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KPNA3 promotes epithelial-mesenchymal transition by regulating TGF-β and AKT signaling pathways in MDA-MB-231, a triple-negative breast cancer cell line

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Karyopherin-α3 (KPNA3), a karyopherin- α isoform, is intimately associated with metastatic progression via epithelial-mesenchymal transition (EMT). However, the molecular mechanism underlying how KPNA3 acts as an EMT inducer remains to be elucidated. In this report, we identified that KPNA3 was significantly upregulated in cancer cells, particularly in triple-negative breast cancer, and its knockdown resulted in the suppression of cell proliferation and metastasis. The comprehensive transcriptome analysis from KPNA3 knockdown cells indicated that KPNA3 is involved in the regulation of numerous EMT-related genes, including the downregulation of GATA3 and E-cadherin and the up-regulation of HAS2. Moreover, it was found that KPNA3 EMT-mediated metastasis can be achieved by TGF-β or AKT signaling pathways; this suggests that the novel independent signaling pathways KPNA3-TGF-β-GATA3-HAS2/E-cadherin and KPNA3-AKT-HAS2/E-cadherin are involved in the EMT-mediated progress of TNBC MDA-MB-231 cells. These findings provide new insights into the divergent EMT inducibility of KPNA3 according to cell and cancer type. [BMB Reports 2023; 56(2): 120-125]

INTRODUCTION

Epithelial-mesenchymal transition (EMT) is a key cellular process in which immotile epithelial cells transform into mesenchymal cells through cell polarity loss, cell-cell junction disassembly, and extracellular matrix (ECM) alteration. EMT endows tumor cells with enhanced migratory and invasive properties necessary for metastasis, the primary cause of cancer-related deaths (1). However, the EMT process activated by the pleiotropic control of intrinsic and extrinsic factors has inherent flexibility and variation across different cancer cells and types (2). Therefore, understanding the intricate network among EMT-related genes in various cancers will provide insight into the differences in EMT-mediated metastatic pathways and lead to the development of advanced antimetastatic therapies.

Karyopherin-α 3 (KPNA3), a member of the nuclear transport protein family, is important in the nucleocytoplasmic trafficking of certain cargoes via a heterodimeric interaction with importin-β1 (also known as KPNB1) (3). KPNA3 is upregulated in colon and liver cancers, and its enhanced expression is associated with poor prognosis and a low survival rate in patients with breast cancer (4). Moreover, it has been reported that EMT can be induced by the KPNA3-serine/threonine kinase (AKT)-extracellular signal-related kinase (ERK)-twist-related protein (TWIST) signaling cascade in hepatocellular carcinoma (HCC) (5). Despite evidence of an EMT-inducing role of KPNA3 in multiple cancers, the function of KPNA3 in EMT-associated transcriptional reprogramming remains to be clarified.

In this study, it was found that KPNA3 regulates numerous EMT-related genes and induces the EMT process via at least two independent signaling pathways in the highly invasive triple-negative breast cancer (TNBC) cell line MDA-MB-231. One pathway is the transforming growth factor-β (TGF-β) signaling pathway that downregulates GATA binding protein 3 (GATA3) to suppress E-cadherin and upregulate hyaluronan synthase 2 (HAS2); the other is the AKT signaling pathway, which is also involved in the up-regulation of HAS2 and down-regulation of E-cadherin. Our findings highlighted that EMT induction by KPNA3 can be achieved by the networking and interplay among many genes involved in several EMT-related signaling pathways. In addition, these results suggest that KPNA3 can trigger EMT via two axes in TNBC MDA-MB-231 cells, KPNA3-TGF-β-GATA3-HAS2/E-cadherin and KPNA3-AKT-HAS2/E-cadherin.

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RESULTS

KPNA3 is highly expressed in TNBC and closely associated with poor patient outcomes

To determine the clinical relevance of KPNA3 in breast cancer, its protein expression was compared in different breast cancer subtypes. The expression level of KPNA3 was significantly higher in the more aggressive TNBCs than in normal and luminal-type breast cancer ($P < 0.05$ and $P < 0.01$, respectively; Fig. 1A, B). Consistent with these findings, the mRNA and protein expression levels of KPNA3 were markedly higher than those of other KPNAs in TNBC cell lines (Hs578T, BT549, and MDA-MB-231) and non-TNBC cell lines (MCF7 and T47D) (Supplementary Fig. 1A, B and Supplementary Table 1). In the analysis of different tumor grades, the expression level of KPNA3 gradually increased with an increase in tumor grade (Supplementary Fig. 1C). Kaplan-Meier survival analysis using GENT2 revealed that KPNA3 expression was closely associated with shortened overall survival ($P = 0.001$, Fig. 1C). Taken together, the results retrieved from various web-based databases clearly suggest that KPNA3 is highly expressed in aggressive breast cancer cells and tissues indicating that KPNA3 may play a key role in breast cancer progression and metastasis.

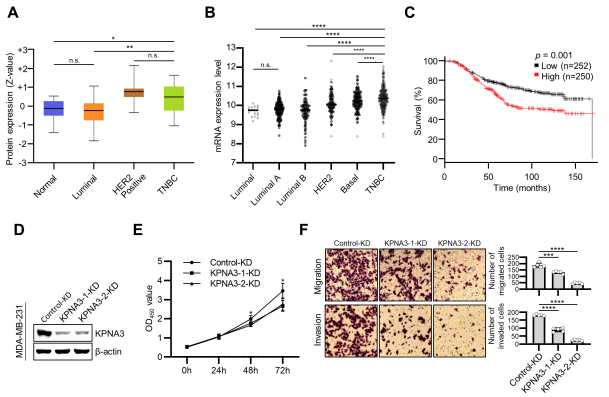

Fig. 1. Karyopherin-α3 (KPNA3) promotes proliferation, migration, and invasion in triple-negative breast cancer. (A) Expression pattern of KPNA3 protein in different breast cancer subtypes obtained from the UALCAN database, consisting of normal (n = 18), luminal (n = 64), HER2 (n = 10), and TNBC (n = 16) subtypes. (B) mRNA expression pattern of KPNA3 in different breast cancer subtypes. Expression data were obtained from the GENT2 database, consisting of luminal (n = 17), luminal A (n = 379), luminal B (n = 244), HER2 (n = 230), basal (n = 363), and TNBC (n = 251) subtypes. For each subtype, the log2 fold change (FC) was calculated. (C) Survival rate analysis of breast cancer patients according to differential expression levels of KPNA3 obtained from the GENT2 database. Low (n = 252) and high (n = 250) expression levels of KPNA3 were divided by median expression. (D) Expression levels of KPNA3 protein in MDA-MB-231 cells exposed to two siRNAs of KPNA3 (KPNA3-KD) for 48 h by western blot analysis using β-actin as a loading control. (E) Proliferation rates in cells exposed to KPNA3-KD, as examined by the WST-1 assay at various time intervals. Data represent the mean ± standard deviation (SD) of three independent experiments. (F) Cell images of migrated or invaded cells under the same conditions as those in (D) (left panel). The number of migrated or invaded cells (right panel). Data represent the mean number of cells per five visual regions (×100) of three replicate wells.

Depletion of KPNA3 inhibits cell proliferation and TNBC metastasis

To investigate the functional significance of KPNA3 expression in TNBC cells, the *KPNA3* gene was knocked down in two TNBC cell lines (MDA-MB-231 and Hs578T) using two siRNAs for KPNA3 (KPNA3-1-knockdown (KD) and KPNA3-2-KD) (Fig. 1D and Supplementary Fig. 2A). WST-1 assays revealed that both KPNA3-1-KD and KPNA3-2-KD inhibited cell proliferation in MDA-MB-231 cells ($P < 0.05$). However, only KPNA3-1-KD reduced cell proliferation in Hs578T cells ($P < 0.01$; Fig. 1E and Supplementary Fig. 2B). Next, we investigated whether KPNA3-KD's inhibitory effect on MDA-MB-231 cells' proliferation was caused by apoptosis or cell cycle delay. The protein expression levels of apoptotic markers were not changed by KPNA3-KD (Supplementary Fig. 3A). Moreover, KPNA3-KD induced cell cycle arrest at the G1/S phase, given the increase and decrease in the cell numbers in the G0/G1 and S phases, respectively (Supplementary Fig. 3B), suggesting that KPNA3-KD inhibits cell proliferation through cell cycle arrest at the G1/S phase and not through apoptosis in MDA-MB-231 cells. Additionally, transwell assays were performed to evaluate the effect of KPNA3 on metastasis. KPNA3-KD inhibited cell migration ($P < 0.001$) and invasion ($P < 0.0001$) in MDA-MB-231 and Hs578T cells (Fig. 1F and Supplementary Fig. 2C).

KPNA3-KD inhibits cell migration through the downregulation of HAS2 in MDA-MB-231 cells

To further elucidate the molecular mechanism whereby KPNA3 silencing decreases the proliferation and metastasis of TNBC, transcriptome analysis using RNA sequencing was performed on KPNA3-KD-231 cells (Supplementary Fig. 4A). A volcano plot and heatmap were constructed to indicate the transcripts' general scattering and to filter the differentially expressed genes from the transcriptome profiles, respectively (Fig. 2A and Supplementary Fig. 4B). Of the total 26,679 transcripts annotated, 2,245 genes (976 upregulated genes and 1,269 downregulated genes) were filtered by applying the criteria of absolute fold change (FC) > 1.5 and adjusted $P < 0.05$.

To identify EMT-related genes regulated by KPNA3, the expression profiles of genes downregulated in the transcriptome profiles retrieved from KPNA3-KD-231 cells were compared to two different bio-informatic data sets, the upregulated gene list in TNBC cells, and the EMT-core gene list, which were derived from at least 10 gene expression study datasets (Supplementary Fig. 4C) (6, 7). Intriguingly, *HAS2* was the only gene common among the three expression profiles (Fig. 2B). The expression of HAS2 was assessed in KPNA3-KD-231 cells to ascertain whether KPNA3 can regulate it. Fig. 2C, D show that KPNA3-KD significantly reduced *HAS2*'s mRNA and protein expression

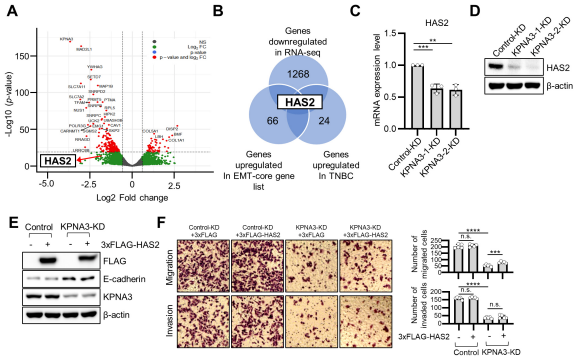

Fig. 2. Knockdown of karyopherin-α3 (KPNA3-KD) inhibits cell migration and invasion through the transcriptional regulation of HAS2 and CDH1 in MDA-MB-231 cells. (A) Volcano plot indicating the general scattering of the transcripts identified by RNA sequencing analysis according to log2 (FC) and −log10 (P-value). Red-colored dots indicate genes filtered with absolute FC > 1.5 and −log10 > 20. Green-colored dots represent genes filtered with absolute FC > 1.5 and P < 0.05. Gray-colored dots indicate genes filtered with absolute FC < 1.5. (B) Venn diagram showing the overlap of genes downregulated in transcriptome profiles, genes upregulated in the EMT-core gene list, and genes upregulated in triple-negative breast cancer cells. (C) Expression level of HAS2 mRNA by RT-qPCR. Data are shown as the mean ± standard deviation (SD) of three independent experiments. (D) Expression level of HAS2 protein in KPNA3-KD-231 cells for 48 h by western blot analysis. (E) Expression levels of FLAG, E-cadherin, and KPNA3 proteins in KPNA3-KD-231 cells co-transfected with or without 3× FLAG-HAS2. (F) Cell images of migrated or invaded cells under the same conditions as those in (D) (left panel).

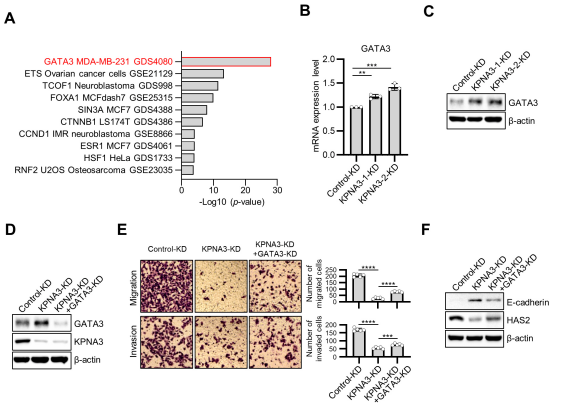

Fig. 3. GATA binding protein 3 (GATA3) is a major downstream transcription factor in karyopherin-α3 (KPNA3)-mediated epithelial-mesenchymal transition (EMT). (A) Transcription factor perturbations from GEO database-based enrichment analysis of KPNA3-KD-induced transcriptional signatures (absolute fold change > 1.5, P < 0.05). The P-values were computed using Fisher's exact test and converted to −log10 (P-value). (B) Expression level of GATA3 mRNA by RT-qPCR. Data represent the mean ± standard deviation (SD) of three independent experiments. (C) Expression level of GATA3 protein in KPNA3-KD-231 cells for 48 h by western blot analysis using β-actin as a loading control. (D) Expression levels of GATA3 and KPNA3 proteins in KPNA3-KD-231 cells co-transfected with or without GATA3 (GATA3-KD) siRNA for 48 h. (E) Cell images of migrated or invaded cells under the same conditions as those in (E) (left panel). The number of migrated or invaded cells (right panel). Data represent the mean number of cells per five visual regions (magnification, ×100) of three replicate wells. (F) Expression levels of E-cadherin and HAS2 proteins in KPNA3-KD-231 cells co-transfected with or without GATA3-KD for 48 h.

levels. In addition, a positive correlation between the expression levels of KPNA3 and HAS2 (P = 3.3e-52; R = 0.44) was confirmed using the GEPIA2 database, based on TCGA retrieved from cancer samples from over 11,000 patients over 12 years (Supplementary Fig. 5A). Furthermore, HAS2-KD exhibited significant reductions in both migratory (P < 0.001) and invasive (P < 0.05) properties and showed that the extent of reduction was greater in migratory properties (Supplementary Fig. 5B, C).

Next, MDA-MB-231 cells were co-transfected with a HAS2 overexpression vector (3×FLAG-HAS2) and KPNA3-KD. As a result, overexpression of HAS2 restored the migratory properties (P < 0.001) of KPNA3-KD-231 cells but not the invasive properties or the expression of E-cadherin, a representative EMT marker (Fig. 2E, F). These results suggest that KPNA3 plays a critical role in EMT induction through HAS2 regulation. These results imply that the EMT or mesenchymal-epithelial transition characteristics of cells whose transcriptional program has already been altered by KPNA3-KD cannot be fully restored by the control of HAS2 alone.

KPNA3 promotes EMT-mediated metastasis via down-regulation of the transcription factor GATA3

To identify which transcription factor plays a critical role in KPNA3-mediated EMT, enrichment analysis of KPNA3-KD-induced transcriptional signatures was performed with the Enrichr tool using gene expression signatures derived from the GEO database for transcription factor perturbations. As shown in Fig. 3A, GATA3 was ranked highest among 265 transcription factors (P < 8.37e-29). Accordingly, western blot and RT-qPCR analyses were performed to determine whether KPNA3 regulates GATA3 expression. The mRNA and protein expression levels of GATA3 were significantly increased by KPNA3-KD (Fig. 3B, C). Transwell chamber assays indicated that the KPNA3-KD-mediated reduction in migratory and invasive properties were partially but significantly restored by an siRNA of GATA3 (GATA3-KD; P < 0.0001 for migration and P < 0.001 for invasion; Fig. 3D, E), suggesting that GATA3 is a major downstream target in KPNA3-mediated EMT.

To determine whether KPNA3-KD-mediated GATA3 upregulation affects the expression of the above-mentioned genes, the expression levels of E-cadherin and HAS2, were assessed after dual knockdown by KPNA3-KD and GATA3-KD. E-cadherin

expression, which was upregulated by KPNA3-KD, was reduced by GATA3-KD (Fig. 3F), indicating that GATA3 is involved in the regulation of E-cadherin; this is consistent with the findings of Yan *et al*. (8). In addition, it was found that GATA3-KD upregulated HAS2 expression, which was downregulated by KPNA3-KD (Fig. 3F), suggesting the involvement of the KPNA3-GATA3-HAS2/E-cadherin cascade. Moreover, the regulatory mechanism of HAS2 expression by GATA3 was assessed by screening the potential binding sites of GATA3 on the *HAS2* promoter region spanning 2,000 bp upstream from the transcription start site using the JASPAR database. Five putative GATA3-binding sites were detected on the *HAS2* promoter, implying that *HAS2* is transcriptionally regulated by direct binding of GATA3 (Supplementary Fig. 6). Collectively, these findings suggest that KPNA3 induces EMT-mediated metastasis by inhibiting GATA3, which regulates E-cadherin and HAS2 in MDA-MB-231 cells.

KPNA3 promotes EMT-mediated metastasis through the regulation of TGF-β and AKT signaling pathways

Signaling pathways associated with genome-wide transcriptional reprogramming in KPNA3-KD-231 cells were analyzed with the Enrichr tool using the BioPlanet 2019 database. Enrichment analysis revealed that the "TGF-β signaling pathway for the regulation of ECM" was the most important pathway out of the 1,658 human pathways in KPNA3-KD-231 cells (Fig. 4A). Furthermore, TGF-β signaling was transduced through small mothers against decapentaplegic (SMAD) and non-SMAD pathways. These pathways are mediated by TGF-β ligands, type 1 and type 2 receptors, and SMAD or non-SMAD proteins, including AKT, ERK1/2, and p38 mitogen-activated protein kinase (9). Therefore, to determine whether TGF-β signaling was inhibited in KPNA3-KD-231 cells, the expression of pSMAD2/3 was assessed by western blot analysis. As shown in Fig. 4B, the expression levels of total SMAD2/3 were unchanged, whereas KPNA3-KD inhibited the expression levels of their phosphorylated forms. The RT-qPCR results revealed that the expression of *CTGF* and *PTHRP*, which are the metastasis-related downstream genes of SMAD signaling, were downregulated by KPNA3-KD (Fig. 4C). These findings suggest that TGF-β/SMAD is a crucial downstream signaling pathway in KPNA3-mediated EMT. As shown in Fig. 4B, the expression levels of phosphorylated AKT (T308 and S473) were downregulated by KPNA3-KD. In contrast, the expression levels of phosphorylated ERK and TWIST1/2 were unchanged (Fig. 4B and Supplementary Fig. 7); this suggests that ERK-TWIST1/2 signaling may not be involved in KPNA3-mediated EMT in MDA-MB-231 cells. 2-(4-Morpholinyl)-8-phenyl-4H-1-benzopyran-4-one (LY294002), an inhibitor of AKT phosphorylation, reduced HAS2 expression and increased E-cadherin expression, whereas the expression of GATA3 was unchanged (Fig. 4D). These results indicate that phosphorylated AKT regulates the expression of HAS2 and E-cadherin but is not involved in GATA3 expression regulation.

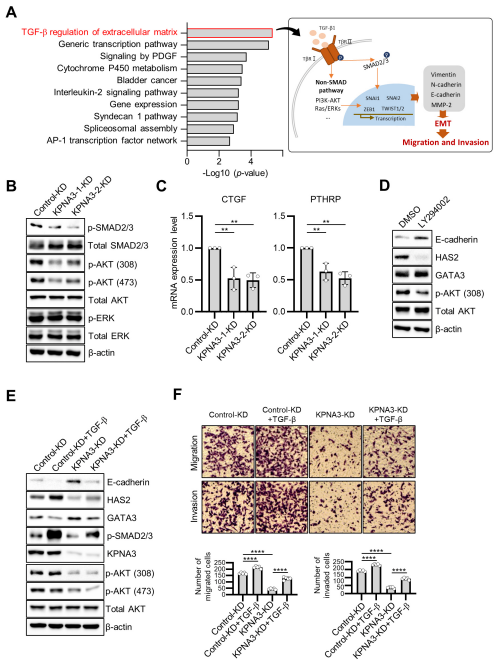

Fig. 4. Karyopherin-α3 (KPNA3) triggers epithelial-mesenchymal transition (EMT) via transforming growth factor-β (TGF-β) and serine/threonine kinase (AKT) signaling pathways. (A) BioPlanet 2019-based enrichment analysis of KPNA3-KD-induced transcriptional signatures (absolute fold change > 2, $P < 0.05$). The P-values were computed using Fisher's exact test and converted to −log10. (B) Expression levels of total SMAD2/3 and phosphorylated SMAD2 (S465/467)/SMAD3 (S423/425), total AKT, phosphorylated AKT (T308/S473), total ERK, and phosphorylated ERK (T202/Y204) proteins in KPNA3-KD-231 cells for 48 h. (C) Expression levels of CTGF and PTHRP mRNAs in KPNA3-KD-231 cells. (D) Expression levels of E-cadherin, HAS2, GATA3, total AKT, and phosphorylated AKT (T308) proteins in MDA-MB-231 cells with DMSO or LY294002 treatment (10 μM) for 24 h. (E) Expression levels of E-cadherin, HAS2, GATA3, phosphorylated SMAD2/3, total AKT, phosphorylated AKT (T308/S473), total ERK, phosphorylated ERK (T202/Y204), and KPNA3 proteins in KPNA3-KD-231 cells treated with or without TGF-β (5 ng/ml) for 24 h. (F) Cell images of migrated or invaded cells under the same conditions as those in (E) (left panel). The number of migrated or invaded cells (right panel).

To determine whether KPNA3 regulates GATA3 through TGF-β signaling, GATA3 expression was evaluated after exposing KPNA3-KD-231 cells to TGF-β. TGF-β clearly reduced GATA3 and E-cadherin expression, and increased HAS2 in MDA-MB-231 cells (Fig. 4E). However, these TGF-β-induced changes were hindered by KPNA3-KD (Fig. 4E), indicating that TGF-β is critical for the regulation of GATA3 expression in KPNA3-mediated EMT. Furthermore, KPNA3-KD suppressed the migratory and invasive properties induced by TGF-β (Fig. 4F). However, TGF-β induced no change in the expression levels of both total and phosphorylated AKT (Fig. 4E), implying that the KPNA3-KD-induced downregulation of phosphorylated AKT may be mediated independently of the TGF-β signaling pathway. Col-

lectively, we conclude that KPNA3 ultimately promotes EMT-mediated metastasis through the independent regulation of the TGF-β and AKT signaling pathways, suggesting two axes: KPNA3-TGF-β-GATA3-HAS2/E-cadherin and KPNA3-AKT-HAS2/E-cadherin.

DISCUSSION

The EMT process involves the loss of cell-cell junctions and remodeling of ECM through genome-wide transcriptional reprogramming induced by several epithelial-mesenchymal transition-associated transcription factors (EMT-TFs) and various relevant signaling pathways, consequently promoting the metastasis of malignant tumors (1, 2). Emerging evidence suggests that KPNA3 is closely related to metastasis via EMT in various types of cancers (4, 5). However, the genome-wide regulatory mechanism of KPNA3-induced EMT in breast cancer remains largely unknown. In the present study, comprehensive bioinformatic data confirmed that among the KPNAs, KPNA3 is highly expressed in aggressive breast cancer cells and tissues, particularly in TNBC, and is closely associated with poor prognosis. Further mechanistic investigation revealed that KPNA3 triggers EMT by inducing cell-cell junction remodeling and ECM through the regulation of two independent signaling pathways, including KPNA3-TGF-β-GATA3-HAS2/E-cadherin and KPNA3-AKT-HAS2/E-cadherin, in TNBC MDA-MB-231 cells (Supplementary Fig. 8).

EMT-inducing capacity of KPNA3 through regulation of cell-cell junctions and ECM organization

The transcriptome profiles retrieved from KPNA3-KD-231 cells showed significant enrichment in comparison with the defined EMT-core gene list. As shown in Supplementary Fig. 4C, several genes shared by our transcriptome profiles and the EMT-core gene list were classified into the cell adhesion and migration category. HAS2 is related to EMT induction through the synthesis of hyaluronic acid (HA), a major component of ECM (10). Versican has HA-binding properties and is an anti-adhesion molecule, implying the cooperative role of versican and HA in ECM remodeling (11). Neuropilin-1 promotes tumor invasion through the up-regulation of vascular endothelial growth factor A, which interacts with ECM components (12). Junction plakoglobin, a member of the catenin protein family, is a cytoplasmic component comprising desmosomes and adherens junctions (13). E-cadherin is the most well-known member of the cadherin family and is closely associated with EMT induction when its expression is significantly reduced (14). In addition, matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) are associated with ECM degradation and remodeling (15). The transcriptome profiles retrieved from KPNA3-KD-231 revealed that the expression of MMP-1 was downregulated (-2.001 FC; $P < 0.0001$), whereas that of TIMPs, which suppress MMP expression and activity, were upregulated (Supplementary Table 2) (16). These data strongly suggest that KPNA3 is a strong EMT inducer in TNBC and that its EMT-inducing capacity is attributed to altered expression levels of numerous genes, which mainly regulate cell-cell junctions and ECM organization.

A novel KPNA3-TGF-β-GATA3-HAS2/E-cadherin signaling cascade that promotes EMT

The TGF-β/SMAD signaling pathway is well known to play an important role in inducing EMT through the up-regulation of EMT-TFs, such as snail family transcriptional repressor 1/2 (SNAI1/2), zinc-finger E-box-binding homeobox 1/2 (ZEB1/2), and TWIST1/2, which have been widely accepted as representative EMT inducers that regulate the expression of EMT-related genes, including *CDH1* and *HAS2* (17). Our results indicated that TGF-β-mediated SMAD2/3 phosphorylation and its downstream target genes, *CTGF* and *PTHRP*, were downregulated in KPNA3-KD-231 cells. Additionally, the migratory and invasive properties induced by extrinsic TGF-β were considerably hindered by KPNA3-KD, suggesting that KPNA3-KD inhibited the TGF-β signaling pathway. However, the protein expression levels of ZEB1/2, SNAI1/2, and TWIST1/2 were either upregulated or unchanged by KPNA3-KDs (Supplementary Fig. 7). In addition, enrichment analysis revealed that ZEB1/2, SNAI1/2, and TWIST1/2 were not included in the top 10 rankings. Rather GATA3, which regulates the expression of E-cadherin and HAS2, ranked highest among transcription factors. As shown in Fig. 3F, changes in E-cadherin and HAS2 expression in GATA3-KD suggest that GATA3 is involved in regulating E-cadherin and HAS2 expression. Furthermore, the exposure of cells to extrinsic TGF-β was confirmed to reduce GATA3 expression, a crucial downstream target of KPNA3; this result agrees with a previous study in T cells (18). However, further studies are required to elucidate the molecular mechanism and whether KPNA3 regulates GATA3 expression directly or indirectly through TGF-β. This finding suggests that KPNA3 triggers EMT through a novel KPNA3-TGF-β-GATA3-HAS2/E-cadherin signaling cascade in TNBC MDA-MB-231 cells.

Additional KPNA3-AKT-HAS2/E-cadherin signaling cascade to promote EMT

It has been reported that TGF-β can trigger many non-canonical pathways, also termed non-SMAD pathways (19). Particularly in MDA-MB-231 cells, exposure of extrinsic TGF-β to cells increased the expression of phosphorylated AKT and enhanced MMP-9 expression and activity via the ITGB1/FAK/Src/AKT/β-catenin/MMP-9 signaling cascade (20). On the contrary, it was also reported that TGF–β inhibited AKT phosphorylation in MDA-MB-231 cells (21). Our results revealed that AKT phosphorylation was unchanged by exposure of extrinsic TGF-β to MDA-MB-231 cells (Fig. 4E). In addition, the transcriptome profiles retrieved from KPNA3-KD-231 cells revealed that the expression of MMP-9 remained unchanged, indicating that AKT cannot be regulated TGF-β by in MDA-MB-231 cells (Supplementary Table 2). The reasons for these discrepancies are not fully understood. However, they may be attri-

buted to the TGF-β concentration and treatment time. Moreover, *HAS2* and *CDH1* were regulated negatively and positively by LY294002, respectively, whereas the expression of GATA3 was unchanged. These results imply that *GATA3* is regulated by the TGF-β and not the AKT signaling pathway. In addition, these results suggest that KPNA3 also induces EMT through the KPNA3-AKT-HAS2/E-cadherin cascade independently of the TGF-β signaling pathway. However, the detailed molecular mechanism of the association between KPNA3 and the EMT-related signaling pathways, TGF-β, and AKT requires further investigation.

In summary, this study reveals the EMT inducibility of KPNA3 in TNBC MDA-MB-231 cells. Moreover, KPNA3 triggers EMT through two axes, TGF-β-GATA3-HAS2/E-cadherin and AKT-HAS2/E-cadherin, to promote tumor progression and metastasis, suggesting that KPNA3 might be a putative target for the treatment of TNBC. However, for the clinical application of KPNA3 as an EMT suppressor or chemotherapy drug, more comprehensive and multidisciplinary studies need to be conducted on the genome-wide transcriptomic modulations induced by KPNA3 in the cells.

MATERIALS AND METHODS

Materials and methods are available in the supplemental material.

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CONFLICTS OF INTEREST

The authors have no conflicting interests.

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