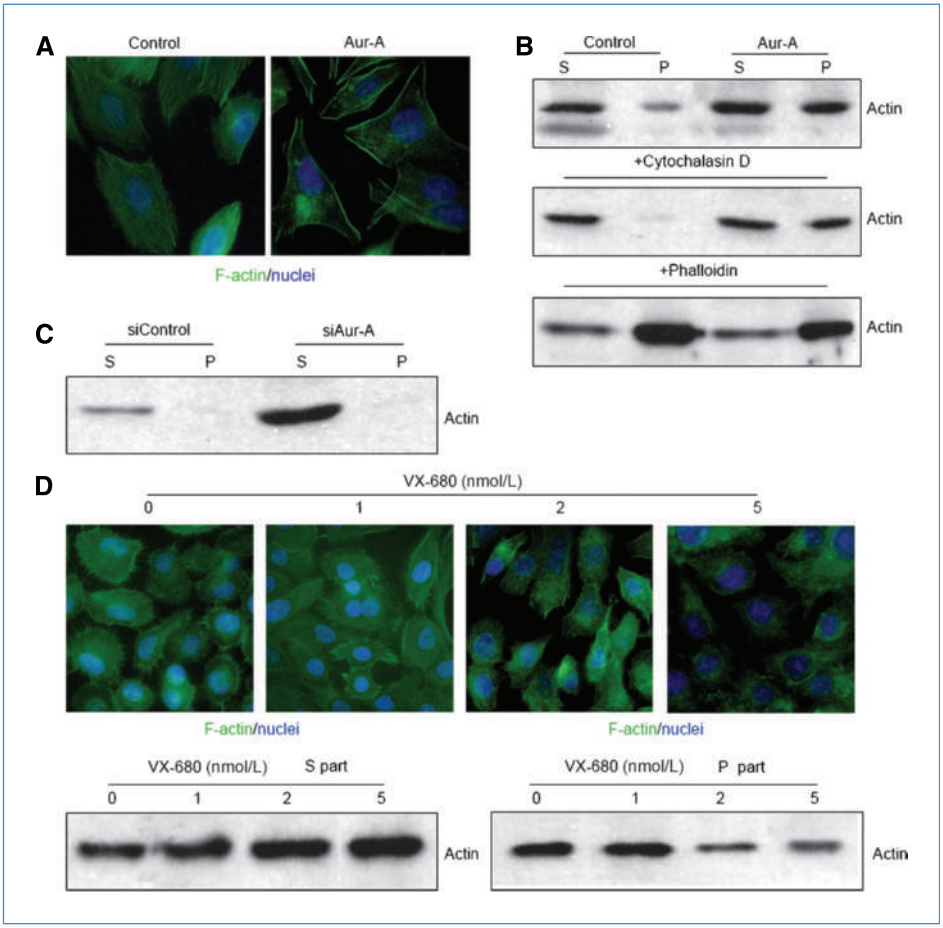

Figure 2. Aur-A regulates actin organization and polymerization. A, immunofluorescence analysis was performed using FITC-labeled phalloidin (F-actin; green). Nuclei were stained with DAPI (blue). An overlay of the two fluorescent signals is shown (×1,000). B, F-actin and G-actin fractions were prepared from MCF-10A-control and MCF-10A-Aur-A cells (top). Two cell lines were treated with F-actin depolymerization factor (cytochalasin D; middle) or F-actin enhancing factor (phalloidin; bottom). F-actin and G-actin fractions were prepared and subjected to Western blot analysis [S, supernatant part (G-actin); P, pellet part (F-actin)]. C, F-actin and G-actin fractions were prepared from control siRNA– or Aur-A siRNA–treated MCF-10A cells and subjected to Western blot analysis. D, MCF-10A cells were treated with increasing dose of VX-680 for 16 h, followed by immunofluorescence analysis. The fluorescent signals of F-actin (green) along with the nuclei (blue) are shown (×1,000; top). F-actin and G-actin fractions were prepared and subjected to Western blot analysis (bottom).

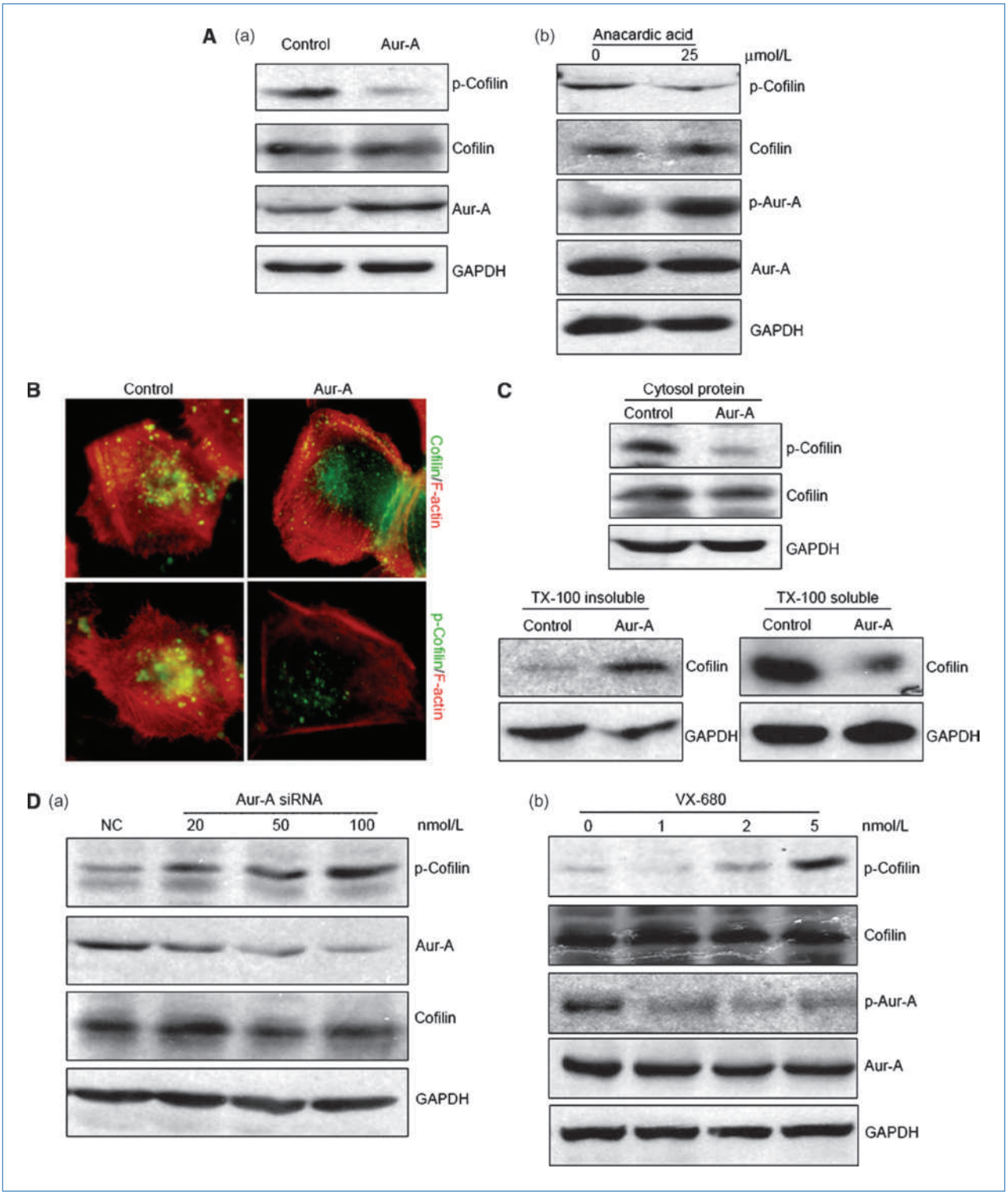

Figure 3. Aur-A regulates activation of the actin binding protein cofilin. A, a, MCF-10A-control and MCF-10A-Aur-A cells were lysed and subjected to Western blot analysis with the indicated antibodies. b, MCF-10A cells were treated with 25 μmol/L anacardic acid for 5 min. Cell lysates were subjected to Western blot analysis with the indicated antibodies. B, MCF-10A-control and MCF-10A-Aur-A cells were coimmunostained with FITC-labeled F-actin and p-cofilin or cofilin antibodies. The fluorescent signals of cofilin or p-cofilin (Green) along with F-actin (red) are shown (×1,000). C, MCF-10A-control and MCF-10A-Aur-A cells were lysed using cytosol buffer (top) and Triton X-100–based solubilization buffer (bottom). Cell lysates were further subjected to Western blot analysis with the indicated antibodies. D, MCF-10A cells were treated with increasing dose of Aur-A siRNA (a) or VX-680 (b). Cell lysates were subjected to Western blot analysis with the indicated antibodies.

migration was related to a particular phase in cell cycle, we synchronized MCF-10A-control and MCF-10A-Aur-A cells at G_1-S and G_2-M phases (Supplementary Fig. S2B). Migration assay showed that Aur-A–enhanced cell migration in all cell cycle phases (Supplementary Fig. S2C and D). We used MDA-MB-435 cells to test our findings in an experimental metastasis model. As shown in Supplementary Fig. S3, the pattern of cell proliferation, as well as Aur-A cellular localization, cell cycle–dependent expression, and specific phosphorylation states, did not show significant differences between MDA-MB-435-control and MDA-MB-435-Aur-A cells. Mice injected with MDA-MB-435-Aur-A cells had a significantly increased metastatic burden as measured by macrography, H&E staining, and MRI (Fig. 1B). MDA-MB-435-control cells formed 3 to 19 metastatic nodules per lung. In contrast, mice injected with MDA-MB-435-Aur-A cells formed 30 to 90 nodules per lung (Fig. 1C), indicating that overexpression of Aur-A significantly increased metastatic spread. Taking these data together, we showed that Aur-A enhances breast cell migration and breast cancer metastasis.

Both reduction of Aur-A expression and inhibition of Aur-A kinase activity decrease cell migration

Next, we found that suppression of Aur-A reduced the ability of cells to migrate (Supplementary Fig. S4A). Cell migration assay showed that VX-680, a small molecular inhibitor of Aurora kinases, effectively prevented cell movement in a dose-dependent manner (Supplementary Fig. S4B). Moreover, control cells migrated into the wound closure within 30 hours, whereas VX-680–treated cells were significantly less motile, as shown by the delay in the mean time of closure (Supplementary Fig. S5).

Aur-A regulates actin organization and polymerization

We next determined if differences in actin cytoskeletal organization could explain the migratory function of Aur-A. In control cells, actin was diffusely distributed throughout the cytoplasm (Fig. 2A, left). In contrast, in Aur-A–overexpressing cells, F-actin structures were formed as large actin-rich lamellipodial protrusions around the periphery of the cell with a few thin stress fibers located within the cell body (Fig. 2A, right). Because the rates of polymerization and depolymerization of actin were important determinants of cell motility, the cellular F-actin/G-actin assay was carried out. Aur-A markedly increased the amount of F-actin (Fig. 2B, top). Moreover, we treated both control and Aur-A–overexpressing cells with the F-actin depolymerization factor cytochalasin D. F-actin in Aur-A–overexpressing cells was not depolymerized as compared with control cells (Fig. 2B, middle). When cells were treated with the F-actin enhancing factor phalloidin, the status of F-actin polymerization in Aur-A–overexpressing cells did not change significantly (Fig. 2B, bottom). The cellular F-actin/G-actin assay also showed that G-actin was more abundant in Aur-A–depleted cells (Fig. 2C). Inhibition of Aur-A activity by VX-680 typically resulted in the disappearance of actin-rich membrane ruffles and caused a decrease in detectable leading-edge protrusions (Fig. 2D, top). The actin polymerization rates were decreased as well (Fig. 2D, bottom). Thus, Aur-A–driven cell movement is associated with dramatic reorganization of actin cytoskeleton and formation of lamellipodia at the leading edge.

Aur-A regulates activation of the actin binding protein cofilin

Cofilin, an actin binding protein, regulates the rates of actin polymerization and depolymerization (32). As shown in Fig. 3A, overexpression of Aur-A or stimulation of Aur-A activity by using the Aur-A kinase activator anacardic acid induced a larger pool of the nonphosphorylated active form of cofilin (total cofilin was unchanged). Next, we found that in control cells, cofilin was distributed diffusely in the cytoplasm without associating with actin stress fibers, whereas in Aur-A–overexpressing cells, cofilin was colocalized with F-actin and recruited to the leading edge (Fig. 3B, top). In contrast, the distribution of phosphorylated cofilin showed no differences between control and Aur-A–overexpressing cells (Fig. 3B, bottom). Additionally, we observed that Aur-A reduced cofilin phosphorylation in the cytoplasm (Fig. 3C, top). Moreover, Aur-A induced enrichment of cofilin in the detergent-insoluble fraction (Fig. 3C, bottom left), which was known to include actin-associated proteins. In control cells, cofilin was mainly in the detergent-soluble part, which largely disappeared following overexpression of Aur-A (Fig. 3C, bottom right). Reduction of Aur-A expression [Fig. 3D(a)] or decrease in its kinase activity by VX-680 [Fig. 3D(b)] induced phosphorylation of cofilin and reduced its activity, which was consistent with the defects observed in actin polymerization (Fig. 2D). Transient overexpression of Aur-A in HeLa and MCF-7 cells also caused reduction of cofilin phosphorylation (Supplementary Fig. S6). These data indicate that Aur-A induces cofilin activity to remodel actin fiber.

Cofilin activity mediates Aur-A–driven mammary cell migration and actin organization

To address the dependence of Aur-A–induced cell migration on cofilin, we depleted endogenous cofilin using specific small interfering RNA (siRNA) and found that both cofilin and p-cofilin decreased in control and Aur-A–overexpressing cells [Fig. 4A(a)]. Following depletion of cofilin, overexpression of Aur-A failed to enhance cell migration [Fig. 4A(b)]. Moreover, cofilin siRNA suppressed Aur-A–induced actin reorganization (Fig. 4B). To analyze if cofilin activity contributed to Aur-A–induced cell migration and actin poly merization, we expressed dominant-active or inactive cofilin mutants in control and Aur-A–overexpressing cells (Fig. 4C). Expression of the active nonphosphorylatable cofilin S3A mutant in control cells had apparent effects on cell migration and actin polymerization, mimicking the Aur-A phenotype [Fig. 4D(a)]. Expression of an inactive phosphorylation-mimic cofilin S3E mutant in Aur-A–overexpressing cells reversed the cell migration and actin polymerization [Fig. 4D(b)]. Furthermore, we ectopically introduced vector

control, the active form of cofilin S3A, or the inactive form of cofilin S3E into Aur-A siRNA–treated cells. Transwell assay showed that the active form of cofilin S3A reversed Aur-A siRNA–induced reduction in cell migration, whereas the inactive form of cofilin S3E did not have any effects (Supplementary Fig. S7), providing additional evidence that cofilin activity has an essential role in Aur-A–driven cell motility.

Aur-A upregulates cofilin activity by increasing the expression of the cofilin phosphatase SSH1

SSH1 is known to regulate actin filament dynamics by dephosphorylating and activating cofilin (18). The expression of SSH1 was increased in Aur-A–overexpressing cells [Fig. 5A (a), left] but was decreased in MCF-10A cells treated with Aur-A siRNA [Fig. 5A(a), right]. Aur-A has been found to phosphorylate cytoplasmic polyadenylation element (CPE) binding protein (CPEB) and promote the mRNA polyadenylation and translation of CDK1 and cyclin B1 (33). We found that the 3′ untranslated region of SSH1 mRNA contained a CPE consensus sequence, UUUUUAU (34), which interacted with CPEB, playing a crucial role in promoting translation (35). As shown in Fig. 5A(b), overexpression of Aur-A elongated the polyadenylate [poly(A)] tail of SSH1 in the PAT assay, indicating that Aur-A increased SSH1 expression by promoting its translation. Additionally, depletion of SSH1 blocked Aur-A–induced cofilin dephosphorylation [Fig. 5B(a)] and cell migration [Fig. 5B(b)]. We next found that inhibition of PI3K with 100 nmol/L wortmanin blocked Akt1 phosphorylation and suppressed Aur-A–induced cofilin dephosphorylation [Fig. 5C(a)] and cell migration [Fig. 5C(b)]. Moreover, Aur-A–induced actin reorganization was totally blocked by wortmannin (100 nmol/L; Fig. 5D). These results suggest that SSH1 as well as PI3K play a critical role in Aur-A–induced cofilin activity and cell migration.

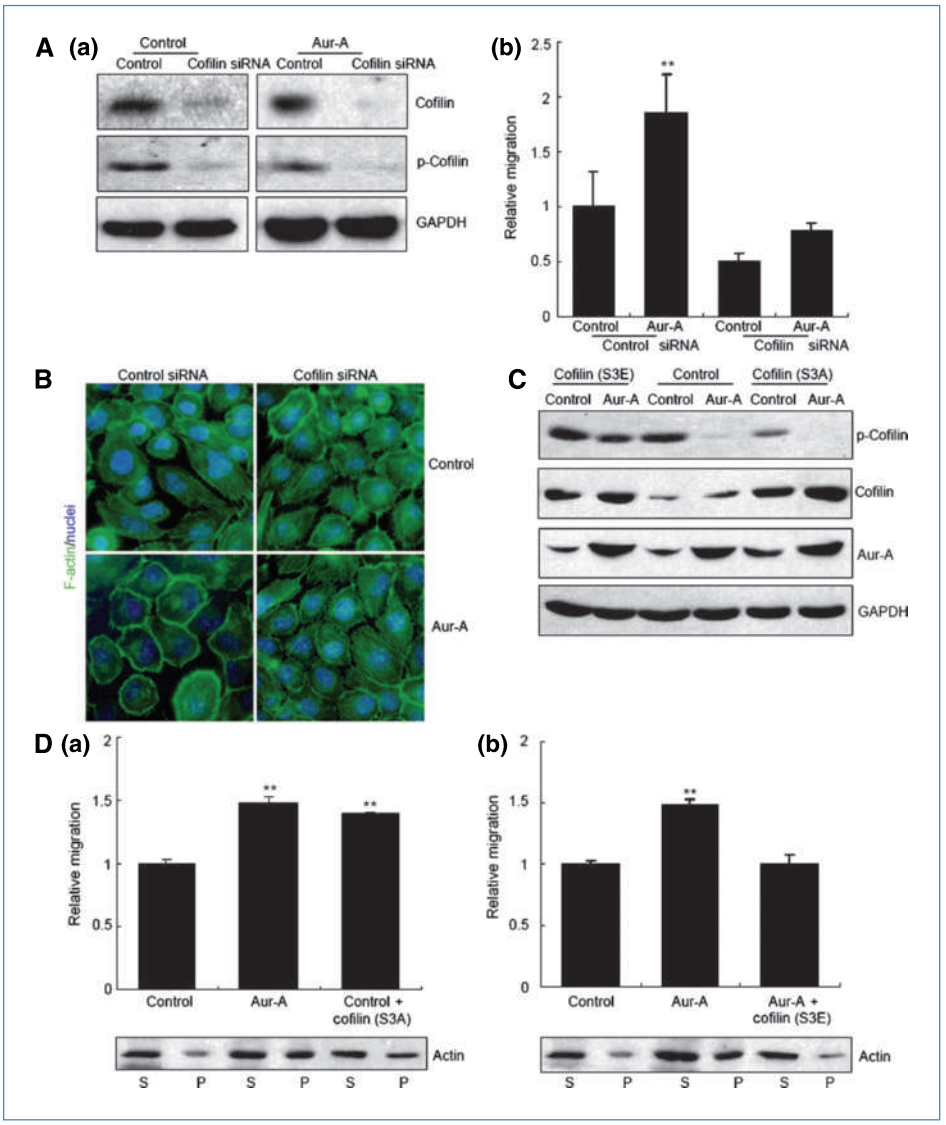

Figure 4. Cofilin activity mediates Aur-A–driven mammary cell migration and actin organization. A, MCF-10A-control and MCF-10A-Aur-A cells were transfected with control siRNA or cofilin siRNA. a, cells were lysed and subjected to Western blot analysis with cofilin and p-cofilin antibodies. b, the migratory properties of the cells were analyzed by transwell assay. Data are averages from three independent experiments. B, the fluorescent signals of F-actin (green) along with the nuclei (blue) are shown (×1,000). C, MCF-10A-control and MCF-10A-Aur-A cells were transfected with cofilin S3A or S3E. Cells were lysed and subjected to Western blot analysis with the indicated antibodies. D, MCF-10A-control cells were transfected with cofilin S3A (a), whereas MCF-10A-Aur-A cells were transfected with cofilin S3E (b). The migratory properties of the cells were analyzed by transwell assay (top). Data were summarized from three independent experiments. F-actin and G-actin fractions were prepared and subjected to Western blot analysis (bottom).

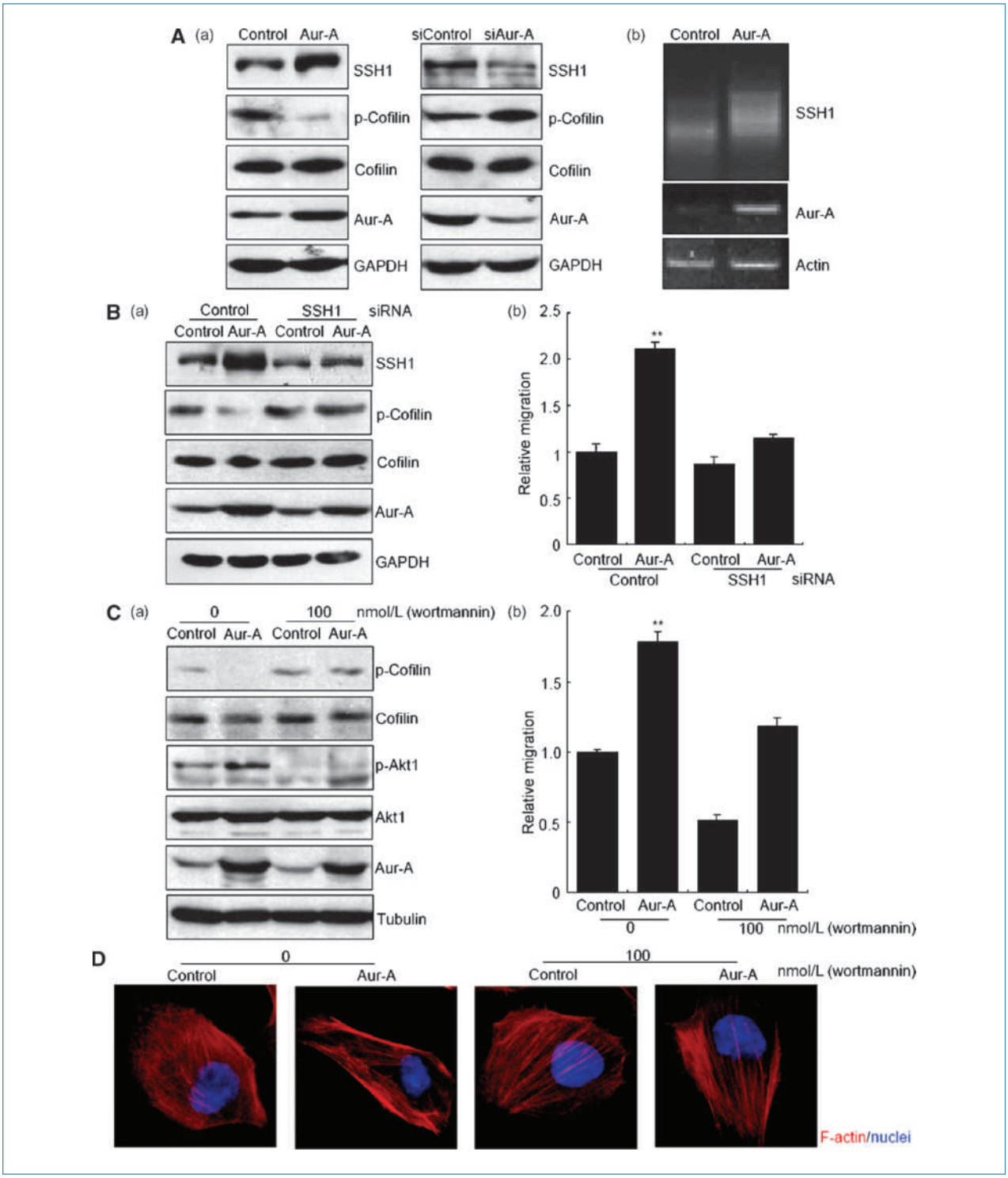

Figure 5. Aur-A upregulates cofilin activity by increasing the expression of the cofilin phosphatase SSH1. A, a, SSH1 expression of control and Aur-A–overexpressing cells was detected by Western blot analysis (left). SSH1 expression of control siRNA or Aur-A siRNA treated cells was detected by Western blot analysis (right). b, a PAT assay was performed using specific primers to SSH1. B, MCF-10A-control and MCF-10A-Aur-A cells were treated with control siRNA or SSH1 siRNA. a, cells were lysed and subjected to Western blot analysis with the indicated antibodies. b, the migratory properties of the cells were analyzed by transwell assay. Data are averages from three independent experiments. C, a, MCF-10A-control and MCF-10A-Aur-A cells were treated with the PI3K inhibitor wortmannin (Cell Signaling; 100 nmol/L). Cells were lysed and subjected to Western blot analysis with the indicated antibodies. b, the migratory properties of the cells were analyzed by transwell assay. Data were summarized from three independent experiments. D, the fluorescent signals of F-actin (red) along with the nuclei (blue) are shown (×1,000).

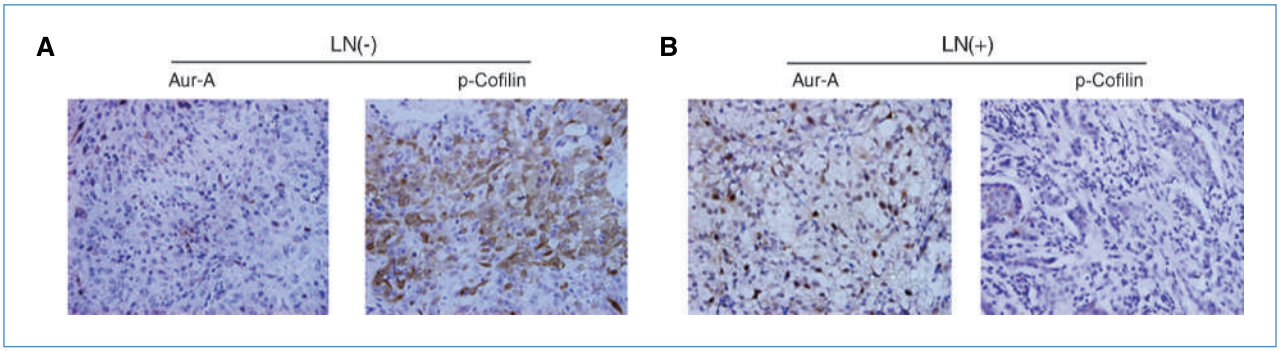

Figure 6. Aur-A expression and cofilin activity are correlated with lymph node metastasis in breast cancer. A, representative immunohistochemical analysis of a tumor sample from a patient without lymph node metastasis [LN(–)], labeled by Aur-A and p-cofilin (Ser3) antibodies (×200). B, representative immunohistochemical analysis of a tumor sample from the same patient with lymph node metastasis [LN(+)], labeled by Aur-A and p-cofilin (Ser3) antibodies (×200).

Aur-A expression and cofilin activity are correlated with lymph node metastasis in breast cancer

We analyzed 99 breast tissue specimens of histologically positive and negative lymph node metastasis by immunohistochemistry. The clinical features are summarized in Supplementary Table S1. Aur-A was expressed at high or low level in specimens with or without lymph node metastasis, respectively (Fig. 6A and B, left; Supplementary Table S2; $P = 0.0013$). Conversely, cofilin was phosphorylated at high or low level in specimens without or with lymph node metastasis, respectively (Fig. 6A and B, right; Supplementary Table S2; $P = 0.0017$). Importantly, we found statistically significant correlations between high Aur-A expression and low cofilin phosphorylation (Supplementary Table S3; $P = 0.01$). In conclusion, our data implicate the Aur-A-cofilin pathway in breast cancer progression and identify them as potential markers of metastatic breast cancer.

Discussion

In this study, we showed that overexpression of Aur-A promoted migration in both breast epithelial noncancerous cells and carcinoma cells. More importantly, we revealed a potentially significant role of Aur-A in breast cancer metastasis, as observed both in the *in vivo* animal model (Fig. 1) and in clinical specimens (Fig. 6). We showed that overexpression of Aur-A regulated actin reorganization, leading to free barbed end formation (Fig. 2). These morphology changes were mediated by SSH1-enhanced cofilin

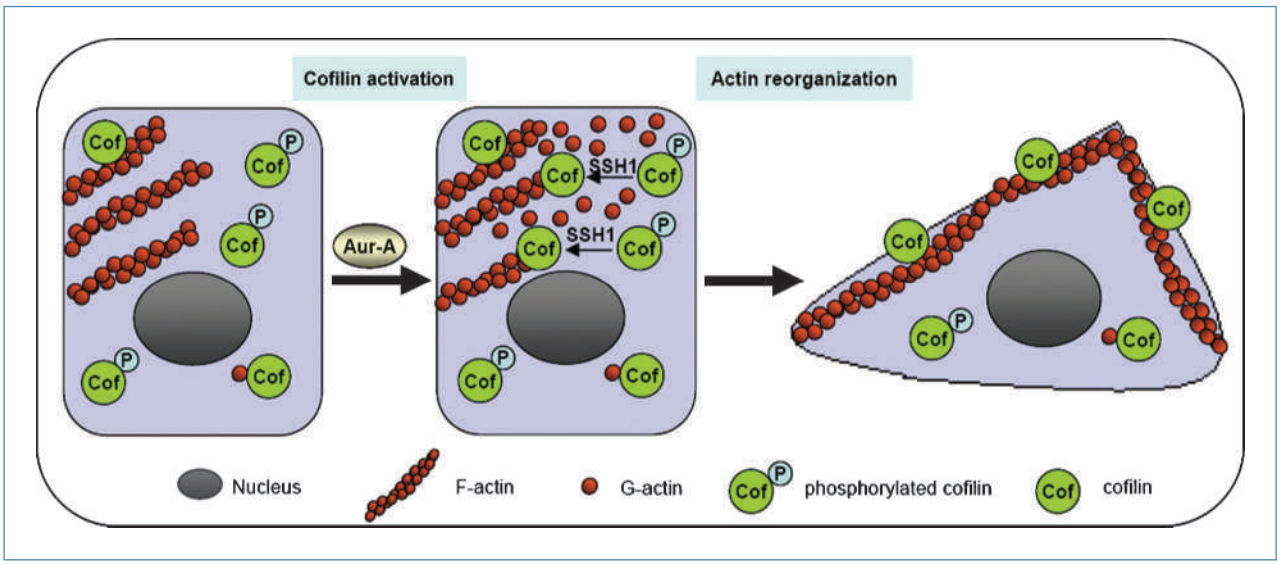

Figure 7. A model of Aur-A-cofilin-actin signaling cascade in cell migration. In resting state, cells exhibited a cobble-like epithelial morphology, and F-actin was diffusely distributed throughout the cell. A few cofilin molecules bound with both F-actin and G-actin, whereas most cofilin molecules were phosphorylated and scattered in the cytoplasm without associating with actin stress fibers. Overexpression of Aur-A increased SSH1 expression and reduced the phosphorylation level of cofilin, resulting in cofilin activation, colocalization with F-actin, and subsequent recruitment to the leading edge. Actin was reorganized and polymerized by activated cofilin, leading to the formation of large actin-rich lamellipodial protrusions around the cell periphery.

dephosphorylation, which was upregulated by Aur-A overexpression (Figs. 3–5). Furthermore, our data confirmed a strong correlation between Aur-A expression and cofilin activation in breast cancer (Fig. 6; Supplementary Table S3). To our knowledge, this is the first study to describe a potential role for Aur-A in tumor metastasis, indicating that Aur-A is a potential target in future studies on the prevention breast cancer progression.

Accumulating evidence has shown that both cancer invasion and metastasis are directly linked to activation of cofilin, which is regulated by phosphorylation status on Ser3 (36). For example, the increased expression of LIMK1 in carcinoma cells significantly reduced cell motility because the phosphorylation of cofilin by LIMK1 abolished epidermal growth factor–stimulated actin nucleation, protrusion, and chemotaxis *in vitro* and invasion, intravasation, and metastasis *in vivo* (37). Here, we provided evidence that Aur-A upregulated cofilin activity by increasing its dephosphorylation (Fig. 3A), resulting in colocalization with actin and recruitment to the leading edge (Fig. 3B). In addition, we found that Aur-A enriched cofilin in the detergent-insoluble fraction (Fig. 3C). These data indicated that Aur-A enhanced cofilin activity, which promoted actin polymerization, membrane protrusion, and cell migration, consistent with a previous study showing that cofilin activity was essential for the localized formation of barbed ends and that determined the direction of cell protrusion and movement (11). The finding that Aur-A activated the cytoskeletal regulator cofilin and enhanced actin polymerization during cell migration has not been reported previously. Our data revealed a novel mechanism whereby Aur-A promoted actin reorganization and subsequently induced mammalian cell migration through cofilin activation, suggesting that inhibition of cofilin activity may prevent the oncogenic effect of Aur-A in breast cancer metastasis.

Cofilin is dephosphorylated and reactivated by SSH1 phosphatase (18). We showed that overexpression of Aur-A increased SSH1 expression [Fig. 5A(a)]. Indeed, we found that the 3′ untranslated region of SSH1 mRNA contained a specific RNA sequence (UUUUUAU) called the cytoplasmic polyadenylation element (CPE; ref. 34). CPE and its binding protein CPEB play an essential role in elongating the 3′ poly(A) tail of mRNA and inducing 5′ cap ribose methylation, thus promoting translation initiation (35). Previous study showed that overexpression of Aur-A activated CPEB and promoted the mRNA polyadenylation and translation of CDK1 and cyclin B1 (33). Here, we showed that overexpression of Aur-A elongated the poly(A) tail of SSH1 [Fig. 5A(b)], suggesting that Aur-A enhanced SSH1 expression by promoting its translation. Additionally, SSH1 reduction almost completely suppressed Aur-A–induced cofilin dephosphorylation [Fig. 5B(a)]. These data indicate that SSH1 is a major cofilin phosphatase in Aur-A–promoted breast epithelial cell migration.

Previous study showed that Aur-A expression was correlated with nuclear grade but not with prognosis in primary breast tumors (38). On the other hand, a recent work found that high Aur-A expression was strongly associated with high nuclear grade and remained as an independent prognostic marker (39). We and others previously showed that Aur-A overexpression was closely correlated with clinic stage and lymph node metastasis in other cancer types (40, 41). Here, we found that Aur-A was highly expressed in breast cancer specimens with positive lymph node metastasis (Fig. 6B). The percentage of Aur-A high expression in total tissues was 61% (Supplementary Table S2). Previously, several studies using breast cancer tissues have statistically analyzed high Aur-A expression, ranging from 26% to 94% as summarized in Supplementary Fig. S8 (38, 42–46). One possible explanation for the discrepancy in the rates of Aur-A overexpression is that the intensity and degree of staining might have been graded by a different standard.

Increased level of cofilin expression is detected in clinical tumor samples and highly invasive cell lines (37). However, the status of phosphorylated cofilin has not been shown in clinical specimens. Here, we found that the level of cofilin phosphorylation was significantly decreased in breast cancer with positive lymph node metastasis (Fig. 6B; Supplementary Table S2). Importantly, we first found a significant correlation between Aur-A overexpression and cofilin dephosphorylation in breast cancer (Supplementary Table S3), further suggesting a potential role of the Aur-A-cofilin pathway in the pathogenesis of breast cancer metastasis.

Taken together, our results show a hitherto unrevealed mechanism of Aur-A–driven cell migration in breast epithelial cells: Overexpresssion of Aur-A increases the expression of the cofilin phosphatase SSH1 and then activates cofilin, resulting in actin reorganization and the formation of lamellipodial protrusions around the cell periphery (Fig. 7). More importantly, we found a correlation between Aur-A overexpression and cofilin activity in breast cancer tissues, suggesting that cofilin may be a novel target for the treatment of metastatic cancers caused by overexpression of Aur-A. Further studies will address the prognostic and predictive significance of the Aur-A-cofilin signaling pathway in cancer development.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Meraldi P, Honda R, Nigg EA. Aurora kinases link chromosome segregation and cell division to cancer susceptibility. Curr Opin Genet Dev 2004;14:29–36.
2. Barr AR, Gergely F. Aurora-A: the maker and breaker of spindle poles. J Cell Sci 2007;120:2987–96.
3. Liu Q, Ruderman JV. Aurora A, mitotic entry, and spindle bipolarity. Proc Natl Acad Sci U S A 2006;103:5811–6.
4. Marumoto T, Zhang D, Saya H. Aurora-A—a guardian of poles. Nat Rev Cancer 2005;5:42–50.
5. Seki A, Coppinger JA, Jang CY, Yates JR, Fang G. Bora and the kinase Aurora a cooperatively activate the kinase Plk1 and control mitotic entry. Science 2008;320:1655–8.
6. Zhou H, Kuang J, Zhong L, et al. Tumour amplified kinase STK15/BTAK induces centrosome amplification, aneuploidy and transformation. Nat Genet 1998;20:189–93.
7. Mountzios G, Terpos E, Dimopoulos MA. Aurora kinases as targets for cancer therapy. Cancer Treat Rev 2008;34:175–82.
8. Guan Z, Wang XR, Zhu XF, et al. Aurora-A, a negative prognostic marker, increases migration and decreases radiosensitivity in cancer cells. Cancer Res 2007;67:10436–44.
9. Gupta GP, Massague J. Cancer metastasis: building a framework. Cell 2006;127:679–95.
10. Bailly M, Condeelis J. Cell motility: insights from the backstage. Nat Cell Biol 2002;4:E292–4.
11. Ghosh M, Song X, Mouneimne G, Sidani M, Lawrence DS, Condeelis JS. Cofilin promotes actin polymerization and defines the direction of cell motility. Science 2004;304:743–6.
12. Dang D, Bamburg JR, Ramos DM. $\alpha_v\beta_3$ integrin and cofilin modulate K1735 melanoma cell invasion. Exp Cell Res 2006;312:468–77.
13. Hotulainen P, Paunola E, Vartiainen MK, Lappalainen P. Actin-depolymerizing factor and cofilin-1 play overlapping roles in promoting rapid F-actin depolymerization in mammalian nonmuscle cells. Mol Biol Cell 2005;16:649–64.
14. Arber S, Barbayannis FA, Hanser H, et al. Regulation of actin dynamics through phosphorylation of cofilin by LIM-kinase. Nature 1998;393:805–9.
15. Bamburg JR. Proteins of the ADF/cofilin family: essential regulators of actin dynamics. Annu Rev Cell Dev Biol 1999;15:185–230.
16. Bernard O. Lim kinases, regulators of actin dynamics. Int J Biochem Cell Biol 2007;39:1071–6.
17. Moriyama K, Iida K, Yahara I. Phosphorylation of Ser-3 of cofilin regulates its essential function on actin. Genes Cells 1996;1:73–86.
18. Niwa R, Nagata-Ohashi K, Takeichi M, Mizuno K, Uemura T. Control of actin reorganization by Slingshot, a family of phosphatases that dephosphorylate ADF/cofilin. Cell 2002;108:233–46.
19. Pak CW, Flynn KC, Bamburg JR. Actin-binding proteins take the reins in growth cones. Nat Rev Neurosci 2008;9:136–47.
20. van Rheenen J, Song X, van Roosmalen W, et al. EGF-induced PIP2 hydrolysis releases and activates cofilin locally in carcinoma cells. J Cell Biol 2007;179:1247–59.
21. Toshima J, Toshima JY, Amano T, Yang N, Narumiya S, Mizuno K. Cofilin phosphorylation by protein kinase testicular protein kinase 1 and its role in integrin-mediated actin reorganization and focal adhesion formation. Mol Biol Cell 2001;12:1131–45.
22. Yang N, Higuchi O, Ohashi K, et al. Cofilin phosphorylation by LIM-kinase 1 and its role in Rac-mediated actin reorganization. Nature 1998;393:809–12.
23. Zebda N, Bernard O, Bailly M, Welti S, Lawrence DS, Condeelis JS. Phosphorylation of ADF/cofilin abolishes EGF-induced actin nucleation at the leading edge and subsequent lamellipod extension. J Cell Biol 2000;151:1119–28.
24. Huang TY, DerMardirossian C, Bokoch GM. Cofilin phosphatases and regulation of actin dynamics. Curr Opin Cell Biol 2006;18:26–31.
25. Nishita M, Wang Y, Tomizawa C, et al. Phosphoinositide 3-kinase-mediated activation of cofilin phosphatase Slingshot and its role for insulin-induced membrane protrusion. J Biol Chem 2004;279:7193–8.
26. Nagata-Ohashi K, Ohta Y, Goto K, et al. A pathway of neuregulin-induced activation of cofilin-phosphatase Slingshot and cofilin in lamellipodia. J Cell Biol 2004;165:465–71.
27. Carson MR, Shasby SS, Lind SE, Shasby DM. Histamine, actin-gelsolin binding, and polyphosphoinositides in human umbilical vein endothelial cells. Am J Physiol 1992;263:L664–9.
28. Salles FJ, Strickland S. Analysis of poly(A) tail lengths by PCR: the PAT assay. Methods Mol Biol 1999;118:441–8.
29. Huang XF, Luo SK, Xu J, et al. Aurora kinase inhibitory VX-680 increases Bax/Bcl-2 ratio and induces apoptosis in Aurora-A-high acute myeloid leukemia. Blood 2008;111:2854–65.
30. Wan XB, Long ZJ, Yan M, et al. Inhibition of Aurora-A suppresses epithelial-mesenchymal transition and invasion by downregulating MAPK in nasopharyngeal carcinoma cells. Carcinogenesis 2008;29:1930–7.
31. Yan B, Yap CT, Wang S, et al. Cofilin immunolabelling correlates with depth of invasion in gastrointestinal endocrine cell tumors. Acta Histochem 2010;112:101–6.
32. Bamburg JR, McGough A, Ono S. Putting a new twist on actin: ADF/cofilins modulate actin dynamics. Trends Cell Biol 1999;9:364–70.
33. Sasayama T, Marumoto T, Kunitoku N, et al. Over-expression of Aurora-A targets cytoplasmic polyadenylation element binding protein and promotes mRNA polyadenylation of Cdk1 and cyclin B1. Genes Cells 2005;10:627–38.
34. Stebbins-Boaz B, Richter JD. Multiple sequence elements and a maternal mRNA product control cdk2 RNA polyadenylation and translation during early *Xenopus* development. Mol Cell Biol 1994;14:5870–80.
35. Stebbins-Boaz B, Hake LE, Richter JD. CPEB controls the cytoplasmic polyadenylation of cyclin, Cdk2 and c-mos mRNAs and is necessary for oocyte maturation in *Xenopus*. EMBO J 1996;15:2582–92.
36. Wang W, Eddy R, Condeelis J. The cofilin pathway in breast cancer invasion and metastasis. Nat Rev Cancer 2007;7:429–40.
37. Wang W, Mouneimne G, Sidani M, et al. The activity status of cofilin is directly related to invasion, intravasation, and metastasis of mammary tumors. J Cell Biol 2006;173:395–404.
38. Royce ME, Xia W, Sahin AA, et al. STK15/Aurora-A expression in primary breast tumors is correlated with nuclear grade but not with prognosis. Cancer 2004;100:12–9.
39. Nadler Y, Camp RL, Schwartz C, Rimm DL, Kluger HM, Kluger Y. Expression of Aurora A (but not Aurora B) is predictive of survival in breast cancer. Clin Cancer Res 2008;14:4455–62.
40. Tanaka E, Hashimoto Y, Ito T, et al. The clinical significance of Aurora-A/STK15/BTAK expression in human esophageal squamous cell carcinoma. Clin Cancer Res 2005;11:1827–34.
41. Yao JE, Yan M, Guan Z, et al. Aurora-A down-regulates IκBα via Akt activation and interacts with insulin-like growth factor-1 induced phosphatidylinositol 3-kinase pathway for cancer cell survival. Mol Cancer 2009;8:95.
42. Conte N, Delaval B, Ginestier C, et al. TACC1-chTOG-Aurora A protein complex in breast cancer. Oncogene 2003;22:8102–16.
43. Hoque A, Carter J, Xia W, et al. Loss of aurora A/STK15/BTAK overexpression correlates with transition of *in situ* to invasive ductal carcinoma of the breast. Cancer Epidemiol Biomarkers Prev 2003;12:1518–22.
44. Miyoshi Y, Iwao K, Egawa C, Noguchi S. Association of centrosomal kinase STK15/BTAK mRNA expression with chromosomal instability in human breast cancers. Int J Cancer 2001;92:370–3.
45. Tanaka T, Kimura M, Matsunaga K, Fukada D, Mori H, Okano Y. Centrosomal kinase AIK1 is overexpressed in invasive ductal carcinoma of the breast. Cancer Res 1999;59:2041–4.
46. Yamamoto Y, Ibusuki M, Nakano M, Kawasoe T, Hiki R, Iwase H. Clinical significance of basal-like subtype in triple-negative breast cancer. Breast Cancer 2009;16:260–7.