Inhibition of STAT5a by Naa10p contributes to decreased breast cancer metastasis

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N-α-Acetyltransferase 10 protein (Naa10p, also called arrest-defective 1), the catalytic subunit of N-α-acetyltransferase A, is a critical regulator of cell death and proliferation. Naa10p is also shown to regulate cancer metastasis by inhibiting cell motility; however, its role in cancer metastasis is not fully understood. In this study, we found that high expression of Naa10p is positively correlated with the survival of patients with breast cancer, whereas negatively correlated with lymph node metastasis. Naa10p inhibits breast cancer cell migration and invasion in vitro and decreases the xenograft growth and metastasis in nude mice. Microarray screening revealed that Naa10p downregulates inhibitors of differentiation 1 (ID1) expression. Naa10p binds to signal transducer and activator of transcription 5α (STAT5α) and decreases STAT5α-stimulated ID1 expression in an acetyltransferase-independent manner. Moreover, Naa10p antagonizes Janus kinase 2-STAT5α signaling by lowering p65-activated interleukin-1β expression. Our results demonstrate a novel mechanism through which Naa10p inhibits the metastasis of breast cancer cells by targeting STAT5α.

Introduction

N-α-Acetyltransferase 10 protein (Naa10p; also known as arrest-defective 1) is encoded by NAA10 gene and catalyzes N-α-acetylation of nascent polypeptides emerging from ribosomes and lysine ε-acetylation of mature proteins, including itself (1–4). Mutation of arginine 82 to alanine greatly impairs its enzymatic activity (1,4). Naa10p regulates cell proliferation (2,6,5), cell cycle (2), apoptosis (7,8), cell motility (3,9), autophagy (10) and 28S proteasome activity (11). Overexpression of Naa10p has been documented in various types of cancer (6,9,12–17); however, downregulation of Naa10p in thyroid neoplasms samples was also reported (18).

Naa10p was shown to associate with androgen receptor (AR) and form a positive feedback loop for AR-dependent prostate tumorigenesis (6). In lung cancer cells, Naa10p potentiates DNMT1’s affinities with promoter regions of Ecadherin and Lats, thereby suppressing their transcription (12). In addition, Naa10p was reported to interact with β-catenin and enhance transcription of Ccnd1 in lung cancer cells (2). By binding to p65 subunit of nuclear factor-kB (NF-kB), Naa10p may increase mcl1 transcription and confer resistance to pro-apoptotic stimuli (19). These findings reveal the role of Naa10p as a transcriptional co-factor (1,2,6,12,19).

Increased expression of inhibitors of differentiation 1 (ID1) significantly correlates with malignant transformation, chemo-resistance and unfavorable prognosis (20,21). ID1 could promote metastasis, and both matrix metalloproteinase 2 and 9 (MMP2 and MMP9) are downstream effectors of ID1 (22,23). Upstream of ID1, ATF3, KLF17 and STAT5a were shown to be essential for driving its expression (24–26). STAT5a belongs to the signal transducer and activator of transcription (STAT) family. Upon hormones and cytokines stimulation, STAT5a is activated by Janus kinases (JAKs)-mediated tyrosine phosphorylation, particularly by JAK2 (27,28). Active STAT5a has enhanced nuclear retention enabling the recruitment to the regulatory elements of its target genes (27–30). Constitutively active STAT5a had been found in several solid tumors and leukemias, but its role in tumorigenesis is complicated (27,30). For instance, STAT5a may serve as a growth promoter in the early stage of breast cancer (30), but could suppress breast cancer invasiveness (31). Such discrepancies may be determined by the factors bound to STAT5a (27,30); however, the whole spectrum of protein–protein binding network modulating STAT5a’s role in tumorigenesis is still largely unknown.

In this study, we characterized Naa10p as a negative regulator of STAT5a in breast cancer cells. Our results highlight a novel mechanism through which Naa10p inhibits the metastasis of breast cancer by targeting p65-interleukin (IL)-1β-STAT5a-ID1 cascade.

Materials and methods

Patients and samples

The collection of tissue specimens was approved and supervised by the research ethics committee of Peking University Cancer Hospital and Institute. Written informed consents were obtained from all patients prior to operation. A total of 421 breast cancer tissues (cohort 1) were obtained from women receiving surgery at Peking University Cancer Hospital and Institute between 1996 and 2002. The tumors were staged according to TNM (Tumor, Node, Metastasis) classification of Union for International Cancer Control (UICC), including 311 Stage III tumors and 110 Stage III tumors. The majority of patients received adjuvant therapy, including chemotherapy, endocrine therapy or combined therapy. The period of follow-up was up to 15 years. Fresh tumor tissues from another 55 breast cancer patients (cohort 2) were immediately transferred to liquid nitrogen and stored at −80°C for subsequent studies. The period of follow-up for this cohort was up to 8 years. Breast cancer tissue microarrays (TMAs, BR030a) containing 77 breast cancer and 3 normal mammary tissues were from Alenabio Technologies (Xi’an, China).

Cell culture

Human breast cancer cell line MDA-MB-231, MCF-7, T47D, ZR75, HS578T, SKBR3, MDA-MB-453 and immortalized human breast cell line MCF-10A were obtained from American Type Culture Collection (Rockville, MD) and cultured with standard settings. BICR breast cancer cells were kindly provided by Dr Z.Zhang and cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (Invitrogen, Carlsbad, CA). Cell line authentication was performed according to United Kingdom Coordinating Committee on Cancer Research Guidelines every 2–3 months, including mycoplasma test by PCR and measurement of cell proliferation by counting.

Antibodies and inhibitors

Primary antibodies used in this study were listed in Supplementary Table S1 (available at Carcinogenesis Online). JAK2 inhibitor AG490 (ab120950) was from Abcam (Cambridge, MA). NF-κB inhibitor Bay 11–7082 (BS556) was from Sigma (St Louis, MO).

Immunohistochemical staining

Procedures for immunohistochemical staining of Naa10p were reported previously (13,14). Naa10p immunoreactivity was evaluated independently by two
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Pathologists without any knowledge of the clinical data. Both the percentage of positive cells and the intensity of cytoplasmic staining in 10 randomly chosen microscopic fields were assessed. The score for Naa10p staining was graded as follows: no staining or staining observed in ≤10% of tumor cells was scored as 0; faint or barely perceptible staining detected in >10% of tumor cells was scored as 1+; moderate or strong complete staining observed in ≥10% of tumor cells was scored as 2+ or 3+, respectively. A score of 0–2+ was considered as low expression, whereas 3+ was considered as high expression.

**Small interfering RNAs, short hairpin RNAs, plasmids and transfection**

Small interfering RNAs (siRNAs; listed in Supplementary Table S2, available at Carcinogenesis Online) were synthesized by GenePharma (Shanghai, China). RNA interference was achieved by transient transfection using 100 nM siRNA plus Lipofectamine 2000 (Invitrogen) for 48 h. Targeted sequence for short hairpin RNA-induced silencing of Naa10p was 5′-CACCUGACCCCUAAUGUUUUA-3′, using psilencer 2.1-U6/neg plasmid. The control short hairpin RNA sequence was 5′-UUACUGGUAAAGCUUUGGUU-3′. The lentiviral vectors LV-NAA10, LV-shNAA10 and LV-NC (control) were from GenePharma. Plasmids encoding truncated forms of GFP-Naa10p were generated by PCR. pCMV6-STAT5A (RC205753) was obtained from Origene (Beijing, China). Plasmids encoding V5-tagged wild-type Naa10p and mutant (R82A) Naa10p were kindly provided by Drs L-J. Juan and S-H. Peng. Plasmids were transfected into cells with Lipofectamine 2000.

**Protein analysis**

Human full-length MAA10 complementary DNA was cloned into pGEX-4T1 vector, and the recombinant Glutathione-S-transferases (GST)-Naa10p protein was expressed in Escherichia coli and purified. About 1 μg of His-STAT5a (purified from Acanthamoeba castellanii) was incubated with 1 μg of GST-Naa10p or GST plasmid for 1 h at room temperature in the binding buffer (50 mM N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid, pH 7.5, 150 mM NaCl, 1 mM ethylenediaminetetraacetic acid, 1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride, 1 mM dithiothreitol, 10% glycerol) at 4°C overnight, followed by washing with the same binding buffer for three times. Proteins were resolved by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and subjected to western blot. Cellular proteins were prepared with buffer containing 50 mM Tris–HCl, pH 7.5, 150 mM NaCl, 1 mM ethylenediaminetetraacetic acid, 1 mM Na2VO4, 20 mM NaF, 1% NP-40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate and 1 μg protease inhibitor cocktail (Roche, Mannheim, Germany). For immunoprecipitation assay, cells were homogenized in 50 mM N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid, pH 7.5, 150 mM NaCl, 1 mM ethylenediaminetetraacetic acid, 1% Triton X-100, 1 mM dithiothreitol, 1 mM Na2VO4, 20 mM NaF, 10% glycerol and 1 μg protease inhibitor cocktail. Western blot and immunoprecipitation were performed according to standard protocols. Phosphorylation of STAT5a was analyzed by immunoprecipitation of STAT5a, followed by western blot with an anti-phospho-STAT5 antibody. Protein bands were visualized using enhanced chemiluminescence detection system (Pierce, Rockford, IL). Analysis of IL-1β in the culture supernatant was performed with an enzyme-linked immunosorbent assay (ELISA) kit (VAL101, R&D Systems, Minneapolis, MN).

**Colony formation, cell migration and invasion assays**

Colony formation, cell migration and invasion assays were carried out according to a previous study (9).

**Tumor xenograft assay and histological examination**

Animal studies were approved by the biomedical ethical committee of Peking University Cancer Hospital and Institute and performed along established institutional animal welfare guidelines concurrent with the United States guidelines (NIH Publication 85-23, revised in 1985). Inbred 6- to 8-week-old female immunocompetent BALB/c mice (Vital-River Laboratories, Beijing, China) were maintained under pathogen-free conditions. MCF-7 cell cultured in 24-well plates were co-transfected with indicated plasmids and pGL3-1DI (500 ng per well) plus an internal control, pRL-SV40 (Promega; 25 ng per well). After 72 h, the luciferase activity was measured using a kit from Promega. The firefly luciferase intensity was normalized based on transfection efficiency measured by Renilla luciferase activity.

**Quantitative chromatin immunoprecipitation assay**

Quantitative chromatin immunoprecipitation (qChIP) was performed as described previously (19). The sequences of specific primers were listed in Supplementary Table S3 (available at Carcinogenesis Online).

**Image acquisition**

X-ray films of western blot were digitized on a Canon CanoScan LiDE100 scanner with linear intensity settings. Fluorescence images from immunofluorescence staining were acquired with a Leica TCS SP5 confocal microscope at fixed exposure setting at room temperature. Breast cancer tissue sections and TMAs were observed under a Nikon Eclipse 80i microscope equipped with Nikon DS-FI1 camera. Digital images were processed with Adobe Photoshop CS (Adobe Systems) by adjusting the linear image intensity display range. Full-length blots were shown in Supplementary Figure S5 (available at Carcinogenesis Online).

**Statistical analysis**

Data analysis was performed using SPSS 13.0 (SPSS, Chicago, IL). A standard χ2 test was performed to assess the association between Naa10p and clinicopathologic characteristics. Survival curves were estimated using the Kaplan–Meier method and compared with the log-rank test. A multivariate analysis was performed using the Cox proportional hazard regression model (a backward selection) to assess whether a factor was an independent predictor of disease-free survival (DFS) or overall survival (OS). Hazard ratios with 95% confidence intervals were estimated. TV and tumor weight results were evaluated by analysis of variance. Correlation bivariate analysis was performed to evaluate the relationship between Naa10p and ID1 expression in breast cancer specimens. A two-tailed Student’s t-test was used to determine the significance of differences between different experimental groups. Values represent the mean ± SD from at least three independent experiments with triplicate wells. A P < 0.05 was considered statistically significant.

**Results**

Naa10p levels inversely correlates with lymph node metastasis in breast cancer tissues

To determine the clinical relevance of Naa10p, we immunohistochemically examined the expression of Naa10p in 421 breast cancer tissues (13,14). As shown in Figure 1A, Naa10p was mainly expressed in the cytoplasm, and partially in the nuclei. Although Naa10p expression in cancerous tissues exhibited no correlation with age, tumor size, clinical stage or adjuvant therapy, significantly inverse correlations with degree of lymph node metastasis and recurrence were revealed (Supplementary Table S4, available at Carcinogenesis Online).
Kaplan–Meier survival curves showed that high expression of Naa10p correlated with DFS and OS (Figure 1B). Univariate and multivariate analysis further confirmed that high Naa10p was an indicator for better prognosis (Supplementary Table S5, available at Carcinogenesis Online). Next, the patients were divided into two groups according to their nodal status. In 187 node-positive patients, high levels of Naa10p were associated with a better prognosis (Figure 1C). Multivariate analysis verified this result (Supplementary Table S6, available at Carcinogenesis Online). In contrast, no significant correlation between the Naa10p expression and DFS or OS was found in node-negative group (Supplementary Figure S1, available at Carcinogenesis Online). With 55 freshly isolated breast cancer tissues (cohort 2), we found that $NAA10$ levels of node-positive patients were lower than those of node-negative patients (Figure 1D). Moreover, patients with low $NAA10$ had shorter DFS and OS (Figure 1E). Taken together, our data suggest that Naa10p may act as a favorable prognostic factor for patients with breast cancer, especially for those with lymph node metastasis.

Naa10p suppresses metastasis of breast cancer cells

There were higher levels of $NAA10$ and Naa10p in breast cancer cell lines than those in immortalized human breast cell line MCF-10A (Supplementary Figure S2A, available at Carcinogenesis Online). To determine the functional roles of Naa10p, Naa10p was transiently knocked down by three siRNAs in MCF-7 and MDA-MB-231 cells (Figure 2A). We found that both cell migration (Figure 2B) and invasion (Figure 2C) were negatively regulated by Naa10p. After transfection of shNAA10, whose target was identical to that of siNAA10-2, in ZR75 cells, both cell migration and invasion were significantly exacerbated (Supplementary Figures S2B and C, available at Carcinogenesis Online). Conversely, ectopic expression of Naa10p in T47D cells strongly decreased the cell migration and invasion (Supplementary Figures S2D and E, available at Carcinogenesis Online). Upon stable knockdown of Naa10p by lentivirus-mediated transduction of shNAA10 (Figure 2D), the potentials of colony formation were markedly enhanced (Figure 2E). Next, cells were inoculated into the mammary fat pads of female nude mice. We noticed that the growth rates of xenografts were elevated by stable knockdown of Naa10p (Figure 2F and G). Furthermore, there were more and larger metastatic foci detected in the lungs of mice inoculated with Naa10p-silenced cells (Supplementary Figure S2F, available at Carcinogenesis Online). Consequently, mice inoculated with Naa10p-silenced cells had shorter survival time (Figure 2H). Therefore, Naa10p suppresses the metastasis of breast cancer cells in vitro and in vivo.
Naa10p inhibits STAT5a in breast cancer

To explore the mechanism underlying Naa10p’s inhibitory effect on breast cancer metastasis, we performed microarray analysis. A total of 849 genes were found to be affected by ablation of Naa10p in MCF-7 cells, including 462 upregulated genes and 387 downregulated genes. Profiles of representative genes were shown in Figure 3A. Some of these genes are linked to apoptosis and cell cycle control, which is consistent with the established roles of Naa10p in these two biological events (1,2,7,8). Interestingly, a subset of adhesion- and metastasis-related genes were revealed, including ID1 (Figure 3A). By qRT–PCR analysis, we validated that ID1 levels were increased by Naa10p knockdown, but were decreased by stable expression of ectopic Naa10p (Figure 3B). To determine whether these results were applicable in breast cancer tissues, we performed qRT–PCR with RNA samples from cohort 2. Previously, we found an inverse correlation between NAA10 and MMP9 in this cohort (32). Herein, we found that NAA10 abundance was negatively associated with that of ID1 (Figure 3C). Western blot analysis of cell lysates also showed the negative effects of Naa10p on protein levels of ID1 in three breast cancer cell lines.

**ID1 is essential for Naa10p’s inhibition on the invasiveness of breast cancer cells**

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Results obtained raised a possibility that Naa10p regulates ID1 expression at transcriptional level. This predication was corroborated by the fact that messenger RNA (mRNA) levels of ID1 were gradually elevated by transfecting MCF-7 cells with increasing amount of shNAA10 or diminished by transfecting with increasing amount of pcDNA3-NAA10 (Supplementary Figure S4A, available at Carcinogenesis Online). Transcriptional factors ATF3, KLF17 and STAT5a control ID1 expression (24–26). Our microarray screening did find Naa10p’s effects on JAK-STAT pathway-related genes (Figure 3A). Besides ID1, expression levels of seven of eight known STAT5a target genes were inversely correlated with Naa10p levels (Supplementary Figure S4B, available at Carcinogenesis Online).

Naa10p interacts with STAT5a

Correlation analysis of NAA10 and ID1 in 55 breast cancer specimens. (D) Stable silencing of endogenous Naa10p resulted in increased expression of ID1 in indicated cells. (E) Stable expression of ectopic Naa10p resulted in decreased expression of ID1 in indicated cells. (F) Immunostaining of Naa10p and ID1 in human breast cancer TMAs. Two representative cases were shown. Magnification, ×100. (G) Correlation analyses of Naa10p and ID1 in breast cancer TMAs.

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Functional interplay between Naa10p and STAT5a, a direct association between GST-Naa10p and His-STAT5a was found (Figure 4B). Results of reciprocal co-immunoprecipitation assays revealed endogenous Naa10p-STAT5a interaction in breast cancer cells (Figure 4C and D). ID1 and other two STAT family members, STAT1 and STAT3, were not detected in anti-Naa10p immunoprecipitates (Figure 4D). By immunofluorescence staining, co-localization between Naa10p and STAT5a was revealed mainly in the nuclei and partially in the cytoplasm (Figure 4E). With a series of truncated forms of GFP-Naa10p, 46–131 region of Naa10p was shown to be critical for its interaction with STAT5a (Figure 4F).

Naa10p suppresses STAT5a-activated ID1 transcription

Overexpression of STAT5a augmented ID1’s promoter activity, however such activity was enhanced by silencing of Naa10p, but was compromised by co-expression of Naa10p (Figure 5A), indicating Naa10p could repress STAT5a-regulated ID1 transcription. The STAT5a consensus binding motif on the pro-B-cell enhancer of ID1 had been characterized and designated as S1 site, whereas S2 site was shown to be a weak binding motif for STAT5a (26). Naa10p was bound to S1 site, but not to S2 site in qCHIP assays (Figure 5B). Once STAT5a was transiently knockdown by a specific siRNA (Figure 5C), binding of Naa10p to S1 site was partially attenuated (Figure 5B), suggesting that Naa10p was targeted to ID1 regulatory site, at least in part, in a STAT5a-dependent manner. Additionally, binding of STAT5a to S1 site was negatively regulated by the cellular levels of Naa10p (Figure 5D). Knockdown of STAT5a had no effect on the protein levels of Naa10p, but resulted in decreased ID1 (Figure 5E), which was consistent with the role of STAT5a in driving ID1 expression (26,33). Importantly, silencing of Naa10p-induced cell migration was significantly decreased by knockdown of STAT5a (Figure 5F). The p21-activated kinase (PAK)-interacting exchange factor (PIX) was previously identified as a mediator of Naa10p-inhibited cell motility (9), which was confirmed by this study with siRNA against βPIX (Figure 5E and F). Now that knockdown of βPIX did not affect ID1 levels (Figure 5E), our results suggest that Naa10p suppresses breast cancer cell migration through two pathways, one by targeting PIX and another by targeting STAT5a-ID1.

Naa10p’s acetyltransferase activity is not required for suppressing ID1 expression or cell metastasis

The acetyltransferase activity of Naa10p is essential for some of Naa10p-regulated biological events (1). Because 46–131 region of Naa10p, which flanks the R82 enzymatic center (1,4), was identified as the domain required for its association with STAT5a (Figure 4F), it is plausible that acetyltransferase activity of Naa10p may play a role in controlling STAT5a-dependent cell metastasis. To verify this assumption, plasmids encoding wild-type and R82A mutant Naa10p were employed to expressed exogenous Naa10p (Figure 5G). We found these two constructs exhibited similar inhibitory effects on ID1 protein levels (Figure 5G). In qCHIP analysis, these two constructs decreased STAT5a’s binding to S1 site to a similar degree (Figure 5H). Consistently, no significant difference was found in the migration assay (Figure 5G). With an antibody specific for acetylated lysine, we showed that STAT5a was not acetylated at lysine residue(s), nor was affected by the exogenous wild-type or mutant Naa10p, despite that we could detect lysine acetylation of β-catenin and autoacetylation of p65.
Naa10p (Figure 5I). Thus, acetyltransferase activity of Naa10p could be dispensable for inhibiting STAT5a-dependent ID1 expression and suppressing invasiveness of breast cancer cells.

**Decreased IL-1β-JAK2-STAT5a signaling contributes to Naa10p-inhibited ID1 expression**

Although we did not find Naa10p-mediated lysine acetylation of STAT5a, we found phosphorylation of STAT5a at tyrosine 694 was inhibited by Naa10p in an acetyltransferase-independent fashion (Figure 5G), whereas silencing of Naa10p resulted in increased phosphorylation of STAT5a (Figure 6A). JAK2 kinase is essential for the tyrosine phosphorylation and transcriptional activity of STAT5a (27–30). We did not detect Naa10p-JAK2 interaction in the co-immunoprecipitation assay (Figure 4D), but we found Naa10p levels also inversely affected phosphorylation of JAK2 (Figure 6A), suggesting that Naa10p is a negative modulator of JAK2-STAT5a signaling. With JAK2 inhibitor AG490 (34), we found that silencing of Naa10p-induced cell migration and JAK2-STAT5a phosphorylations were attenuated (Figure 6B), underscoring the critical role of...
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JAK2-STAT5a in mediating Naa10p’s suppression on cell metastasis. JAK2-STAT5a is regulated by several mechanisms, in particular by cytokine signalings (27,30). Microarray analysis identified IL1B as a gene potentially restrained by Naa10p (Figure 3A), which was validated by qRT–PCR (Figure 3B). ELISA assay of culture supernatant further confirmed negative effects of Naa10p on IL-1β levels (Figure 6A). By knocking down endogenous IL-1β, JAK2-STAT5a signaling was weakened (Figure 6C), confirming IL-1β as an upstream activator of JAK2-STAT5a. By administrating cells with neutralizing antibody against IL-1β, silencing of Naa10p-induced JAK2-STAT5a activation was reversed (Figure 6D). Conversely, administration of IL-3, a cytokine shown previously to activate STAT5a-dependent ID1 transcription (26), was shown to override Naa10p-inhibited STAT5a phosphorylation (Figure 6E). Meanwhile, expression of ID1 at both mRNA and protein levels were restored (Figure 6E), emphasizing the role of cytokine-activated STAT5a phosphorylation in mediating Naa10p-inhibited ID1 expression.

Naa10p inhibits IL-1β expression through NF-κB-dependent mechanism

Our results imply that Naa10p inhibits STAT5a phosphorylation by limiting IL-1β autocrine, but how Naa10p controls IL1B expression is unresolved. Data of expression profiling and qRT–PCR suggest that Naa10p regulates IL1B at transcriptional level (Figure 3A and B). NF-κB signaling is one of key mechanisms determining IL1B transcription (35). Previously, we found a Naa10p–p65 interaction in colon cancer and lung cancer cells (19), herein we confirmed this finding in breast cancer cells (Figure 4D). We then proposed that Naa10p may repress
**Discussion**

It had been proposed that Naa10p’s role in tumorigenesis is cancer type- and cell type-dependent (1). As for Naa10p’s contribution to breast cancer development, initial study revealed that Naa10p gene expression correlated with better clinical outcome (16). This finding was supported by a later study showing that Naa10p levels were higher in breast cancer samples from patients with longer relapse-free survival, smaller tumor size and fewer lymph node metastases (10). At protein level, one study found that breast cancer patients with high Naa10p had lower incidence of lymph node metastasis (9). However, another study reported opposite results (17). The inconsistency between these two studies could be due to small sample size (n = 53 and n = 82, respectively) without clinical follow-up data, difference in the compositions of cohorts and/or antibody specificity. To understand the clinical significance of Naa10p in breast cancer, we recently examined Naa10p levels in 253 specimens and showed that high Naa10p expression predicted longer survival and decreased lymph node metastasis (32). These results were further validated by this study using two independent cohort of breast cancer specimens (n = 421 and n = 55, respectively). Based on above-mentioned results, we conclude that high Naa10p is a good prognostic factor for breast cancer patients.

This conclusion is consistent with results from Hua et al. (9) using 318 lung cancer specimens. In that study, Naa10p was demonstrated to decrease GIT-assisted localization of PX on membrane protrusions, thus alleviating CDC42/RAC1-dependent cell motility (9). In addition, Naa10p was found to suppress cell motility by inhibiting MLCK (3). On the other hand, overexpression of a murine Naa10p variant in B16F10 cells drastically reduced lung metastasis (32). The contributions of STATs in normal biological settings. Further studies are warranted to test this predication. In addition, the contributions of Naa10p-regulated STAT5a phosphorylation and Naa10p-STAT5a interaction to expression of other STAT5a target genes also need to be investigated.

Both N-α- and N-ε-acetyltransferase activity of Naa10p had been reported (1). For instance, DNMT1 and TSC2 were shown to be N-α-acetylated (10,12), whereas AR, β-catenin and MLCK were found to be N-ε-acetylated by Naa10p (2,3,6). Recent studies suggested that both acetylation-dependent and -independent mechanisms may contribute to the function of Naa10p (1,9,11). In this study, we provided evidence that wild-type and R82A mutant Naa10p exhibited similar inhibition on ID1 protein expression, cell migration, STAT5a’s occupancy on ID1 promoter and p65’s occupancy on IL1B promoter, suggesting an acetyltransferase-independent role was involved in Naa10p-suppressed breast cancer metastasis. Furthermore, we confirmed previous findings showing Naa10p promotes N-ε-acetylation of β-catenin and itself (2,4), but we did not find any lysine-acetylated STAT5a in cells overexpressing exogenous Naa10p, implying that STAT5a may not be N-ε-acetylated by Naa10p.

We found JAK2-STAT5a pathway was negatively affected by Naa10p. JAK2-STAT5a pathway transmits signals downstream of multiple cytokine receptors and is tightly associated with tumor development (27,28,30). Through chemical inhibition of JAK2, we confirmed that this pathway was indeed associated with motility of breast cancer cells. Whether or not Naa10p affects other JAK2-STAT5a-dependent malignant phenotypes merits further explorations. Diverse factors had been found to negate JAK2-STAT5a signaling, such as phosphatases, caveolin-1 and cytokine-inducible suppressors of cytokine signaling proteins (27,30). Although we cannot rule out the possible involvement of these factors, our data revealed that Naa10p indirectly exerted its inhibition on phosphorylations of JAK2-STAT5a by decreasing p65-dependent expression of proinflammatory cytokine IL-1β. Because IL-1β is potent in promoting tumorigenesis, angiogenesis and metastasis (46), our results suggested that Naa10p may modulate the tumor micro-environment via IL-1β. It is noteworthy that Naa10p functions as both co-repressor (for IL1B) and co-activator (for MCL1) of p65, again emphasizing the context-dependent nature of Naa10p.

Our data collectively demonstrated that high expression of Naa10p is associated with increased survival and decreased metastasis for breast cancer.
breast cancer patients. Naa10p may function as a suppressor of breast cancer metastasis by blocking p65-IL-1β-JAK2-STAT5a-ID1 cascade, further shedding light on the therapeutic potential of Naa10p in antagonizing tumor development.

Supplementary material
Supplementary Tables 1–6 and Figures 1–5 can be found at http://carcin.oxfordjournals.org/

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References

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