Article

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MEX3A Drives Triple-Negative Breast Cancer Migration, Invasion, and Metastasis by Interacting with NTRK1

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Background: Triple-negative breast cancer (TNBC) stands out as the most aggressive form of breast cancer due to its significant propensity for metastasis. Exploring the molecular mechanisms of TNBC metastasis is essential to develop TNBC therapeutic strategies. Mex-3 RNA Binding Family Member A (MEX3A) has been recognized to promote TNBC proliferation. However, its role in TNBC metastasis hasn't been investigated.

Methods: We investigated MEX3A levels in breast cancer cells, normal mammary tissues, and breast cancer tissues using Western blot analysis. Moreover, immunohistochemistry (IHC) analysis was used to determine MEX3A levels in TNBC tissues. Cell migration and invasion assays and lung metastasis assay were performed to investigate the role of MEX3A in TNBC metastasis *in vitro* and *in vivo*. Additionally, Co-immunoprecipitation was used to determine the interacting proteins of MEX3A.

Results: MEX3A was upregulated in TNBC tissues compared to the normal mammary tissues, especially in TNBC tissues. Patients with high MEX3A expression exhibited shorter overall survival, distant metastasis-free-, relapse-free-, and post-progression survival time than those with low MEX3A expression ($p < 0.05$). MEX3A knockdown inhibited TNBC cell migration and invasion *in vitro* ($p < 0.05$). MEX3A overexpression promoted TNBC metastasis *in vivo* ($p < 0.05$). Mechanistically, MEX3A could interact with Neurotrophic Receptor Tyrosine Kinase 1 (NTRK1), resulting in activation of Ras GTPase (Ras-GTP), AKT Serine/Threonine Kinase (AKT), and Extracellular signal-regulated kinase (ERK) pathways, thereby promoting TNBC metastasis. Additionally, Ras pathway inhibitor MCP110 reversed the effect of MEX3A overexpression on TNBC cell migration and invasion.

Conclusion: MEX3A is upregulated in TNBC tissues and can serve as an independent prognostic factor for TNBC patients. MEX3A promotes TNBC cell migration, invasion, and metastasis by interacting with NTRK1, resulting in the activation of Ras, AKT, and ERK pathways. These findings offer a potential therapeutic target for patients with TNBC.

Keywords: MEX3A; triple-negative breast cancer; migration; invasion; metastasis

Introduction

Breast cancer ranks as the most commonly diagnosed type of tumor and remains the leading cause of cancer-related mortality among women worldwide. The classification of breast cancer relies on the expression levels of three key receptors: Estrogen Receptor (ER), Progesterone Receptor (PR), and Human Epidermal Growth Factor Receptor 2 (HER2). This classification system divides breast cancer into four distinct subtypes: Luminal A, Luminal B, HER2 positive, and triple-negative breast cancer (TNBC) [1,2]. TNBC is the most aggressive and deadly breast cancer subtype. It exhibits highly aggressive biological characteristics and poses a higher risk of recurrence and distant metastasis ability. With the development of molecular classification and molecular mechanisms associated with TNBC, some targets have been found for clinical application, including Poly (ADP-Ribose) Polymerase 1 (PARP), AKT Serine/Threonine Kinase (AKT), and immune checkpoints. Moreover, two PARP inhibitors, olaparib, and talazoparib, have been approved for treating TNBC patients. However, the effectiveness of these treatments remains unsatisfactory, underscoring the lack of effective targeted therapy for TNBC. Thus, there is an urgent need to identify novel targets to improve TNBC therapy [3–5].

Mex-3 RNA Binding Family Member A (MEX3A), an RNA-binding protein, is recognized to colocalize with Dcp1 and Agronaute in processing bodies (P bodies) [6]. Its role in regulating the progression of various tumors has been demonstrated. For example, MEX3A promotes

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growth, migration, apoptosis, and sorafenib resistance in hepatocellular carcinoma by activating Hippo signalling [7]. Furthermore, MEX3A plays a crucial role in suppressing ferroptosis by degrading p53 and also regulates the alternative splicing of Timeless Circadian Regulator (TIMELESS) to enhance tumorigenesis of ovarian cancer [8,9]. MEX3A marks a subpopulation of chemoresistant cancer stem cells in colorectal cancer that reactivate after drug treatment [10]. In glioblastoma relapse, MEX3A directly interacts with MutS Homolog 2 (*MSH2*) mRNA, leading to recruit CCR4-NOT complexes, subsequently inducing deadenylation and degradation of *MSH2* mRNA [11]. Recently, two studies have indicated that MEX3A interacts with and promotes the degradation of mRNA encoding Insulin-Like Growth Factor Binding Protein 4 (IGFBP4), which in turn activates the phosphatidylinositol 3-kinase (PI3K)/AKT pathway and promotes breast cancer progression [12,13]. Other studies have demonstrated that MEX3A promotes breast cancer by regulating Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha (PIK3CA) and Ras GTPase (Ras-GTP) Homolog Family Member A (RhoA)/Rho Associated Coiled-Coil Containing Protein Kinase 1 (ROCK1) LIM Domain Kinase 1 (LIMK1) signalling pathways [14,15]. However, the precise role and regulatory mechanisms of MEX3A in the development of TNBC remain poorly understood. Therefore, in this study, we assessed the prognostic significance of MEX3A in TNBC patients and elucidated its role and the underlying regulatory mechanisms affecting TNBC prognosis.

Materials and Methods

Cell Culture

Human breast cancer cell lines BT-20 (cat. HTB-19, ATCC, Manassas, VA, USA), BT-549 (cat. HTB-122, ATCC, Manassas, VA, USA), HCC1806 (cat. CRL-2335, ATCC, Manassas, VA, USA), HCC1937 (cat. TCHu148, NCACC, Shanghai, China), Hs 578T (cat. TCHu127, NCACC, Shanghai, China), MDA-MB-231 (cat. TCHu227, NCACC, Shanghai, China), MDA-MB-468 (cat. TCHu136, NCACC, Shanghai, China), and 293T (cat. SCSP-5209, NCACC, Shanghai, China) were cultured in Dulbecco's Modified Eagle Medium (DMEM)/high glucose (cat. SH30249.01, Hyclone, Logan, UT, USA) supplemented with 10% Fetal bovine serum (FBS) (cat. SH30406.05, Hyclone, Logan, UT, USA). The cultures were incubated in a humidified atmosphere at 37 °C in the presence of 5% CO_2. The cell lines were mycoplasma-free and underwent STR authentication.

Specimens and Immunohistochemistry (IHC)

The individuals diagnosed with TNBC (n = 30) at Guangdong Provincial People's Hospital, China were retrospectively included in this study. TNBC tissues and normal breast tissues were collected at Guangdong Provincial People's Hospital between 2010-2015. The study was conducted with the approval of the Guangdong Provincial People's Hospital Ethics Committee (No. KY-Z-2021-439-01). We assessed the correlation between MEX3A expression and clinicopathological characteristics in TNBC patients (Table 1). Furthermore, TNBC tissue slides were stained with anti-MEX3A antibody (1:50, ab79046, Abcam, Cambridge, UK). These slides were observed by two independent pathologists, and their photographs were obtained using a microscope (cat. DM4 B, Lecia, Berlin, Germany). The IHC procedure was adopted from a previously described method [16].

Table 1. Clinicopathological features and MEX3A expression of TNBC patients.

Characteristics		No. of cases (n = 30)
Age (years)		
	≤55	17
	>55	13
T stage		
	T1	8
	T2	16
	T3	4
	T4	2
N stage		
	N0	14
	N1	9
	N2	4
	N3	3
M stage		
	M0	28
	M1	2
Clinical stage		
	I	4
	IIa	11
	IIb	6
	IIIa	5
	IIIb	1
	IIIc	2
	IV	1
Status (at follow-up)		
	Alive	8
	Death because of TNBC cancer	22
	Death because of other than TNBC cancer	0
MEX3A expression		
	Negative	2
	Positive	28
	Low expression	10
	High expression	20

TNBC, triple-negative breast cancer; MEX3A, Mex-3 RNA Binding Family Member A.

Plasmids

To overexpress MEX3A in TNBC cells, the MEX3A cDNA was amplified from 293T using polymerase chain reaction (PCR) and subcloned into the lentiviral vector pSin-EF2-Puro. However, to knockdown MEX3A in TNBC cells, two sh*RNA* sequences were cloned to PLKO.1 vector. The sh*RNA* target sequences of MEX3A were as follows: sh*RNA*#1: 5′-AGCTCTGCGCTCTCTACAAAG-3′, sh*RNA*#2: 5′-CACGCAAGCCATCCGAATATT-3′. For the generation of lentiviruses, 293T cells were seeded in 10 cm dishes. Transfection was conducted utilizing Lipofectamine 3000 reagent (cat. L300008, Thermo, Waltham, MA, USA) along with 2 µg pM2.G, 6 µg PsPAX2, and 8 µg plasmids following 24 hours of plating. Following transfections, the medium containing recombinant lentiviruses was harvested and filtered through 0.45 µm sterilization filters. Subsequently, the cells underwent incubation with the filtered lentivirus supernatants in a fresh medium. Moreover, puromycin (cat. S7417, Selleck, Houston, TX, USA) was used to select stable cell lines.

RNA Isolation and RT-qPCR

Total RNA was isolated from cells and tissues using Trizol reagent (cat. RN190, MRC, Cincinnati, OH, USA) and subsequently reverse transcribed into cDNA utilizing HiScript Q Select RT SuperMix for qPCR (cat. R233, Vazyme, Nanjing, China). Real-Time reverse transcription polymerase chain reaction (RT-PCR) was performed using AceQ Universal SYBR qPCR Master Mix (cat. Q511, Vazyme, Nanjing, China) on a CFX-96 PCR system (Bio-Rad, Hercules, CA, USA). The expression data were normalized relative to the housekeeping gene Glyceraldehyde-3-Phosphate dehydrogenase (*GAPDH*) using the formula: $2^{-(\text{Ct of gene} - \text{Ct of } GAPDH)}$. The primers used in RT-PCR were as follows: Q-*MEX3A* forward: 5′-ACTTCTCCATGATCCGTGCC-3′, Q-*MEX3A* reverse: 5′-GCTTGATGGTTGCCCCTTTG-3′. Q-*GAPDH* forward: 5′-TTGGCTACAGCAACAGGGT-3′, Q-*GAPDH* reverse: 5′-GGGGAGATTCAGTGTGGTGG-3′.

Western Blot

Total proteins were extracted using RIPA buffer (cat. 20-188, Millipore, Billerica, MA, USA) coupled with protease inhibitors (cat. 11836170001, Roche, Basel, Switzerland). Subsequently, protein concentration was determined using a BCA assay kit (cat. 23227, Thermo, Waltham, MA, USA) following the manufacturer's instructions. The cell lysate was resolved through 10% SDS-PAGE and subsequently transferred onto the PDVF membrane. After blocking, the membranes were incubated overnight with primary antibodies against MEX3A (1:1000, ab79046, Abcam, Cambridge, UK), Ras (1:1000, #67648, CST, Danvers, MA, USA), GTPase (1:1000, ab32417, Abcam, Cambridge, UK), phospho-AKT (1:2000, #4060, CST, Danvers, MA, USA), AKT (1:2000, #4685, CST, Danvers, MA , USA), Extracellular signal-regulated kinase (ERK) (1:2000, #4696, CST, Danvers, MA , USA), phospho-ERK (1:2000, #4370, CST, Danvers, MA , USA), and GAPDH (1:5000, ab181602, Abcam, Cambridge, UK). The following day, membranes were incubated with secondary antibodies HRP Donkey Anti-Mouse IgG (H+L) (1:5000, AS033, Thermo, Waltham, MA, USA) and Goat Anti-Rabbit IgG (H+L) (1:5000, AS070, Thermo, Waltham, MA, USA), which were purchased from Abclonal. Finally, the immunoblots were developed and visualized, and the grayscale values of the protein bands were assessed.

Cell Migration and Invasion Assay

For migration assay, 5×10^4 cells were plated in the Transwell chamber (cat. 3422, Corning, New York, NY, USA) and incubated for 16 hours. However, for invasion assay, the chamber was pre-coated with Matrigel (cat. 354234, Corning, New York, NY, USA). Subsequently, 1×10^5 cells were seeded into the upper chamber containing DMEM/high glucose medium without serum, and the lower chamber was filled with DMEM/high glucose medium supplemented with 10% FBS. After 24 h, non-invasive cells were removed using a cotton swab. However, the cells that migrated through the membrane and adhered to the lower surface were fixed with 70% ethanol and subsequently stained with 0.05% crystal violet solution (C8470, Solarbio, Beijing, China). Finally, the cells were examined using an inverted microscope (cat. DMi1, Lecia, Berlin, Germany).

Lung Metastasis Model

All experimental protocols involving animals received approval from the Institutional Animal Care and Use Committee of the Guangdong Provincial People's Hospital and adhered to the Guidelines for the Care and Use of Laboratory Animals (No. KY-2020-198-01-01). Female BALB/c-nu mice (5 weeks old and weighing 16–18 g) were purchased from the Model Animal Research Centre of Nanjing University. The mice were randomly divided into two groups, each containing 6 mice. Subsequently, 5×10^6 Hs578T cells with or without MEX3A overexpression were inoculated subcutaneously into the mammary fat pad of the mice. Finally, the bioluminescence signals were determined by an IVIS imaging system (PerkinElmer, Waltham, MA, USA). The mice were euthanized via intraperitoneal injection of pentobarbital sodium at a dosage of 150 mg/kg.

Co-Immunoprecipitation (Co-IP)

The Co-IP was conducted using Pierce Co-immunoprecipitation Kit (cat. 26149, Thermo, Waltham, MA, USA) following the guidelines provided by the manufacturer. We utilized 293T cells and Anti-Neurotrophic Receptor Tyrosine Kinase 1 (NTRK1) antibody (1:100, cat. 06-574, Millipore, Boston, MA, USA) in this assay.

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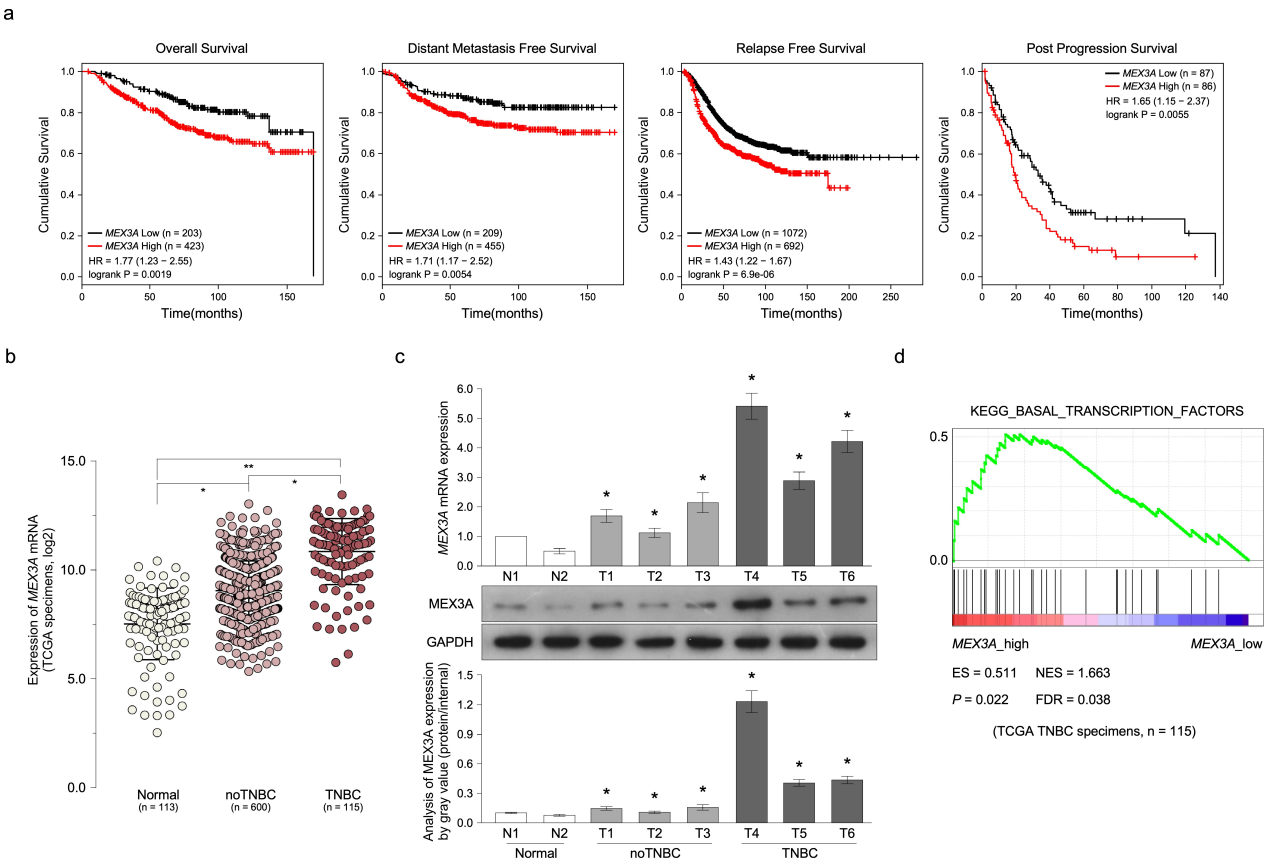

Fig. 1. MEX3A is upregulated in TNBC tissues and associated with poor outcomes. (a) Kaplan-Meier analysis on the overall survival, distant metastasis-free survival, relapse-free survival, and post-progression survival of breast cancer patients stratified based on their MEX3A expression levels (high or low). The data were obtained from the The Cancer Genome Atlas (TCGA) dataset. (b) The MEX3A levels in normal breast tissues, non-TNBC tissues, and TNBC tissues. The data were downloaded from the TCGA dataset. (c) qRT-PCR and Western blot analysis of MEX3A levels in normal breast tissues, non-TNBC tissues, and TNBC tissues. n = 3. (d) The correlation between MEX3A expression and transcriptional activity using GSEA. Data are expressed as the mean ± SEM; *$p < 0.05$. **$p < 0.01$. *GAPDH*, Glyceraldehyde-3-Phosphate dehydrogenase; HR, hazard ratio; ES, enrichment score; P, Probability-value; NES, normalized enrichment score; FDR, false discovery rate; SEM, Standard Error of Mean; GSEA, Gene set enrichment analysis.

Enzyme-Linked Immunosorbent Assay

The levels of Ras expression were assessed using the Ras GTPase Enzyme-linked immunosorbent assay (ELISA) Kit (ab134640, Abcam, Cambridge, UK) according to the guidelines provided by the manufacturer. We used HCC1806 and Hs578T cells in ELISA analysis.

Luciferase Reporter Assay

Forkhead Box O (FOXO) transcription and ERK pathway activities were observed using a Dual-Luciferase Reporter Assay. The 3× DBE_RE Renilla (#124535) and 3× ERRE/ERE-luciferase (#37852) plasmids utilized in this assay were purchased from Addgene (Watertown, MA, USA). The cells were co-transfected with pRL-TK Renilla plasmid using Lipofectamine 3000 reagent (cat. L300008, Thermo, Waltham, MA, USA). After 48 hours of transfection, luciferase activity was assessed following the manufacturer's instructions provided by the Dual-Glo Luciferase Assay Kit (cat. E2920, Promega, Madison, WI, USA). Each experiment was performed in triplicates. We utilized HCC1806 and Hs578T cells in a luciferase reporter assay.

Statistical Analysis

Statistical analyses were performed using GraphPad Prism 8 (GraphPad Software, Inc., San Diego, CA, USA) and SPSS Stat 20 (IBM, Armonk, NY, USA). Each experiment was repeated three times. The data were presented as the mean ± Standard Error of Mean (SEM). Statistical comparison between the two groups was conducted through a two-tailed Student *t*-test. The correlation between MEX3A expression and the clinicopathological characteristics of the patients was determined using the Fisher's Exact test. Moreover, survival data were determined using Univariate and multivariate Cox regression analysis. Gene set enrichment analysis (GSEA) was performed using GSEA 2.0.9 software (http://www.broadinstitute.org/gsea/). A *p*-value < 0.05 was considered statistically significant.

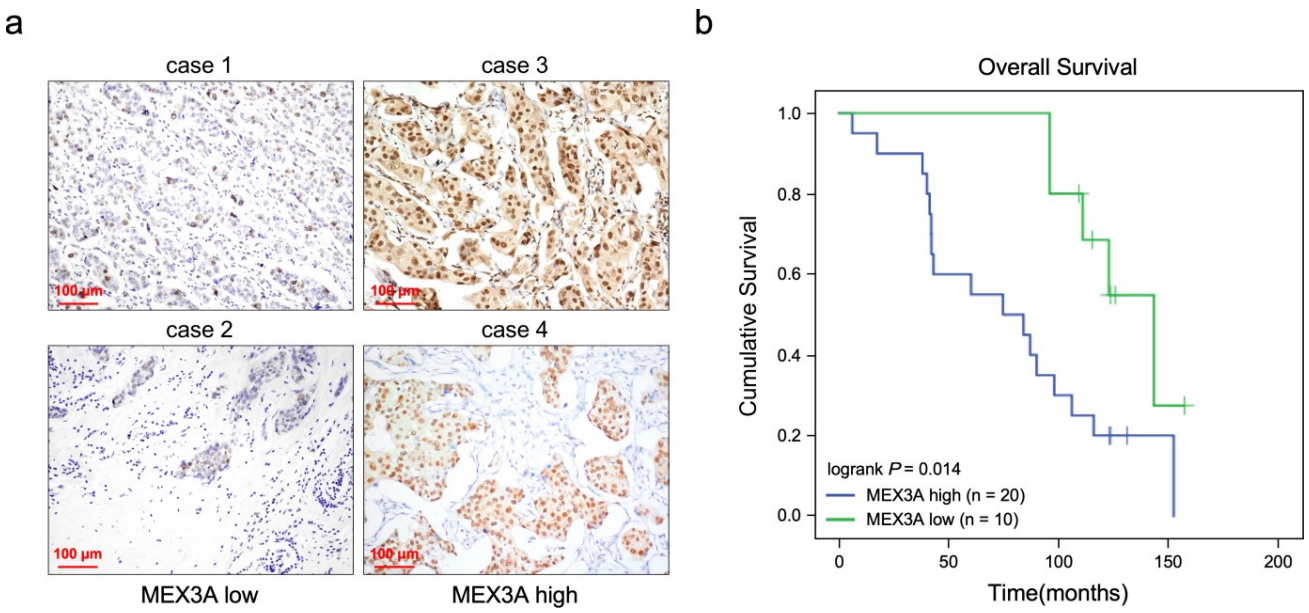

Fig. 2. MEX3A is an independent factor prognostic factor for TNBC patients. (a) Representative immunohistochemistry (IHC) staining images of MEX3A expression in TNBC tissues. (b) Kaplan-Meier analysis of overall survival in TNBC patients stratified as MEX3A high or MEX3A low.

Table 2. The correlation between MEX3A expression and clinicopathologic characteristics of TNBC.

Characteristics		MEX3A		Fisher's Exact test *p*-value
		Low No.	High No.	
Age (years)	≤55	6	11	0.794
	>55	4	9	
T stage	T1	2	6	0.861
	T2	6	10	
	T3	1	3	
	T4	1	1	
N stage	N0	9	5	0.03
	N1	1	8	
	N2	0	4	
	N3	0	3	
M stage	M0	10	18	0.193
	M1	0	2	
Clinical stage	I	2	2	0.276
	II	7	10	
	III	2	6	
	IV	0	1	
Vital status	Alive	5	3	0.045
	Death	5	17	

Table 3. Univariate analyses of various prognostic parameters in patients with triple-negative breast cancer using Cox regression analysis.

	Univariate analysis		
	Number	*p*	Relative risk
Age			
≤55	17	0.25	1.675
>55	13		
Clinical stage			
I	4		
II	17	0.08	1.644
III	8		
IV	1		
T stage			
T1	8		
T2	16	0.964	1.014
T3	4		
T4	2		
N stage			
N0	14		
N1	9	0.027	1.608
N2	4		
N3	3		
M stage			
M0	28	0.002	44.715
M1	2		
Expression of MEX3A			
Low expression	10	0.02	3.3
High expression	20		

Results

MEX3A is Upregulated in TNBC Tissues and Associated with Poor Outcomes

To assess the role of MEX3A in breast cancer development, we investigated the correlation between MEX3A expression and clinic outcome using The Cancer Genome Atlas (TCGA) dataset. Breast cancer patients with high

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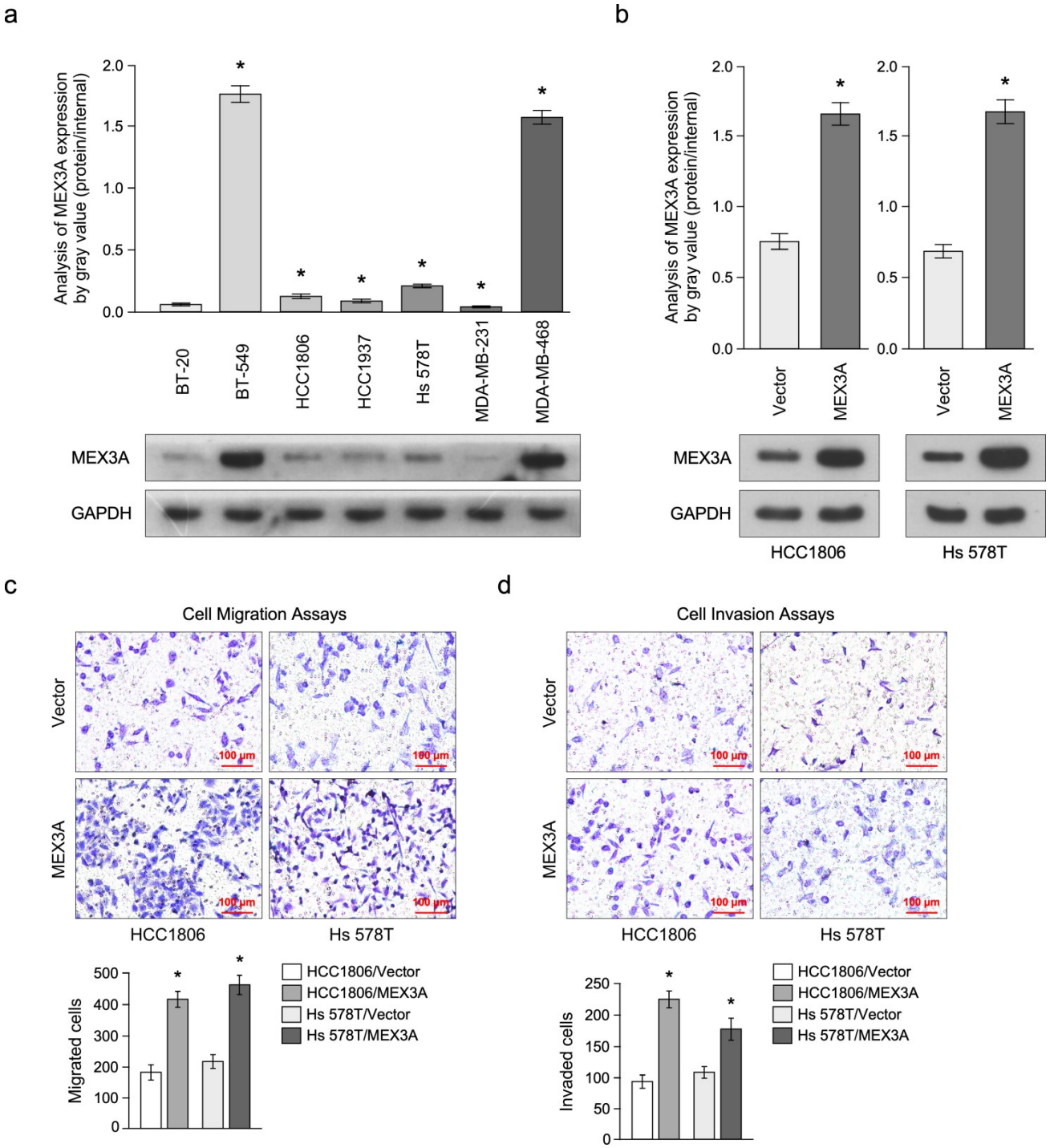

Fig. 3. MEX3A overexpression promotes TNBC invasion and migration. (a) Western blot analysis of MEX3A expression in TNBC cell lines. (b) Western blot analysis of MEX3A expression in TNBC cells infecting with MEX3A overexpression lentivirus. (c) The effect of MEX3A overexpression on TNBC cell migration. Representative micrographs (Upper) and quantification of migration cells (Bottom). n = 3. (d) The promoting effect of MEX3A overexpression on TNBC cell invasion. Representative micrographs (Upper) and quantification of invasion cells. n = 3. Data are expressed as the mean ± SEM; *$p < 0.05$.

MEX3A expression exhibited shorter overall survival, distant metastasis, relapse-free or post-progression survival time than those with low MEX3A expression Fig. 1a. Additionally, analysis of MEX3A expression in normal breast tissues, non-TNBC tissues, and TNBC tissues employing the TCGA dataset revealed a significant upregulation of MEX3A in breast cancer tissues. Furthermore, MEX3A expression was significantly higher in TNBC tissues compared to the non-TNBC tissues ($p < 0.05$, Fig. 1b). In summary, qRT-PCR and Western blot analyses revealed that

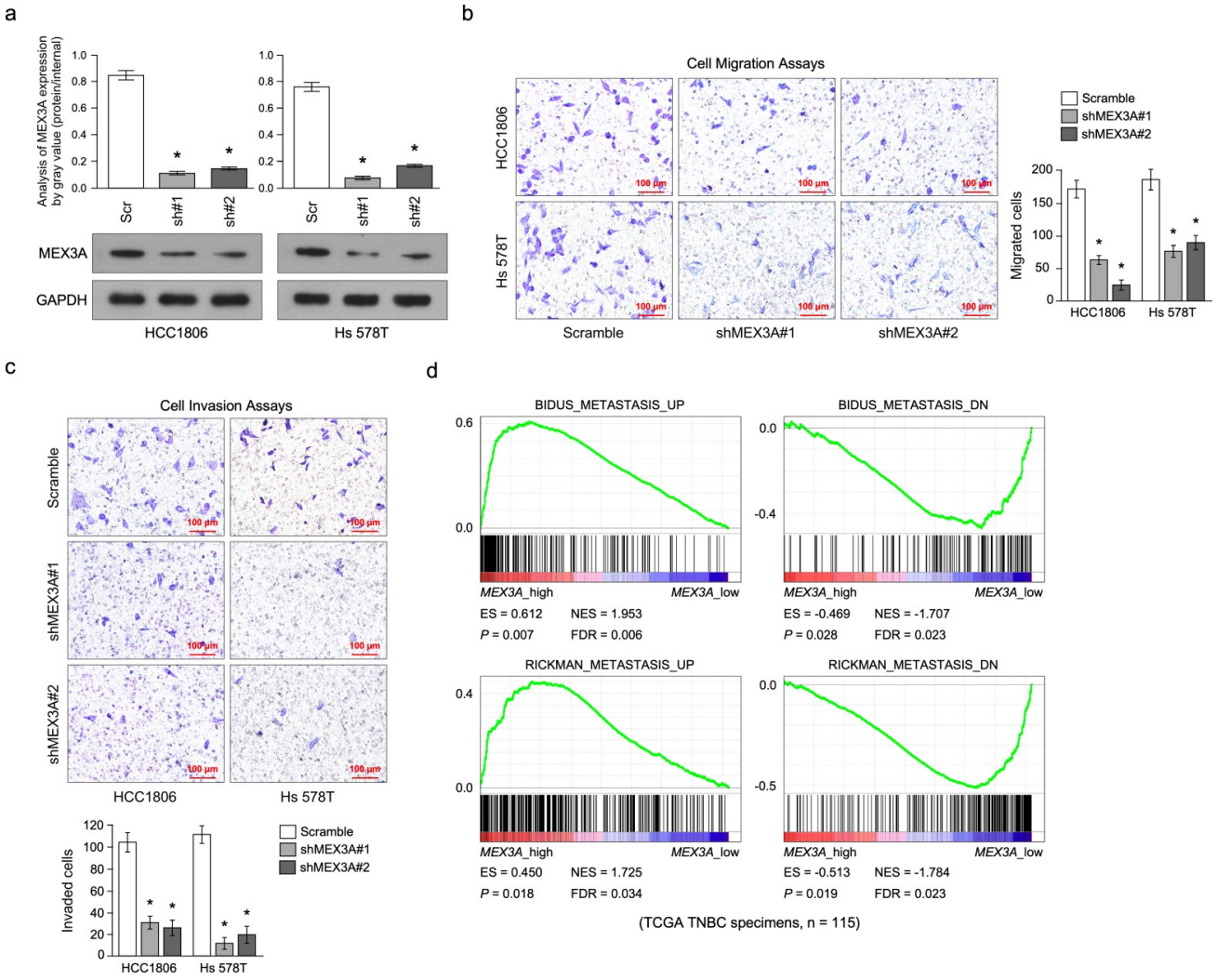

Fig. 4. MEX3A knockdown inhibits TNBC migration and invasion. (a) Western blot analysis of MEX3A expression in TNBC cells knocking down MEX3A. (b) The effect of MEX3A knockdown on TNBC cell migration. Representative micrographs (Left) and quantification of migration cells (Right). n = 3. (c) The impact of MEX3A knockdown on TNBC cell migration. Representative micrographs depicting cell migration (Left) and quantitative analysis of migrated cells (Right). n = 3. (d) The relationship between MEX3A expression and tumor metastasis ability using Gene set enrichment analysis (GSEA). Data are expressed as the mean ± SEM; $*p < 0.05$.

MEX3A expression levels were significantly elevated in breast cancer tissues compared to the normal breast tissues.

Additionally, we found that MEX3A expression levels were higher in TNBC tissues compared to the non-TNBC tissues (Fig. 1c). Furthermore, GSEA suggested that MEX3A was positively correlated with transcription activity in TNBC tissues (Fig. 1d). These findings indicated that MEX3A was upregulated in TNBC tissues and associated with unfavourable clinical outcomes.

MEX3A is An Independent Factor for TNBC Patients

The TNBC tissues (n = 30) underwent IHC to assess MEX3A expression levels (Fig. 2a). We conducted a comparative analysis of the clinical characteristics of TNBC patients with high MEX3A expression versus those with low MEX3A expression. As shown in Table 2, significant differences were observed in the N stage and vital status of the patients. Conversely, no significant correlations were observed between the two groups of patients at the age, T stage, M stage, and clinical stage. Furthermore, Kaplan-Meier survival curves revealed that TNBC patients with high MEX3A expression exhibited significantly shorter overall survival compared to those with low MEX3A expression ($p < 0.05$, Fig. 2b). Additionally, Univariate and Multivariate Cox progression analysis indicated MEX3A as an independent prognostic factor for TNBC patients (Tables 3,4).

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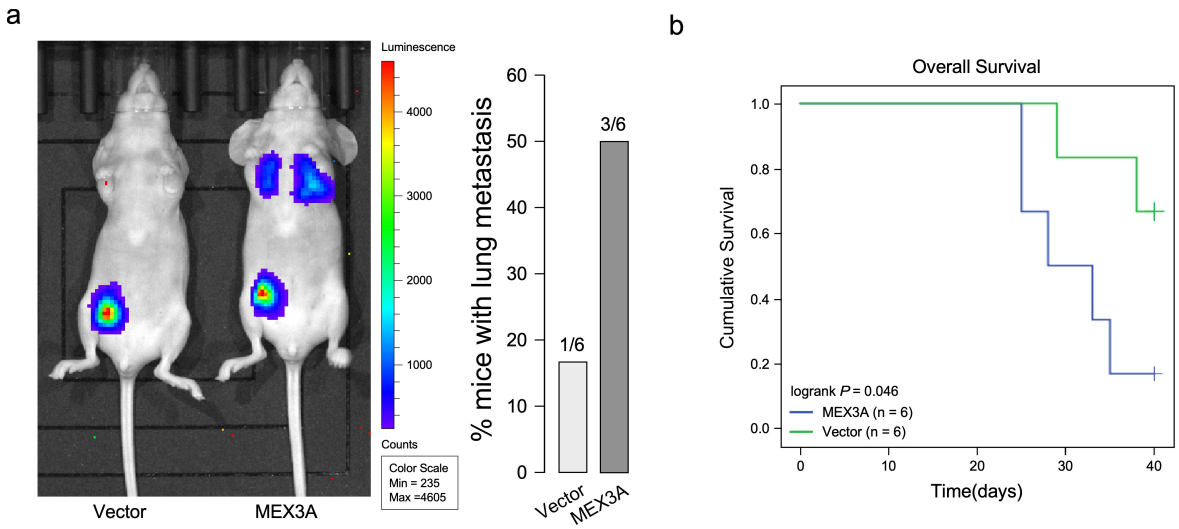

Fig. 5. MEX3A overexpression promotes TNBC cell metastasis. (a) Bioluminescence images of mice showing lung metastases derived from Hs578T with MEX3A overexpression and vector control cells. n = 6. (b) Kaplan-Meier analysis of overall survival in mice transplanted with MEX3A overexpressing-TNBC cells.

Table 4. Multivariate analyses of various prognostic parameters in TNBC patients using Cox regression analysis.

	Multivariate analysis			
	Number	*p*	Relative risk	95% confidence interval
M stage				
M0	28	0.005	33.812	2.976–384.111
M1	2			
Expression of MEX3A				
Low expression	10	0.032	3.065	1.102–8.523
High expression	20			

MEX3A Promotes TNBC Migration, Invasion, and Metastasis

We determined MEX3A expression in TNBC cells using Western blot analysis (Fig. 3a). To investigate the role of MEX3A in TNBC progression, we overexpressed MEX3A in HCC1806 and Hs578T cells, which have low MEX3A expression (Fig. 3b). Cellular migration and invasion assay revealed that MEX3A overexpression significantly promoted TNBC cell migration and invasion ($p < 0.05$, Fig. 3c,d). However, MEX3A knockdown substantially inhibited migration and invasion in both HCC1806 and Hs578T cells ($p < 0.05$, Fig. 4a–c). Similarly, GSEA indicated a positive correlation between high MEX3A expression and metastasis ability ($p < 0.05$, Fig. 4d), suggesting that MEX3A can promote TNBC metastasis. Furthermore, lung metastasis assay showed that MEX3A overexpression increased lung metastasis ability in breast cancer (Fig. 5a). Additionally, Kaplan-Meier survival curves demonstrated that mice transplanted with TNBC cells with MEX3A overexpression exhibited shorter survival time compared to those transplanted with TNBC with control vector (Fig. 5b). These findings indicated that MEX3A overexpression can promote TNBC migration, invasion, and metastasis.

MEX3A Promotes TNBC Migration and Invasion by Interacting with NTRK1

To investigate the regulatory mechanism of MEX3A promoting TNBC progression, we conducted a Co-IP assay and observed the interaction between MEX3A and NTRK1 (Fig. 6a). NTRK1 belongs to the tropomyosin receptor kinase (TRK) family. The interaction between neurotrophins and NTRK1 induces receptor dimerization, phosphorylation, and activation of PI3K, Ras, Mitogen-Activated Protein Kinase (MAPK) and ERK signaling pathways, promoting the progression of various tumors [17,18]. To determine whether MEX3A regulates the Ras pathway, we performed Ras GTPase ELISA assay and revealed that MEX3A overexpression activated the Ras pathway, while MEX3A knockdown inhibited this pathway (Fig. 6b). Moreover, we assessed the impact of MEX3A in regulating Ras, AKT, and ERK signaling pathways using Western blot analysis and found that MEX3A overexpression increased the GTPase HRas expression. However, MEX3A knockdown inhibited GTPase HRas expression, suggesting that MEX3A activates Ras pathway. Furthermore, MEX3A overexpression resulted in increased levels of AKT phosphorylation, while MEX3A knockdown reduced AKT phosphorylation, suggesting that MEX3A

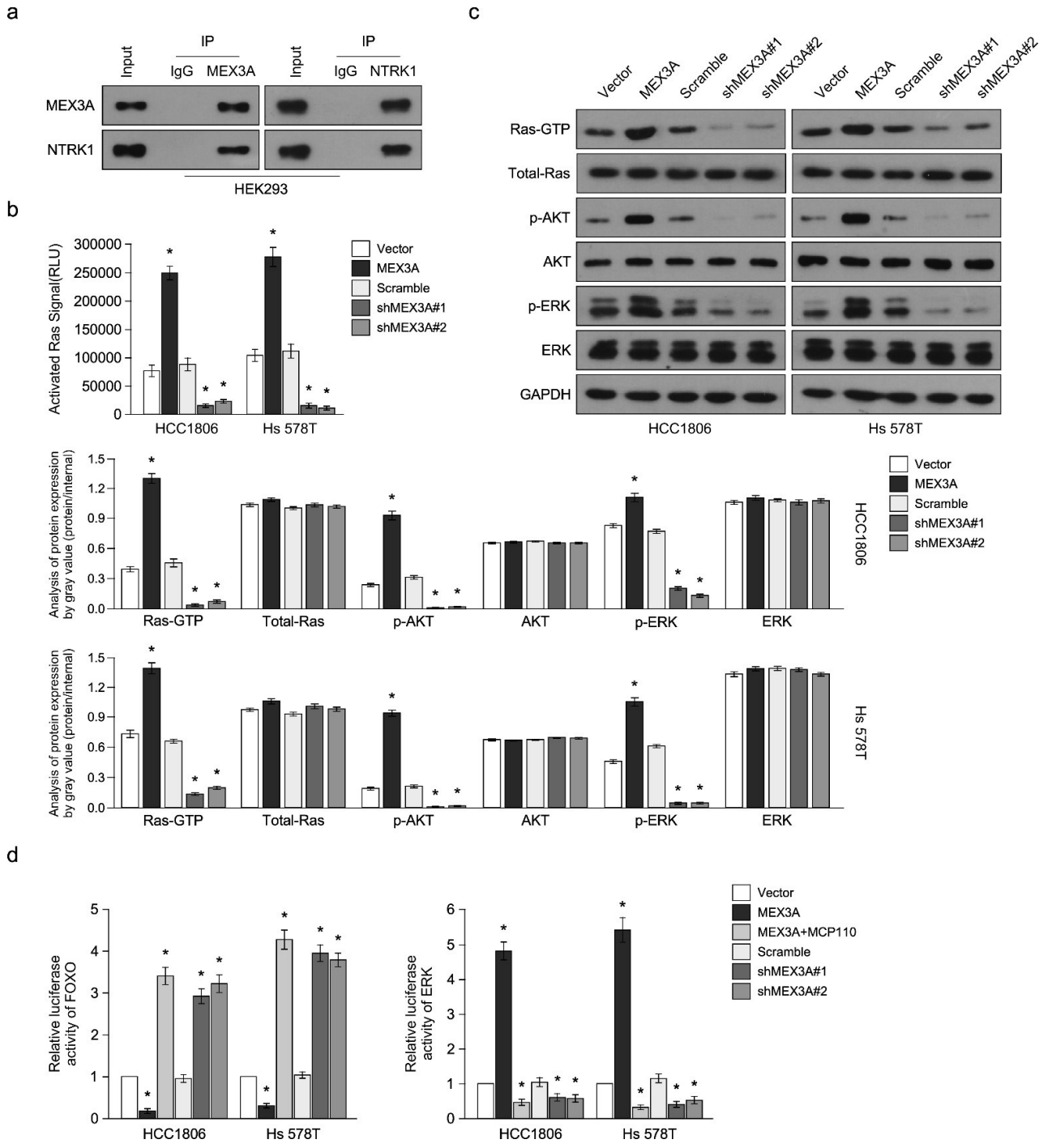

Fig. 6. MEX3A interacts with NTRK1 to activate AKT, Ras, and ERK pathways. (a) Co-immunoprecipitation (Co-IP) analysis of MEX3A interacting with NTRK1. n = 3. (b) Enzyme-linked immunosorbent assay (ELISA) analysis of the effect of MEX3A on Ras pathway activation. n = 6. (c) Western blot analysis of Ras GTPase, p-AKT, AKT, p-ERK and ERK. n = 3. (d) Luciferase reporter assay for the role of MEX3A in AKT and ERK pathway activity. n = 6. Data are expressed as the mean ± SEM; *$p < 0.05$. FOXO, Forkhead Box O; Ras-GTP, Ras GTPase; IgG, Immunoglobulin G; ERK, Extracellular signal-regulated kinase; NTRK1, Neurotrophic Receptor Tyrosine Kinase 1; AKT, AKT Serine/Threonine Kinase.

could activate the AKT pathway. MEX3A overexpression increased the level of ERK phosphorylation, while MEX3A knockdown inhibited this phosphorylation (Fig. 6c). These findings revealed that MEX3A can activate Ras, AKT, and ERK pathways (Fig. 6c).

Subsequently, we overexpressed NTRK1 in MEX3A-knocking down TNBC cells and observed that overexpression of NTRK1 reversed the effect of MEX3A knockdown on Ras, AKT, and ERK pathway activation (**Supplementary Fig. 1**). Ras pathway has been demon-

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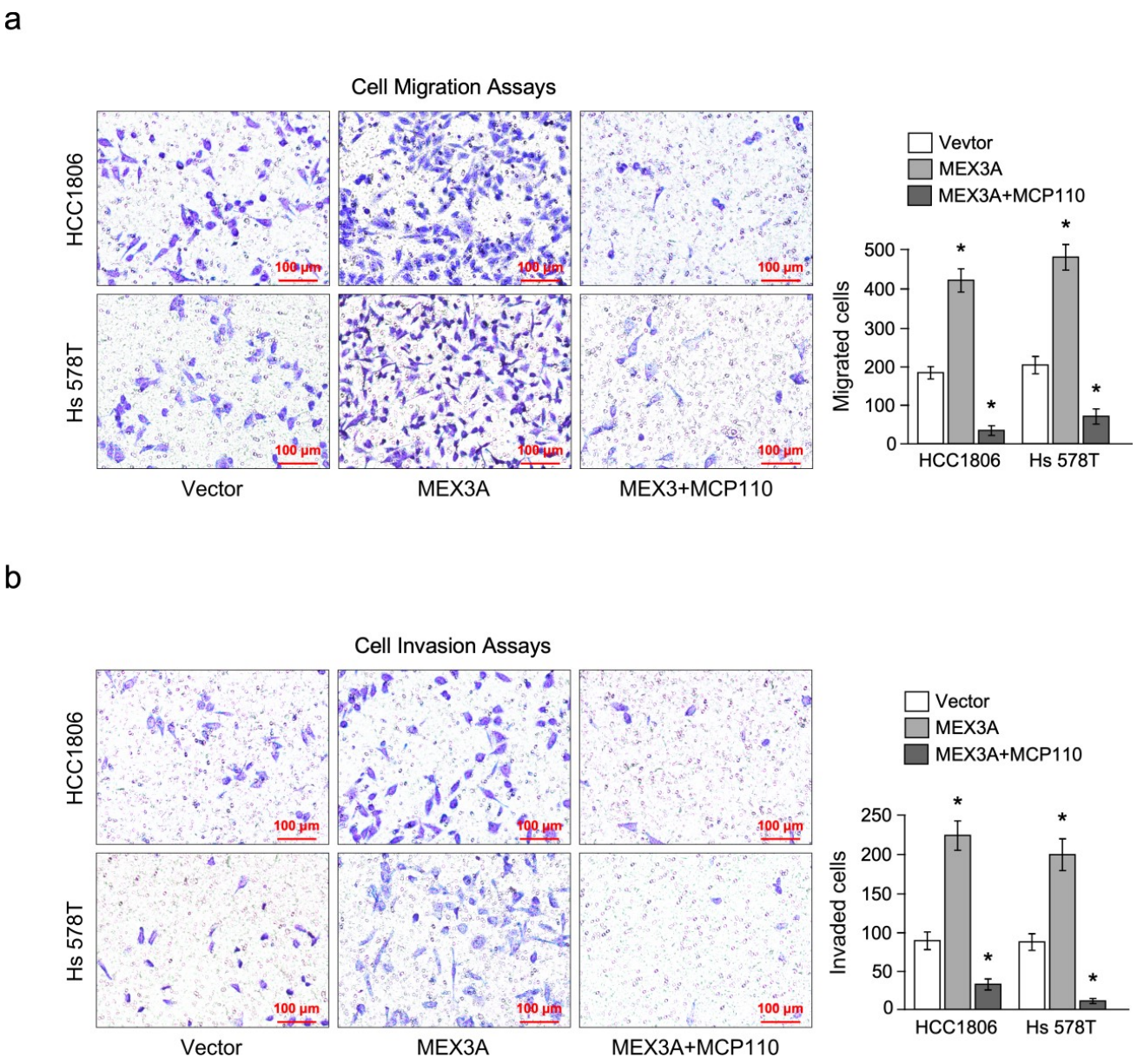

Fig. 7. MEX3A promotes TNBC migration and invasion by activating the Ras pathway. (a) The effect of Ras pathway inhibitor MCP110 on MEX3A-overexpressing TNBC cell migration. Representative micrographs (left) and quantification of migration cells (right). n = 3. (b) The effect of Ras pathway inhibitor MCP110 on MEX3A-overexpressing TNBC cell invasion. Representative micrographs (left) and quantification of migration cells (right). n = 3. Data are expressed as the mean ± SEM; *$p < 0.05$.

strated to regulate AKT and ERK pathway [19]. We used a Ras/Raf-1 interaction inhibitor, MCP110, to treat MEX3A-Overexpressing TNBC cells. The findings revealed that MCP110 treatment significantly reversed the effect of MEX3A overexpression on FOXO transcription activity and ERK activity ($p < 0.05$, Fig. 6d). These outcomes indicated that MEX3A activates Ras, AKT, and ERK pathways through NTRK1. Furthermore, cellular migration and invasion assay showed that inhibiting the Ras pathway in MEX3A-overexpressing TNBC cells suppressed TNBC cell migration and invasion (Fig. 7a,b). This result suggests that MEX3A promotes TNBC cell migration and invasion by stimulating the Ras pathway.

Discussion

In this study, we observed an upregulation of MEX3A level in TNBC, which emerged as an independent prognostic indicator for TNBC patients. Those with elevated MEX3A expression exhibited shorter survival durations compared to individuals with lower MEX3A expression levels. These findings suggest that MEX3A acts as an oncogene in TNBC progression by promoting migration, invasion, and metastasis.

RNA binding proteins (RBPs) have been demonstrated to regulate the development of various tumors [20,21]. In both pancreatic ductal adenocarcinomas and

glioblastomas, the elevated expression of Cytoplasmic Polyadenylation Element Binding Protein 4 (CPEB4) has been associated with increased tumor growth, enhanced vascularization, and augmented invasion capabilities [22]. Additionally, Epithelial Splicing Regulatory Protein 1 (ESRP1) and Epithelial Splicing Regulatory Protein 2 (ESRP2) are upregulated in oral squamous cell carcinogenesis. ESPR1 promotes cell motility by inducing Rac1b to affect the dynamics of the actin cytoskeleton, while ESPR1 promotes cell motility by increasing the expression of epithelial-mesenchymal transition-associated factors [23]. Moreover, increasing evidence demonstrates that RBPs undergo changes in expression and function in tumors. Our findings also show that MEX3A promotes TNBC progression, suggesting the significance of RBPs in tumor progression.

MEX3A has been demonstrated to regulate the progression of various tumors [24–26]. Additionally, MEX3A serves as a marker for $Lgr5^+$ intestinal stem cells characterized by slow proliferation, with $MEX3A^+$ intestinal stem cells generating all intestinal lineages [27]. Moreover, MEX3A is a maker of colon cancer stem cells [28]. However, whether MEX3A regulates breast cancer stem cells remains unknown and needs additional investigations. Recently, several reports have indicated that MEX3A promotes breast cancer progression by regulating PIK3CA, IGFBP4, β-catenin pathway, PI3K/AKT pathway, and RhoA/ROCK1/LIMK1 pathway [14,24,29]. However, the detailed mechanism couldn't be thoroughly studied. In our study, we found that MEX3A directly interacts with NTRK1 to activate multiple pathways that promote TNBC progression. NTRK1 belongs to the tropomyosin receptor kinase (TRK) family. TRK family could induce receptor dimerization and phosphorylation, thereby activating multiple signaling pathways, such as PI3K/AKT and RAS/MAPK/ERK [17]. TRK inhibitors could be potential drugs for TNBC therapy, such as Larotrectinb, Entrectinib, Repotrectinib, LOXO-195, and ONO-5390556 [18].

Conclusion

MEX3A is associated with TNBC progression by interacting with NTRK1, resulting in activation of Ras, AKT, and ERK pathways. Moreover, MEX3A emerged as an independent prognostic factor for patients with TNBC. Its promotion of TNBC migration, invasion, and metastasis suggests its potential as a promising novel target for TNBC therapy.

Availability of Data and Materials

The data used to support the findings of this study are available from the corresponding author upon request.

Author Contributions

FPX designed the experiment, wrote the manuscript. FPX, ZHL, SSL, JX, YG performed the experiment and analyzed the data. XLL, JH, KPZ and DYL performed the experiment and collected the samples. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

For human specimens, all procedures involving human participants were performed in compliance with the relevant ethical standards. Moreover, TNBC tissues were collected from each study participant after getting informed consent. The study was conducted with the approval of the Guangdong Provincial People's Hospital Ethics Committee (No. KY-Z-2021-439-01). All experimental procedures were approved by the Institutional Animal Care and Use committee of Guangdong Provincial People's Hospital and complied with the Guidelines for the Welfare and Use of Animals. All experimental protocols involving animals received approval from the Institutional Animal Care and Use Committee of the Guangdong Provincial People's Hospital and adhered to the Guidelines for the Care and Use of Laboratory Animals (No. KY-2020-198-01-01).

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.23812/j.biol.regul.homeost.agents.20243805.343.

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