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RAS Inhibition Suppresses the Progression and Metastasis of Triple-Negative Breast Cancer

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Abstract

Triple-negative breast cancer (TNBC) is the most lethal subtype of breast cancer and currently lacks effective targeted therapies. Therefore, there is an urgent need to develop new therapeutic strategies for patients with TNBC; the poor prognosis of TNBC patients is linked to the overexpression of epidermal growth factor receptor (EGFR), which is involved in tumor progression, and lysyl oxidase (LOX), which is associated with metastasis. We previously reported that RAS inhibition with small-molecule pan-RAS inhibitors, such as Kobe0065, exerts antitumor and antimetastatic effects on xenografts derived from human cancer cell lines with activating *RAS* mutations via the RAS-RAF-MEK-ERK and RAS-phosphatidylinositol 3-kinase (PI3K)-AKT-hypoxia-inducible factor (HIF)-1α-LOX pathways, respectively. Furthermore, EGFR is located upstream of RAS. However, the role of RAS in breast cancer where *RAS* mutations are not frequently reported, particularly in TNBC with overexpression of EGFR and LOX, has not yet been evaluated. Here, we show that treatment of TNBC with Kobe0065 or a RAS-targeting siRNA inhibits cell proliferation and migration by preventing ERK and AKT phosphorylation and LOX expression. Furthermore, Kobe0065 effectively inhibits not only tumor progression but also bone metastasis in TNBC. Collectively, these findings suggest that RAS is a key molecule involved in TNBC progression and metastasis and that drug therapies targeting RAS may be effective in TNBC patients.

Keywords: Tumor progression; Tumor metastasis; RAS; RAS inhibitors; LOX; EGFR

Abbreviations: TNBC: Triple-Negative Breast Cancer; LOX: Lysyl Oxidase; EGFR: Epidermal Growth Factor Receptor; PI3K: Phosphatidylinositol 3-Kinase; HIF: Hypoxia-Inducible Factor; DMSO: Dimethyl Sulfoxide; FBS: Fetal Bovine Serum; GAPDH: Glyceraldehyde-3-Phosphate Dehydrogenase; siRNA: Small Interfering RNA; qRT–PCR: Quantitative Reverse Transcription–Polymerase Chain Reaction; mpk: mg/kg

Introduction

TNBC is the most aggressive subtype of breast cancer, accounting for approximately 10-20% of all breast cancer cases [1,2]. It is characterized by a lack of estrogen receptor, progesterone receptor and epidermal growth factor receptor 2 expression [2,3]. TNBC is mostly dependent on chemotherapy, while other subtypes can be treated with targeted therapies. In addition, patients with TNBC have a greater incidence of distant metastasis and poorer prognosis than patients with other subtypes [4,5]. Therefore, there is an urgent need to identify novel molecular targets to improve the therapeutic benefit in TNBC patients. One potential therapeutic target for TNBC is EGFR. EGFR, which is involved in tumor progression, is overexpressed in approximately 80% of TNBC patients compared to non-TNBC patients, and overexpression of EGFR has been reported to be associated with a poor prognosis [6,7]. Despite the development of various EGFR inhibitors, such as anti-EGFR monoclonal antibodies (e.g., cetuximab) and EGFR tyrosine kinase inhibitors [8], EGFR inhibitors have performed poorly in clinical trials for TNBC patients [9,10]. LOX is another potential therapeutic target for TNBC. Bone metastasis is a major obstacle to survival for breast cancer patients, with as many as 70% of advanced breast cancers, including TNBC, metastasizing to bone [11,12]. Bone metastasis is regulated by various factors, including LOX, a protein involved in extracellular matrix remodeling and the promotion of cell invasion and metastasis [13]. LOX overexpression has been reported to be strongly associated with bone metastasis in TNBC [14,15] and is considered a crucial factor in breast cancer

metastasis [16]. However, cancer treatment targeting LOX remains a major challenge, and effective therapies have yet to be developed. Mutational activation of *RAS* family genes is observed in approximately 25% of all human cancers. Thus, the RAS is one of the main targets for antitumor drug development [17-20]. We previously showed that RAS plays a crucial role in the progression and metastasis of cancer cells with activated *RAS* by enhancing the RAS-RAF-MEK-ERK and RAS-phosphatidylinositol 3-kinase (PI3K)-AKT-hypoxia-inducible factor (HIF)-1α-LOX signaling pathways, respectively [21]. Furthermore, we showed that pan-RAS inhibitors such as Kobe0065, which prevents the interaction between a broad variety of RAS and RAF proteins, thereby inhibiting downstream signal propagation [22], exhibited antitumor and antimetastatic activity against tumors with activated *RAS* mutations [21,22].

RAS mutations are uncommon in breast cancer and occur in only approximately 5% of cases; thus, RAS signaling is thought to play no significant etiological role in the development of breast cancer [23]. Nevertheless, because EGFR, an upstream molecule of the RAS signaling pathway, and LOX, a downstream molecule, are overexpressed in TNBC patients, we hypothesized that therapies targeting RAS may block the effects of both EGFR and LOX in TNBC. In this study, we aimed to investigate the role of the RAS in TNBC to assess the regulation of EGFR-related tumor progression and LOX-related tumor metastasis by Kobe0065, a RAS signaling blocker. We found that the compound and a RAS-targeting siRNA were effective at suppressing the proliferation and migration of TNBC cells through the inhibition of both the RAS-RAF-MEK-ERK pathway and the RAS-PI3K-AKT-HIF-1α-LOX signaling pathway. In addition, the compound was found to prevent not only tumor progression but also bone metastasis of TNBC in a well-established mouse xenograft model that mimics tumor progression and bone metastasis in humans. These findings suggest that cancer therapies targeting the RAS may be beneficial for TNBC patients.

Methods

Reagents

Kobe0065 was obtained as described previously [22]. For *in vitro* studies, the compound was dissolved in 100% dimethyl sulfoxide (DMSO; Sigma–Aldrich, USA) to prepare a 10 mM solution, which was stored at -20 °C until use. For *in vivo* animal studies, the compound was suspended in different vehicles [HCO-40 (8.75%), Cremophor EL (17.5%), ethanol (8.75%), DMSO (15%), and phosphate-buffered saline (50%)]. Antibodies against phosphorylated ERK1/2 (9101S), total ERK1/2 (9102S), phosphorylated AKT (9271S), total AKT (9272S), and EGFR (4267S) were purchased from Cell Signaling Technology (USA). An antibody against LOX (MAB20452) was purchased from Abnova (Taiwan). An antibody against Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH, sc-47724) was purchased from Santa Cruz Biotechnology (USA). Horseradish peroxidase-conjugated secondary antibodies against rabbit (NA934V) and mouse (NA931V) immunoglobulin G (IgG) were purchased from GE Healthcare (USA).

Cell lines

The human TNBC cell lines BT-20, HCC1937, MDA-MB-157, MDA-MB-231, MDA-MB-468 and Hs578T and the human non-TNBC cell lines T47D and MCF-7 were obtained from the American Type Culture Collection (USA)

and cultured in the media recommended by the supplier. All cell lines used in this study were confirmed to be free of mycoplasma contamination.

Small interfering RNA (siRNA)-mediated gene silencing

Cells were transfected with three sets of Stealth™ siRNAs (against LOX, EGFR, or KRAS at final concentrations of 20 nM) or with a nontargeting control (Invitrogen, USA) by using Lipofectamine RNAiMAX (Invitrogen) according to the manufacturer's instructions. After transfection, the cells were cultured for 24 h at 37 °C and subjected to further experiments. The sequences of the Stealth™ siRNAs used are listed in **Supplementary Table 1**.

Supplementary Table1: The sequences of the Stealth siRNAs.

Name	Species	Primer	Sequence
LOX-1	Human	Sense	AUCAUAAUCUCUGACAUCUGCCCUG
		Antisense	CAGGGCAGAUCUCAGCACUUAUGAU
LOX-2		Sense	UGGGUAAGAAAUCUGAUGUCCCUUG
		Antisense	CAAGGGACAUCAGAUUUCUUACCCA
LOX-3		Sense	GGAUUGAGUCCUGGCUGUUAUGAUA
		Antisense	UAUCAUAACAGCCAGGACUCAAUCC
EGFR-1	Human	Sense	GCAGUCUUAUCUAACUAUGAUGCAA
		Antisense	UUGCAUCAUAGUUAGAUAAGACUGC
EGFR-2		Sense	GCAAAGUGUGUAACGGAAUAGGUAU
		Antisense	AUACCUAUUCCGUUACACACUUUGC
EGFR-3		Sense	GGAGAUAAGUGAUGGAGAUGUGAUA
		Antisense	UAUCACAUCUCCAUCACUUAUCUCC
KRAS-1	Human	Sense	UGUGGACGAAUAUGAUCCAACAAUA
		Antisense	UAUUGUUGGAUCAUAUUCGUCCACA
KRAS-2		Sense	AUAACUUCUUGCUAAGUCCUGAGCC
		Antisense	GGCUCAGGACUUAGCAAGAAGUUAU
KRAS-3		Sense	CAAGACAGAGAGUGGAGGAUGCUUU
		Antisense	AAAGCAUCCUCCACUCUCUGUCUUG

Anchorage-dependent cell proliferation assay

After siRNA treatment, the cells were seeded in 96-well plates (5 × 103 cells per well) and treated with 20 μM Kobe0065 or 0.2% DMSO in 5% fetal bovine serum (FBS)-containing medium for 72 h. Cell viability was determined by the addition of Cell Counting Reagent SF (Nacalai Tesque, Japan) according to the manufacturer's

instructions. Cell counts were normalized against those of control siRNA- and DMSO-treated cells. The experiments were performed three times in quadruplicate.

RNA isolation and quantitative reverse transcription–polymerase chain reaction (qRT–PCR)

Total cellular RNA was isolated from cells treated with siRNA or Kobe0065 using TRIzol (Invitrogen) according to the manufacturer's instructions. qRT–PCR was conducted by using the SYBR Premix Ex Taq II Kit (Takara Bio, Japan) with a Thermal Cycler Dice Real Time System (Takara Bio). Relative mRNA expression levels were determined via the comparative *Ct* method and then normalized to the β-*actin* mRNA expression level. The sequences of the 130 primers used are listed in **Supplementary Table 2**.

Table 2: The primers used for qRT–PCR.

Name	Species	Primer	Sequence
LOX	Human	FWD	CCAGAGGAGAGTGGCT
		REV	CCAGGTAGCTGGGGTT
EGFR	Human	FWD	CAGCGCTACCTTGTCATTCA
		REV	AGCTTTGCAGCCCATTTCTA
KRAS	Human	FWD	TGTGGTAGTTGGAGCTGGTG
		REV	CCCAGTCCTCATGTACTGGTC
β-Actin	Human	FWD	ATGAAGATCAAGATCATTGCTCCTC
		REV	ACATCTGCTGGAAGGTGGACAG

Wound healing migration assay

After siRNA treatment, 5 × 106 cells were seeded into 12-well plates. Once the cells reached confluence, the cells were treated with 20 μM Kobe0065 or 0.2% DMSO in 5% FBS-containing medium, and a “wound” was generated on the cells in each well by scratching with a 200 μL pipette tip. The cells that migrated into the wounded area were observed and imaged under a microscope at 0 h and 48 h after wounding.

Western blot analysis

After treatment with siRNAs or Kobe0065, the cells were solubilized, separated via SDS–PAGE and subjected to western blotting as described previously **[21]**.

Animals

All procedures in this study were conducted in accordance with the "Guidelines for the Care and Use of Laboratory Animals", and all studies were approved by the Institutional Animal Care and Use Committee of Kobe University. Female athymic nude (nu/nu) mice (6-8 weeks old; CLEA Japan) were housed in a temperature (22±1 °C)- and humidity (45-65%)-controlled environment with a 12 h light–dark cycle.

Animal models and drug administration

For the experimental tumor progression assay, MDA-MB-231 cells (5 × 106) were subcutaneously implanted into the right flank of nude mice. After the average tumor volume reached approximately 100 mm^3, the mice were randomly assigned to each group (n=3) and given solvent-suspended Kobe0065 at 40 or 120 mg/kg (mpk) intraperitoneally 5 consecutive days per week for a total of 21 days. The tumor volume (V) was calculated once a week as previously described [22]. After the final dose, the tumor volume was measured, and the tumors were dissected and weighed. For the experimental bone metastasis model, the methods were conducted as described previously [24]. Briefly, MDA-MB-231 cells (1 × 106 cells) suspended in 100 µl of PBS were injected into the caudal artery of nude mice using a syringe with a 29 G needle. The mice were subsequently randomized into groups (n=3) and given solvent-suspended Kobe0065 (120 mpk) intraperitoneally 5 consecutive days per week for a total of 30 days. After the final dose, the mice were sacrificed, and the isolated bone of each hind limb was fixed, decalcified, and embedded in paraffin. The sectioned bones (10 µm thickness) were then subjected to Masson's trichrome staining to histologically visualize collagen connective tissue fibers in the bone sections.

Statistics

The data are presented as the mean ± SEM. The statistical significance of differences among groups of three or more was determined using an unpaired *t test* or one-way ANOVA. Differences were considered statistically significant when the *p* value was less than 0.05. *, $p<0.05$; **, $p<0.01$; and ***, $p<0.005$.

Results

Compared with non-TNBC cells, TNBC cells exhibit increased EGFR and LOX expression

Since it has been reported that EGFR and LOX are overexpressed in TNBC [6,7,15,16], we first validated their expression levels in several non-TNBC and TNBC cell lines. For these experiments, we extracted RNA from human non-TNBC cell lines, such as T47D and MCF-7 cells, and human TNBC cell lines, such as BT-20, HCC1937, MDA-MB-231, MDA-MB-468, MDA-MB-157 and Hs578T cells, and performed qRT–PCR analysis. All TNBC cell lines and four of the six TNBC cell lines (HCC1937, MDA-MB-231, MDA-MB-157 and Hs578T) exhibited higher expression levels of EGFR and LOX than non-TNBC cells **(Figure 1A and B)**.

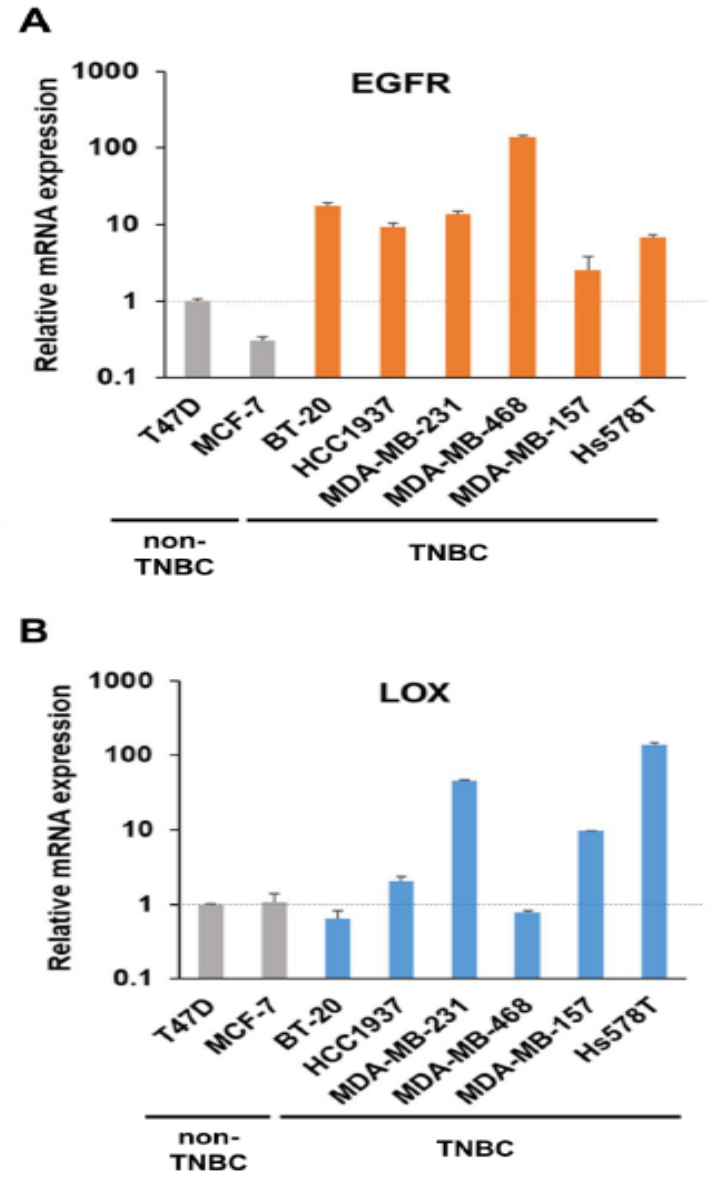

Figure 1: EGFR and LOX expression in TNBC.

Expression of EGFR (A) and LOX (B) mRNA in TNBC cells. Total cellular RNA was prepared, and EGFR and LOX mRNA relative to β−actin mRNA were measured via qRT–PCR. The gray bars, and the orange (A) and blue (B) bars indicate the mRNA expression levels in non-TNBC cells, and TNBC cells, respectively.

RAS regulates TNBC cell proliferation and cell migration

Based on the finding that four TNBC cell lines exhibit increased expression of EGFR and LOX, we used these TNBC cell lines to evaluate the role of RAS in TNBC cell proliferation and cell migration by knockdown with siRNAs or treatment with the pan-RAS inhibitor Kobe0065. We first investigated the role of the RAS in TNBC cell proliferation. Treatment with siRNAs against KRAS or EGFR significantly inhibited the anchorage-dependent proliferation of TNBC cells but not that of non-TNBC cells (p = 0.0018 ~ 0.0048; **Figure 2A**). Treatment with

Kobe0065 also significantly inhibited the proliferation of TNBC cells only (p = 0.0022 ~ 0.025; **Figure 2A**). HCC1937 and MDA-MB-157 cells do not harbor activating *RAS* mutations, suggesting that RAS may play a key role in TNBC cell proliferation, regardless of *RAS* mutational activation **(Figure 2A)**. In contrast, treatment with siRNA against LOX had no significant effect on cell proliferation, suggesting that LOX does not regulate breast cancer cell proliferation **(Figure 2A)**. We next investigated the role of the RAS in TNBC cell migration. Cell migration is a crucial process involved in tumor metastasis. Treatment with siRNA against LOX markedly attenuated the post wound cell migration of both non-TNBC and TNBC cells (p = 0.0022 ~ 0.0194), suggesting that LOX plays a significant role in breast cancer cell migration **(Figure 2B and C)**. Treatment with siRNAs against KRAS and EGFR and treatment with Kobe0065 significantly inhibited the migration of TNBC cells (p = 0.0011 ~ 0.0416) but not non-TNBC cells **(Figure 2B and C)**. This inhibition was also independent of *RAS* mutational activation, as was the inhibition of cell proliferation **(Figure 2B and C)**. Since we have previously demonstrated that the RAS-PI3K-AKT signaling pathway regulates LOX expression **[21]**, these results suggest that RAS and possibly EGFR, which is located upstream of RAS, may regulate cell migration via 199 the induction of LOX expression in TNBC.

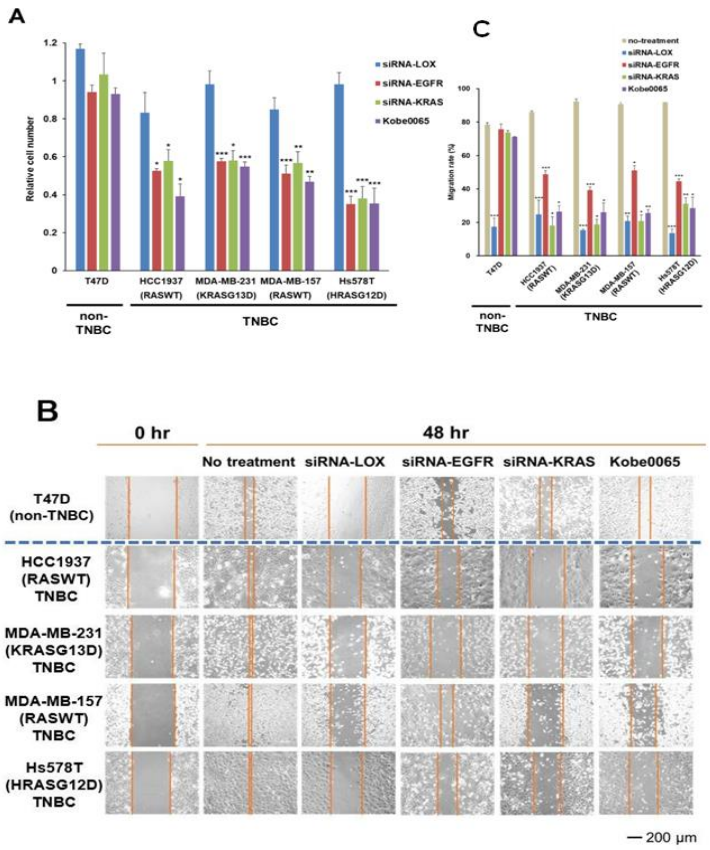

Figure 2: The role of RAS in TNBC cell proliferation and cell migration. A) Role of the RAS in TNBC cell proliferation. The methods used for the detection and quantification of viable cells after the indicated treatment are described in the Materials and Methods. Unpaired t tests were used for statistical analysis: *, $p < 0.05$; **, $p < 0.01$; and ***, $p < 0.005$ compared to the vehicle control. B) Migration images. Representative images from three repeated experiments of cells migrating to the wounded area at 0 and 48 h after wound creation are shown. C) Migration rate. The wound areas at 0 h and 48 h were calculated and recorded as D0 and D48, respectively. The migration rate was calculated as follows: Migration (%) = (D0-D48)/D0 × 100. Unpaired t tests were used for statistical analysis: *, $p < 0.05$; **, $p < 0.01$; and ***, $p < 0.005$ 380 compared to the vehicle control.

RAS regulates the phosphorylation of ERK and AKT and the expression of LOX in TNBC

Given that RAS may play an important role in TNBC cell proliferation and cell migration, we further assessed the expression levels of molecules involved in the RAS signaling pathway in cells treated with siRNAs against LOX, EGFR, or KRAS or in cells treated with Kobe0065. Treatment with siRNAs against EGFR and KRAS, as well as treatment with Kobe0065, decreased ERK and AKT phosphorylation and LOX expression in TNBC cells with or without *RAS* mutational activation **(Figure 3A)**. In contrast, the treatment did not affect any proteins in non-TNBC cells **(Figure 3A)**, suggesting that EGFR-RAS signaling regulates cell proliferation and migration in TNBC via the EGFR-RAS-RAF-MEK-ERK and EGFR-RAS-PI3K-AKT-LOX signaling pathways, regardless of *RAS* mutational

activation **(Figure 3A and B)**. Furthermore, no proteins were affected by siRNA against LOX in any type of breast cancer, indicating that LOX is located downstream of the EGFR-RAS signaling pathway **(Figure 3A and B)**.

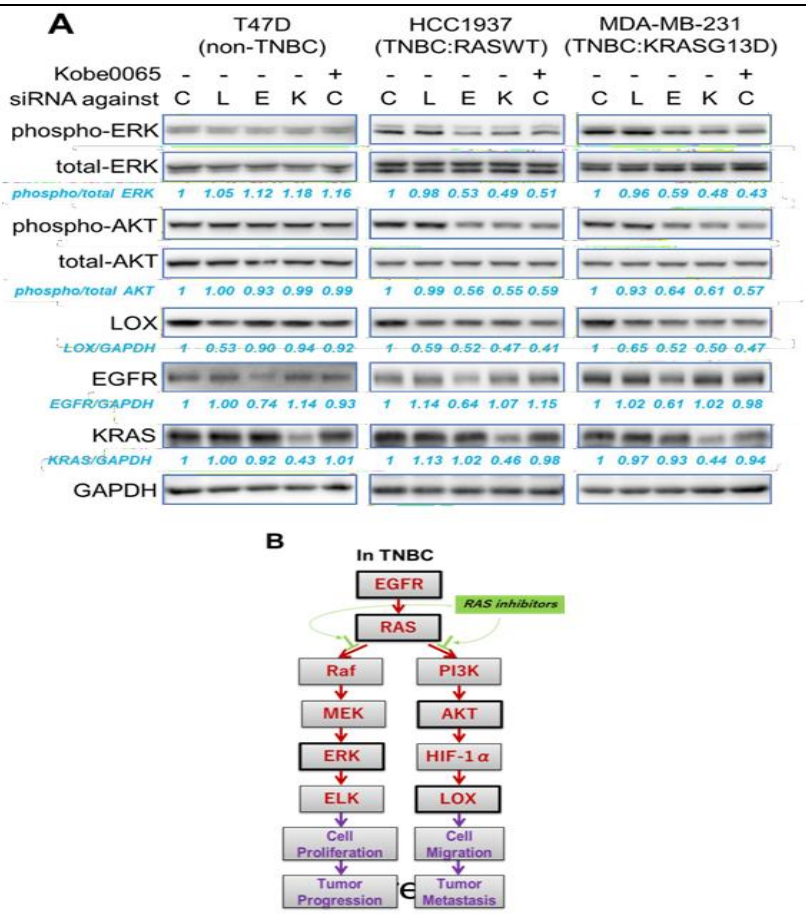

Figure 3: The role of the RAS in regulating protein expression and phosphorylation in TNBC cells. A) After treatment with siRNA or Kobe0065, LOX, EGFR, GAPDH, phosphorylated (phospho) ERK, total ERK, phospho-AKT and total AKT were detected in cell lysates via western blotting with the appropriate antibodies. GAPDH was used as an internal control; the values of LOX/GAPDH, EGFR/GAPDH, phospho/total ERK, and phospho/total AKT relative to those in vehicle-treated cells were calculated using pixel counts. The results shown are representative of three independent experiments. B) Scheme of the RAS signaling pathway regulating tumor progression and metastasis.

Kobe0065 prevents TNBC tumor progression and tumor metastasis in xenograft models

The results described above prompted us to evaluate the antitumor and antimetastatic activity of Kobe0065 in a mouse experimental model of TNBC tumor progression and bone metastasis. First, we evaluated the antitumor activity of Kobe0065 in a mouse xenograft model in which TNBC MDA-MB-231 cells with an activating *KRAS*G13D mutation were subcutaneously injected into the right flank of nude mice. Kobe0065 (40 or 120 mpk) was administered for five consecutive days per week for 21 days and was well tolerated; this treatment resulted in

dose-dependent inhibition of tumor growth **(Figure 4A-C)**. Furthermore, dose-dependent inhibition of ERK phosphorylation was observed in tumors at 2 h after compound administration **(Figure 4D)**. Finally, we evaluated the antimetastatic activity of Kobe0065 in a well-established bone metastasis model in which TNBC MDA-MB-231 cells were injected into the caudal artery of nude mice **[24]**. Breast cancer tends to metastasize to the bone, causing osteolysis **[25]**. Thirty days after caudal arterial inoculation of TNBC cells, the tumors metastasized to the femoral bone region and exhibited aberrant bone formation compared with that in control mice (**Figure 4E**, left and middle images); the proportion of osteoclasts, multinucleated giant cells responsible for bone destruction, was greater in the femurs of mice injected with TNBC cells than in those of control mice (**Figure 4E**, upper enlarged image). Kobe0065 treatment (120 mpk) for five consecutive days per week for 30 days dramatically reduced the tumor size and inhibited tumor metastasis into the femoral bone region (**Figure 4E**, right image). Taken together, these findings suggest that Kobe0065 prevents tumor progression and tumor metastasis to bone in TNBC.

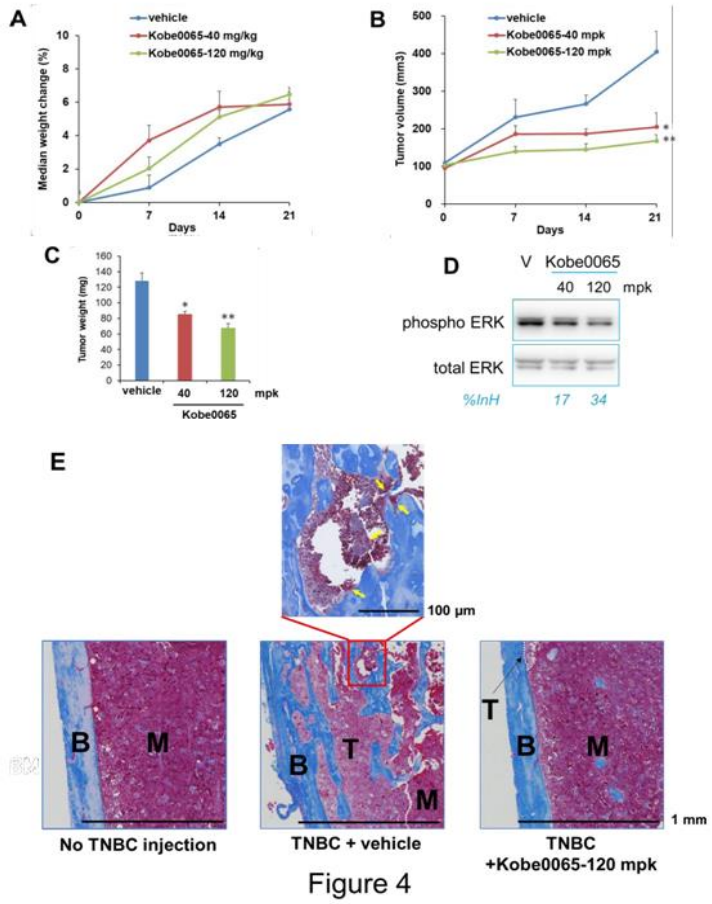

Figure 4

Figure 4: Kobe0065 inhibits TNBC progression and bone metastasis. **A)** Median changes in the body weight of nude mice bearing subcutaneous MDA-MB-231 xenografts were calculated as described in the *Materials and Methods*. **B)** Antitumor effects of Kobe0065 in an MDA-MB-231 xenograft model. Tumor volume was monitored continuously for 21 days. An unpaired *t* test was used for statistical analysis: *, $p < 0.05$ and **, $p < 0.01$ compared

to the vehicle control. **C)** Tumor weights after the final dose of Kobe0065 or vehicle administration. One-way ANOVA was used for statistical analysis: *, $p < 0.05$ and **, $p < 0.01$ compared to the vehicle control. **D)** Kobe0065-mediated inhibition of ERK phosphorylation in MDA-MB-231 xenograft tumors. After intraperitoneal administration of Kobe0065 to mice bearing xenograft tumors for 2 h, the tumors were surgically removed and dissected to prepare the tumor extracts. The expression of phospho- and total ERK in the tumors was detected by western blotting with the appropriate antibodies. The inhibition values of phospho/total ERK relative to those in vehicle-treated tumors were calculated using pixel counts. The results shown are representative of three independent experiments. **E)** Antimetastatic effects of Kobe0065 in an MDA-MB-231 xenograft bone metastasis model. The experimental procedure is described in the *Materials and Methods*. Images were obtained 30 days after intra-arterial inoculation of vehicle (left panel) or MDA-MB-231 cells (middle and right panels). The right image shows a bone section from an MDA-MB-231 tumor-bearing mouse that received 120 mpk of Kobe0065 for 30 days. The upper magnified image shows representative osteoclasts in a bone section (yellow arrow). B: bone, M: marrow, T: tumor.

Discussion

Translational research in TNBC has been limited by the absence of effective anticancer drug targets for clinical therapy. Nevertheless, the observed overexpression of EGFR and LOX, which are factors involved in RAS signaling, supports the hypothesis that targeting the RAS could be a promising therapeutic strategy for TNBC. The results of this study demonstrated that RAS regulates the proliferation and migration of TNBC cells through the EGFR-RAS-MEK-ERK pathway and RAS-PI3K-AKT-LOX pathway, respectively. In addition, TNBC treatment with RAS inhibitors such as Kobe0065 [22], effectively inhibited both the proliferation and migration of TNBC cells. Furthermore, in a well-established mouse model, the compound successfully and effectively suppressed TNBC tumor progression and bone metastasis. Although *RAS* mutations are reportedly rare in breast cancer, this study suggested that targeting RAS was effective in the treatment of TNBC patients with high expression of EGFR and LOX. This may be due to the upregulation of the RAS by EGFR. In fact, Kobe0065 was able to efficiently inhibit the proliferation and migration of TNBC cells, suggesting that anticancer agents targeting the RAS may be potent therapeutic agents for TNBC. However, the compound failed to completely inhibit the ERK and AKT phosphorylation and LOX expression associated with cell proliferation and migration in TNBC. Therefore, RAS inhibitors may be more effective as therapeutic agents in combination with other agents, such as anti-EGFR inhibitors, than as single agents. In addition, it will be important to examine the efficacy of novel pan-RAS inhibitors that are currently in clinical trials and are more potent than Kobe0065 [26]. This study also demonstrated that LOX is an important molecule for cell migration, regardless of breast cancer subtype; unlike in TNBC, treatment of non-TNBC cells with Kobe0065, or siRNAs targeting EGFR and RAS did not affect LOX expression, AKT phosphorylation, or cell migration. Since LOX expression via AKT phosphorylation has been shown to be regulated by several signaling pathways in addition to RAS signaling [27], LOX expression in non-TNBC may be less dependent on RAS signaling due to low EGFR expression.

Our working hypothesis is that in TNBC with elevated expression of EGFR and LOX, RAS functions as a critical molecule in tumor progression and metastasis, regardless of the presence of *RAS* mutations. Based on this

hypothesis, we investigated the potential of the RAS inhibitor Kobe0065 to prevent tumor progression and metastasis in animal models using MDA-MB-231 cells with an activated *KRAS*G13D mutation. We also anticipate that the compound would have similar inhibitory effects on TNBCs without *RAS* mutations but with high expression of EGFR and LOX. We propose that therapeutic strategies targeting the RAS may be effective in treating patients with TNBC. However, it is essential to confirm the expression levels of EGFR and LOX in TNBC before administering RAS inhibitors. Otherwise, the compounds may cause cytotoxicity. Since there are currently no effective treatment options for TNBC, the novel molecules proposed in this study may prove useful in the development of future therapeutic strategies for patients with TNBC.

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