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Ketogenesis promotes triple-negative breast cancer metastasis via calpastatin β-hydroxybutyrylation

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Abstract

Triple-negative breast cancer (TNBC) continues to pose a significant obstacle in the field of oncology. Dysregulation of lipid metabolism, notably upregulated ketogenesis, has emerged as a hallmark of TNBC, yet its role in metastasis has been elusive. Here, by utilizing clinical specimens and experimental models, the study demonstrates that increased ketogenesis fosters TNBC metastasis by promoting the up-regulation of β-hydroxybutyrate (β-OHB), a key ketone body. Mechanistically, β-OHB facilitates β-hydroxybutyrylation (Kbhb) of Calpastatin (CAST), an endogenous calpain (CAPN) inhibitor, at K43, blocking the interaction with CAPN and subsequently promoting FAK phosphorylation and epithelial–mesenchymal transition (EMT). In conclusion, the study reveals a novel regulatory axis linking ketogenesis to TNBC metastasis, shedding light on the intricate interplay between metabolic reprogramming and tumor progression.

Keywords Breast cancer, Metastasis, Ketogenesis, β-hydroxybutyrylation, Epithelial–mesenchymal transition

Introduction

Breast cancer is leathal malignant tumor affecting women [1]. Despite notable advancements in localized tumor treatments, metastatic breast cancer continues to pose a significant therapeutic challenge [2, 3]. Among all the molecular subtypes, triple-negative breast cancer (TNBC) stands out with the poorest prognosis and is often associated with a high propensity for metastasis [4, 5].

Dysregulation of lipid metabolism has emerged as a fundamental hallmark of TNBC [6, 7]. TNBC is associated with increased oxidative phosphorylation [7–9] and ketogenesis [10–12], Ketogenesis is a metabolic pathway primarily involved in the production of ketone bodies. Beyond its traditional role in energy metabolism during states of low carbohydrate availability, ketogenesis has an emerging role in cancer biology [13]. Recent studies have highlighted its importance in cancer cell proliferation,

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survival, metastasis, and shaping of the tumor microenvironment. Tumor cells, particularly under nutrient-deprived conditions, can exploit ketogenesis to meet their metabolic demands, thereby promoting sustained proliferation and survival. For instance, in pancreatic ductal adenocarcinoma, ketogenesis fuels tumor proliferation and survival by supplying ample carbon sources via the tricarboxylic acid cycle (TCA), supporting tumor invasion and metastasis [14, 15]. Moreover, ketogenesis has been implicated in the aberrant activation of certain oncogenes [16] and resistance to endocrine therapies [10]. Interestingly, recent research also suggests a tumor-suppressive role for ketogenesis in colorectal and liver cancers [17–19]. These contradictory results highlight the necessity for more detailed and thorough investigations into the role of ketogenesis in cancer.

3-Hydroxy-3-methylglutaryl-CoA synthase 2 (HMGCS2) and 3-Hydroxybutyrate dehydrogenase 1 (BDH1) play crucial roles as enzymes in the ketogenesis pathway. HMGCS2 catalyzes the rate-limiting step of ketogenesis, converting acetyl-CoA into HMG-CoA, a precursor of ketone bodies [13]. It has been associated with various cancers, and studies suggest that its upregulation may enhance tumor growth and metastasis by promoting ketone body production and meeting the energy needs of the tumor [14, 15]. On the other hand, BDH1, which converts acetoacetate into the most abundant ketone body, β-hydroxybutyrate (β-OHB), has been shown to regulate histone modifications involved in cellular gene regulation [20]. However, the biological role of ketogenesis and these enzymes in metastatic breast cancer remains to be elucidated.

Here, the study revealed that upregulated ketogenesis promotes TNBC metastasis. Mechanistically, upregulated ketogenesis prompts the up-regulation of the ketone body β-hydroxybutyrate, thereby facilitating β-hydroxybutyrylation (Kbhb) of calpastatin (CAST) at K43, subsequently activating calpain (CPAN) to promote FAK phosphorylation and epithelial–mesenchymal transition (EMT). This study not only expands the significance of metabolic reprogramming in the biology of tumor metastasis but also reveals the crosstalk between the ketogenic pathway and the CAST–CAPN system, providing a paradigm for metabolite-mediated regulation of enzyme activity.

Method

Cell culture

MDA-MB-231 cell line was purchased from American Type Culture Collection (ATCC). Cells preserved in cryopreservation solution was thawed by warming them in a 37 °C water bath. The cells were then resuscitated and cultured in complete growth medium. The base medium used is Leibovitz's L-15 medium (ATCC, Maryland, USA). Fetal bovine serum (FBS; Gibco, California, USA) and penicillin-streptomycin (PS; NCM Biotech, Shanghai, PRC) are diluted into the base medium at ratios of 1:9 and 1:99, respectively. Cells were cultured at 37 °C in an environment supplemented with 5% carbon dioxide (CO_2). Cells were passaged when they reached approximately 80% confluence during the logarithmic growth phase.

Clinical specimens

The ethical statement can be found in the Declaration section. Forty patients were enrolled in the study, with their clinical characteristics summarized in Supplementary Table 1. Informed consent was obtained for the use of human subjects in the research. Paraffin-embedded clinical samples were sourced from the Pathology Department. For tissue immunofluorescence staining, samples were progressively rehydrated through xylene, absolute ethanol, graded ethanol solutions, double-distilled water, and PBS. First, a retrieval solution containing 0.01 M citrate was prepared and the pH was adjusted to 6.0. Then the prepared solution was heated to 100 °C, maintaining a gentle boil. After heating the tissue sections in the retrieval solution for 20 min, endogenous peroxidase activity was blocked using a peroxidase inhibitor, followed by antigen blocking with goat serum. The primary antibody was carefully applied to the tissue sections and they were incubated overnight at 4 °C. After incubation, the slides were allowed to equilibrate at room temperature for 30 min. The sections was washed on a shaker using PBS buffer for three cycles of 5 min each. Following the washes, the slides was incubated with the secondary antibody and DAPI staining solution at room temperature for 2 h. Another set of three 5-min washes with PBS buffer was performed on the shaker. The sections was mounted with an anti-fade reagent and immediately observed using an upright fluorescence microscope (Leica, Wetzlar, Germany).

siRNA and plasmid transfection

Cells in the logarithmic growth phase were digested with trypsin and resuspended to create a cell suspension. The cell suspension is then seeded into a 6-well plate (Nest, Jiangsu, PRC) and mixed thoroughly. They were incubated for 24 h to fully adhere. Following the manufacturer's instructions, siRNA (2 μg) and Xfect RNA Transfection Polymer or Xfect™ Transfection Reagent (10 μL; Takara, Kyoto, JPN) were gently mixed in an Eppendorf tube. Subsequently, the mixture was incubated in the dark at 18–20 °C for 20 min, after which it was added to the 6-well plate containing the seeded cells and cultured for 48 h. The necessary siRNAs and plasmids were synthesized by Shanghai Sangon Biotech Co. Ltd. (Sangon,

Shanghai, PRC), with sequence details available in Supplementary Table 2.

To create a stable OE-HMGCS2 cell line, lentiviruses containing HMGCS2 cDNA were synthesized and packaged by GeneChem Biotechnology Co., Ltd. (GeneChem, Shanghai, China). The detailed workflow can be seen in [17]. The packaged lentiviruses were transported to the laboratory using dry ice. The lentivirus was added to the target cells in the presence of polybrene, followed by a 24-h incubation. The used multiplicity of infection (MOI) was 10. Selection was performed using 2.5 μg/ml puromycin (NCM, Shanghai, China).

Protein extraction

For protein extraction, RIPA lysis buffer were added to the cells, and sonicated on ice for 15 s and sitten for 5 min. This process was repeated for a total of three cycles. Phenylmethanesulfonyl fluoride (PMSF; Fude, Zhejiang, PRC) was added to the RIPA lysis buffer at a concentration of 1:100. After extraction, the protein concentration was measured using a BCA assay kit (Glpbio, California, USA), and the samples with loading buffer (Fude, Zhejiang, PRC) were heated at 100 °C for 20 min.

Immunoprecipitation

The anti-Flag antibody was diluted to 1:1000, and the agarose gel was exposed to the prepared working solution before incubation with protein samples overnight at 4 °C. The subsequent steps involved heating the gel-precipitated samples in loading buffer at 95 °C for 5 min.

Immunoblotting

Prepared protein samples were separated on 12.5% polyacrylamide gels (Yamei, Shanghai, PRC) and transferred onto methanol-activated polyvinylidene fluoride (PVDF) membranes (Millipore, Massachusetts, USA). The membrane was placed, after transfer, in silk milk and incubated on a shaker at room temperature for 2 h. After washing off the silk milk with TBST, it was incubated with the primary antibody at 4 °C. After washing off the primary antibody with TBST, the membrane was incubated with the secondary antibody for 2 h, followed by washing with TBST. The horseradish peroxidase signal intensity was detected using the Omni-ECL™ Enhanced Pico Light Chemiluminescence Kit (Yamei, Shanghai, PRC). The specific antibodies and concentrations are shown in Supplementary Table 3.

Xenograft model

The ethical statement can be found in the Declaration section. BALB/c-nude mice (Vital River, Wenzhou, PRC) received subcutaneous injections of MDA-MB-231 cells to establish a spontaneous metastasis model ($n=6$, including 3 male and 3 female mice). At the study endpoint, the lungs were harvested for primary cell extraction and imaging.

For primary lung metastasis MDA-MB-231 cell extraction, isolation of lung metastatic foci were processed through collagenase/hyaluronidase (Sigma, New Jersey, USA) digestion, followed by resuspension and filtration steps.

Statistical analysis

Statistical analyses were conducted by GraphPad Prism 8.0.2 (GraphPad, California, USA), with the data expressed as means ± standard deviations. Student's t-test was used for pairwise comparisons, while Dunnett's test following one-way ANOVA was applied for multiple group comparisons. (*: $P<0.05$, **: $P<0.01$; ***: $P<0.001$, n.s.: no significance).

Results

Expression of ketogenic genes are significantly correlated with lymph node metastasis

To clarify the specific role of ketogenesis in TNBC metastasis, the expression levels of key enzymes in ketogenesis, such as HMGCS2 and BDH1, were evaluated (Fig. 1A) in a clinical cohort of 40 patients (Supplementary Table 1). It was found that the expression of HMGCS2 and BDH1 varies significantly based on the presence of lymph node (Fig. 1B) and distant metastasis (Fig. 1C), rather than tumor grade (Fig. 1D). More importantly, in patients in the top 25% of HMGCS2 expression, focal adhesions and integrins were significantly greater than those in patients in the bottom 25% of HMGCS2 expression (Fig. 1E-F), suggesting that tumor cells with high HMGCS2 expression are in a state of high metastatic ability.

Furthermore, utilizing a nude mouse model of lung metastasis induced by MDA-MB-231 mammary fat pad injection, spontaneous pulmonary metastases were developed. And primary MDA-MB-231 cells from both primary tumors (pMDA-MB-231) and lung metastatic lesions (mMDA-MB-231) were isolated (Fig. 2A). Transwell assays demonstrated that, compared with primary tumors, TNBC cells in lung metastatic lesions presented increased migration and invasion abilities (Fig. 2B-D). Crucially, these cells presented elevated levels of HMGCS2 and BDH1 expression (Fig. 2E-F) and increasing of β-hydroxybutyrate (β-OHB) and acetoacetate (AcAc) (Fig. 2G). These findings suggest a significant association between the expression of ketogenic genes and lymph node and distant metastasis. However, the underlying mechanism remains elusive.

β-OHB facilitates TNBC metastasis

To explore this phenomenon, silencing of HMGCS2 and BDH1/2 were employed in the MDA-MB-231 cell line (Fig. 3A). Transwell assays revealed that

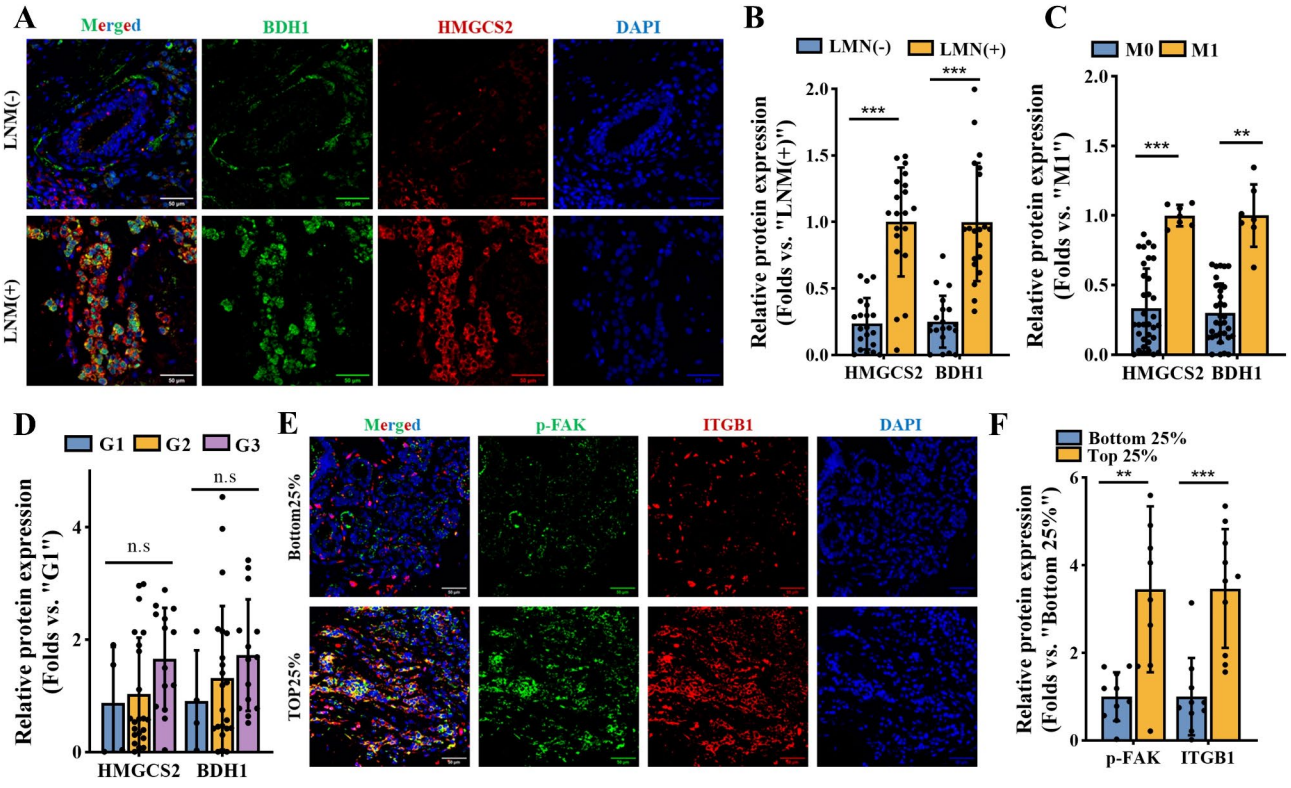

Fig. 1 The expression of ketogenic genes significantly correlates with lymph node metastasis. The expression profiles of HMGCS2 and BDH1 were detected in patients' primary tumor specimens (**A**), and the correlations with lymph node metastasis (**B**), distant metastasis (**C**) and tumor grade (**D**) were analyzed. P-FAK and ITGB expression levels were detected in the 25% of patients with the highest HMGCS2 expression and the 25% with the lowest expression ($n = 10$) (**E**, **F**). The data are presented as the means ± SDs, and each scatter plot point represents an independent replicate

silencing ketogenic-related genes effectively dampened the invasion and migration abilities of the cells (Fig. 3B-D), whereas overexpressing HMGCS2 (Fig. 3A) increased their invasion and migration capabilities (Fig. 3E-G).

Metabolites serve not only as substrates in metabolic reactions but also regulators of biological processes [21, 22]. Therefore, in cell lines where both HMGCS2 and BDH1 were silenced to block actyl-acetone (AcAc) - β-OHB transformation, supplementation with β-OHB or AcAc was performed. The results revealed that β-OHB, rather than AcAc, facilitated the invasion and metastasis of TNBC (Fig. 3H-J). Therefore, the promotion of invasion and migration via the ketogenic pathway in TNBC appears to be independent of specific gene expression and does not involve the ketogenesis-independent biological functions of HMGCS2 and BDH1/2. Therefore, enrichment of the ketogenic product β-OHB predominantly underpins the enhanced metastatic capacity of TNBC.

β-OHB stimulates β-hydroxybutyrylation of CAST at K43

β-OHB, a classic histone deacetylase inhibitor, prompted us to initially investigate whether metastasis-related histone acetylation sites play a role in β-OHB-induced TNBC metastasis [23–25]. Interestingly, H3K27ac or H3K9ac expression showed no significant difference between TNBC cell lines derived from primary tumors and those derived from lung metastases (Fig. 4A).

Lysine β-hydroxybutyrylation (Kbhb), a recently discovered posttranslational protein modification [26], is responsive to upregulated β-OHB and regulates various biological functions in pathological processes [27]. Evaluation of pan-Kbhb modification in pMDA-MB-231 and mMDA-MB-231 using a pan-Kbhb antibody revealed a significant upregulation of pan-Kbhb modification in lung metastases (Fig. 4A-B). Consequently, a mass shift of 86.05 Da at Calpastatin (CAST) K43 was observed (Fig. 4C). To further validate whether CAST undergoes Kbhb modification, the CAST mutant K43R was generated and transfected into MDA-MB-231 cells supplemented with β-OHB. The results showed that K43R blocked CAST Kbhb modification and interaction with Kbhb-modified enzymes (Fig. 4D-E).

Previous reports have indicated that CAST is significantly associated with lymph node metastasis in breast cancer [28]. Further elucidation of whether CAST Kbhb modification is involved in β-OHB-induced metastasis led to the generation of MDA-MB-231 CAST-KO cell lines using the CRISPR/CAS system (Fig. 4F). Deletion of CAST significantly increased migration and invasion

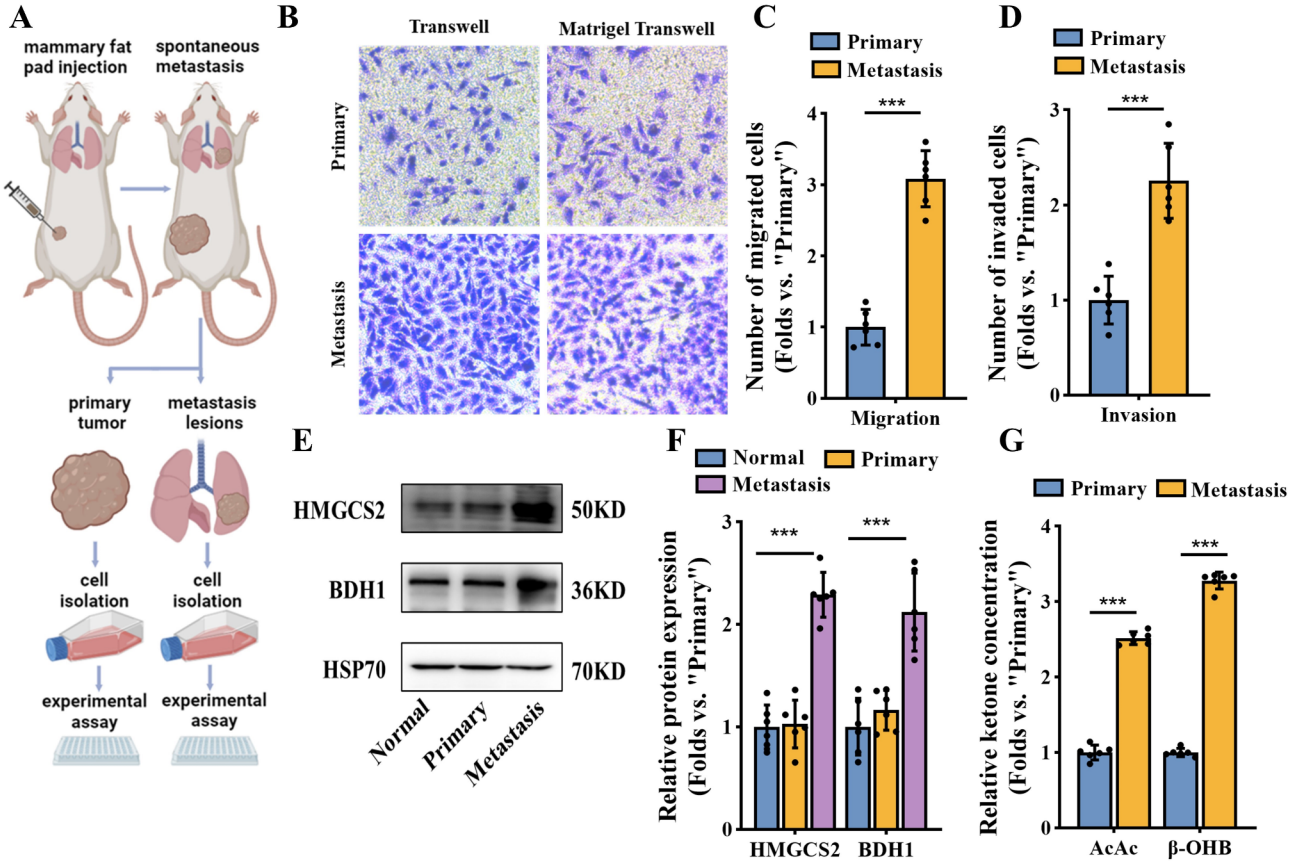

Fig. 2 MB-MDA-231 cell line with high metastatic potential is extracted. Tumor cells were extracted from mouse primary tumors ("Primary"), metastatic lesions ("Metastasis") and HMEC cell line ("Normal") (**A**). Transwell and Matrigel transwell experiments were performed (**B**-**D**). WB detection was performed on "Primary" and "Metastasis" samples (**E**-**F**), and HSP70 was used as a loading control. Assays were adopted to detect the ketone body content (**G**). The data are presented as the means ± SDs, and each scatter plot point represents an independent replicate

capability (Fig. 4G-I) and abrogated the regulatory effects of β-OHB on migration and invasion (Fig. 4G-I). Importantly, wild-type CAST, but not CAST K43R, rescued the regulatory effects of β-OHB on CAST-KO cell lines (Fig. 4G-I). More importantly, the silencing of CAPN, which is the main target of CAST, negated the regulatory effects of K43 Kbhb on cell migration and invasion (Fig. 4G-I). These findings suggest that CAST K43 Kbhb modification plays an important role in β-OHB-mediated regulation of migration and invasion.

CAST β-hydroxybutyrylation activates CAPN to promote FAK phosphorylation and EMT

CAST, a classic endogenous inhibitor of CAPN [29, 30], prompted us to examine whether CAST K43 Kbhb inhibited the interaction between CAST and CAPN. The results demonstrated that β-OHB significantly blocked the colocalization (Fig. 5A) and interaction (Fig. 5B) between wild-type CAST and CAPN but not mutant CAST. Additionally, elevated CAST K43 Kbhb increased phosphorylation of FAK (Fig. 5C, S1A) and promoted the expression of the mesenchymal marker vimentin (Fig. 5C, S1A) as well as extracellular matrix proteases MMP2 and MMP9 (Fig. 5C, S1A).

In addition, stable cell lines expressing wild-type and mutant CAST were generated from both primary- and metastasis-isolated MDA-MB-231 cell suspension were injected into the mammary fat pads. Overexpression of HMGCS2 in the wild-type CAST pMDA-MB-231 strongly mimicked the biological phenotype of mMDA-MB-231 and promoted tumor growth and metastasis (Fig. 5D-G). The CAST K43R mutant did not affect the growth of either pMDA-MB-231 or mMDA-MB-231 cells (Fig. 5E, H), nor did it affect the number or area of lung metastases induced by pMDA-MB-231 cells (Fig. 5D-G). However, K43R markedly decreased both the number and size of lung metastases caused by mMDA-MB-231 cells. (Fig. 5D, I, J).

Finally, utilizing the clinical samples, it was found that in patients whose HMGCS2 expression levels were in the bottom 25%, the interaction between CAST and CAPN was significantly greater than that in patients whose HMGCS2 expression levels were in the top 25% (Figure S1B). Taken together, these data indicate that CAST

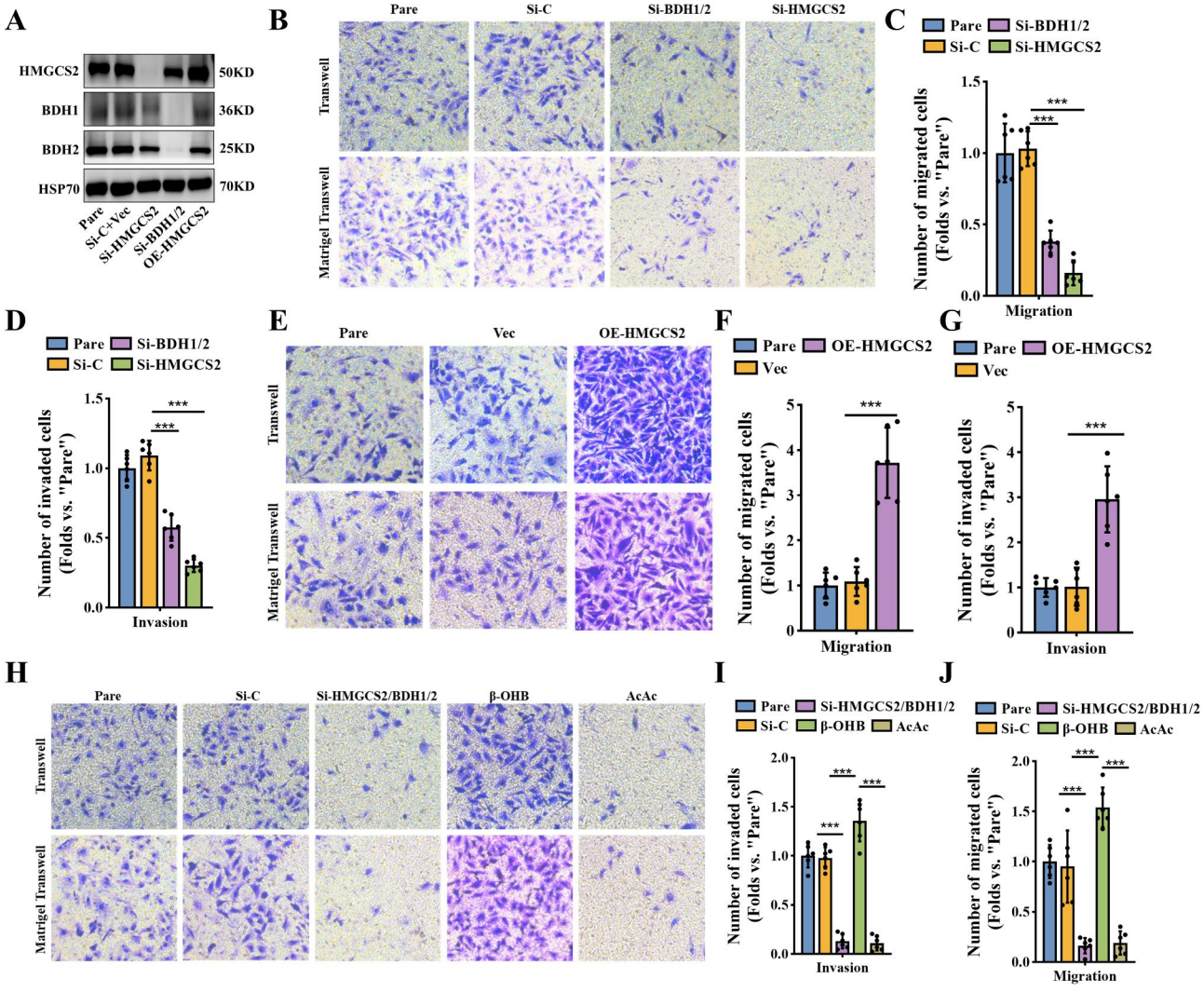

Fig. 3 β-OHB facilitates TNBC metastasis. The cultured MDA-MB-231 cell line ("Pare") was transfected with HMGCS2 siRNA ("Si-HMGCS2"), BDH1/2 siRNA ("Si-BDH1/2"), or HMGCS2 overexpression plasmid ("OE- HMGMCS2") or nonsense siRNA and overexpression plasmid vector ("Si-C + Vec"). WB was used to detect the silencing and overexpression efficiency (**A**), and HSP70 was used as a loading control. Metastasis and invasion abilities were detected in the "Pare", "Si-C", "Si-BDH1/2" and "Si-HMGCS2" groups by transwell (**B**, **C**) and Matrigel transwell (**B**, **D**) assays, respectively. The metastasis (**E**, **F**) and invasion (**E**, **G**) abilities of "Pare", "Vec" and "OE-HMGCS2" cells were detected. HMGCS2, BDH1, and BDH2 siRNAs were simultaneously transfected into cultured MDA-MB-231 cells, which were then supplemented with PBS ("Si-HMGCS2/BDH1/2"), β-OHB ("β-OHB") or AcAc ("AcAc"). Metastasis (**H**, **I**) and invasion (**H**, **J**) abilities were detected for the "Pare", "Si-C", "Si-HMGCS2/BDH1/2", "β-OHB" and "AcAc" groups. All the experiments presented here were repeated 6 times. The data are presented as the means ± SDs, and each scatter plot point represents an independent replicate

K43 Kbhb promotes the development and progression of TNBC lung metastasis by regulating the interaction between CAST and CAPN.

Discussion

Metastasis is the major cause of mortality in patients with TNBC. Through a combination of in vivo and in vitro models and clinical specimens, the study demonstrated that increased ketogenesis serves as a risk factor for TNBC metastasis. Mechanistically, upregulated ketogenesis promotes TNBC metastasis by enhancing the biosynthesis of endogenous β-OHB, which in turn promotes β-hydroxybutyrylation of CAST at the K43 site, thereby attenuating the inhibitory effect of CAST on CAPN. The subsequent upregulation of CAPN then promotes TNBC invasion and metastasis by increasing FAK phosphorylation and EMT (Figure S2).

The study broadens the scope of metabolic reprogramming in tumor metastasis biology. Previous studies have confirmed that TNBC promotes lymphatic and distant metastasis by inducing metabolic reprogramming [31–33]; the study introduces the novel concept that increased ketogenesis induces TNBC invasion and metastasis. Traditionally, lipid metabolism has been widely considered the switch for tumor metastasis [34, 35]. The study revealed that both endogenous and exogenous

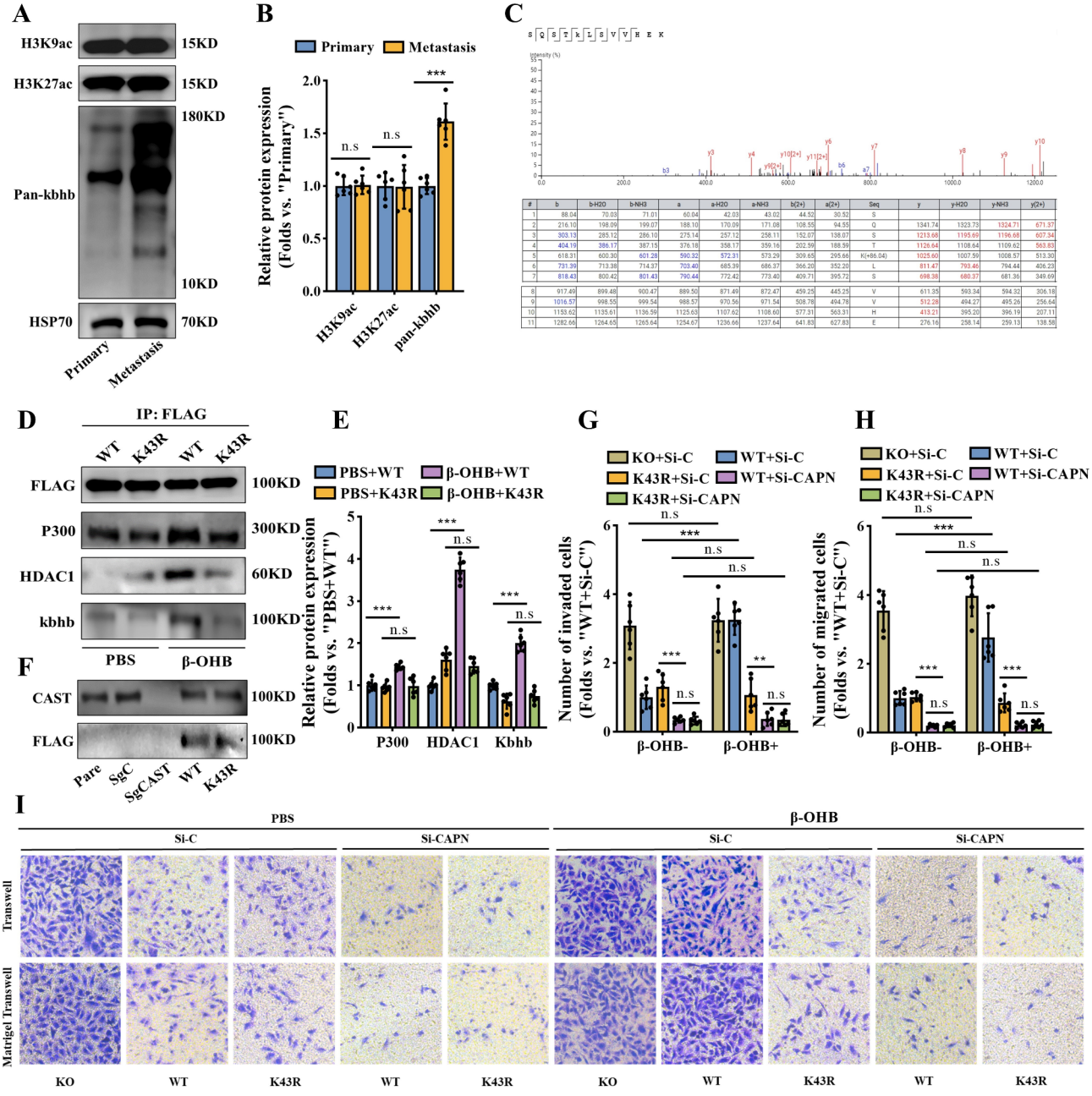

Fig. 4 β-OHB stimulates β-hydroxybutyrylation of CAPN at K43. WB was used to detect the expression levels of H3K9ac, H3K27ac and overall Kbhb modification in the "Primary" and "Metastasis" groups (**A**, **B**), and HSP70 was used as a loading control. Wild-type CAST ("WT") and CASTK43R ("K43R") were transfected into the MDA-MB-231 cell line. Detection of Kbhb modifications in the "Metastasis" group (**C**). A FLAG antibody was used for pull-down, and immunoblotting was used to detect P300, HDAC1 and Kbhb expression levels (**D**, **E**). Based on the MDA-MB-231 cell line ("Pare"), stable expression cell lines carrying the lentiviral CRISPR/Cas9 control construct ("SgC") or the CRISPR/Cas9-CAST knockout construct ("SgCAST") were constructed, after which wild-type CAST ("WT") or CASTK43R ("K43R") was transfected into "SgCAST" cells. WB was used to detect the expression of CAST and FLAG (**F**). A representative image of CAST at K43 Kbhb. Nonsense control siRNA ("WT + Si-C" or "K43R + Si-C") or CAPN siRNA ("WT + Si-CAPN" or "K43R + Si-CAPN") in "WT" and "K43R") were transfected into "SgCAST" cells to detect metastasis (**I**, **G**) and invasion (**I**, **H**) abilities. All the experiments presented here were repeated 6 times. The data are presented as the means ± SDs, and each scatter plot point represents an independent replicate

supplementation with ketone bodies promoted the invasion and metastasis capabilities of MDA-MB-231 cells in vitro, suggesting that increased ketogenesis can serve as a target for inhibiting tumor metastasis. This study thus provides a theoretical foundation for developing novel metabolism-targeted therapies for TNBC.

Moreover, the study revealed the crosstalk between the ketogenic pathway and the CAST–CAPN system. Previous studies demonstrated that the CAST–CAPN

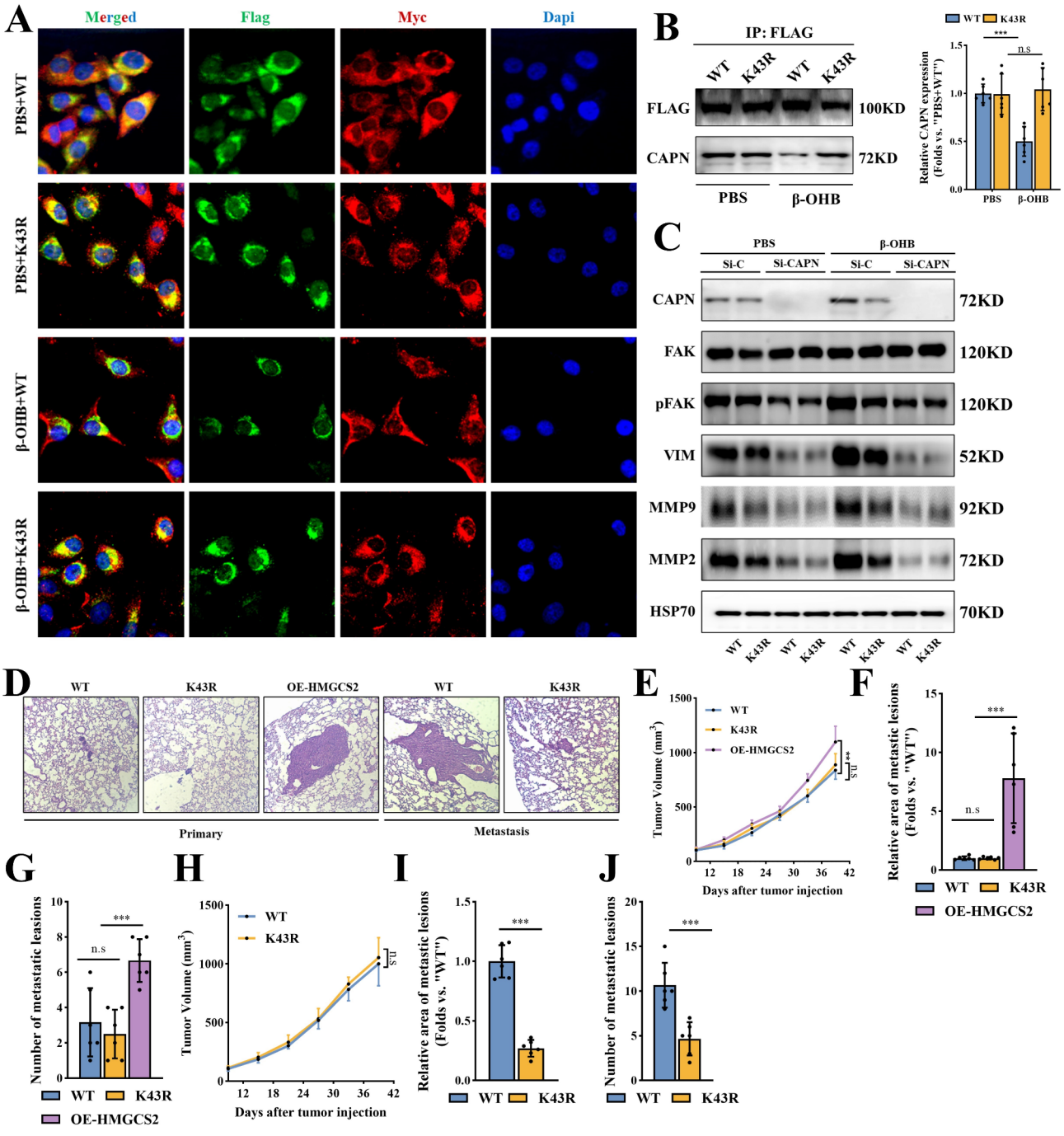

Fig. 5 CAST β-hydroxybutyrylation activates CAPN to promote FAK phosphorylation and EMT. In the CAST-knockout MDA-MB-231 cell line, the Flag-CAST lentiviral construct ("WT") or CASTK43R ("K43R") lentiviral construct and Myc-CAPN were cotransfected, and supplemented with PBS ("PBS + WT" or "PBS + K43R") or β-OHB ("β-OHB + WT" or "β-OHB + K43R"), and FLAG and Myc were stained by immunofluorescence (**A**). The complexes were pulled down with an anti-FLAG antibody, and the interaction between CAPN and CAST was detected by immunoblotting (**B**). CAPN siRNA ("WT + Si-CAPN" or "K43R + Si-CAPN") or nonsense siRNA ("WT + Si-C or "K43R + Si-C") was transfected into "WT" and "K43R" cells, WB was used to detect transfection-related protein expression (**C**), and HSP70 was used as a loading control. After CAST was knocked out using CRISPR/CAS9 in "Primary" and "Metastasis" cells, lentiviral constructs expressing wild-type CAST ("WT") or CASTK43R ("K43R"), or simultaneously expressing wild-type CAST and HMGCS2 ("OE-HMGCS2") were transfected, and mouse primary tumor sizes (**D**-**J**) and the number of lung metastases (**D**, **G**, **J**) and tumor sizes (**D**, **F**, **I**) were detected. The data are presented as the means ± SDs, and each scatter plot point represents an independent replicate

system acts as a regulator of TNBC invasion and metastasis [36–38]; the study revealed a novel mechanism of CAST–CAPN regulation. Enriched β-OHB impedes the endogenous inhibition of CAPN by CAST, thereby activating a series of downstream signaling pathways, including EMT and FAK. The study thus reveals a novel role of ketone bodies in TNBC.

Furthermore, the study provides a quintessential example of how metabolic products influence enzyme activity. The results demonstrate that β-OHB inhibits the endogenous inhibition and interaction of CAST with CAPN via β-hydroxybutyrylation modification of the 43rd lysine residue of CAST, thereby enriching the theoretical model that metabolic products act as substrates for posttranslational modifications to modulate cellular enzyme activity.

Study strengths and limitations

This study possesses several key strengths. Firstly, it provides a novel insight into the role of ketogenesis in TNBC metastasis, demonstrating that increased ketogenesis, through enhanced β-OHB production, promotes TNBC metastasis by disrupting the regulatory relationship between CAST and CAPN. The use of comprehensive in vivo, in vitro, and clinical approaches solidifies these findings, establishing ketogenesis as a potential risk factor for TNBC metastasis. Additionally, the study identifies a new metabolic target for TNBC intervention, highlighting how endogenous ketone bodies drive TNBC cell invasion and metastasis, thus offering a foundation for targeted metabolic therapies. Lastly, this work contributes a significant advancement in the understanding of metabolic regulation mechanisms, illustrating how β-OHB modulates enzyme activity through β-hydroxybutyrylation, particularly at CAST's K43 site, setting a precedent for the role of metabolic products in cellular regulation and enzyme activity.

However, the study also presents certain limitations. Firstly, only a few acetylation modification sites that may be involved in cell invasion and metastasis were discussed. Considering that β-OHB is a broad-spectrum histone deacetylase inhibitor, there may be more acetylation sites regulated by β-OHB that remain undetected and involved in the invasion and metastasis of TNBC. Also, the effects of kbhb on other modifications such as phosphorylation of CAST and other kbhb sites on other proteins in the invasion and metastasis induced by β-OHB cannot be ruled out. In addition, due to the retrospective nature of the study, it were unable to obtain longitudinal data to evaluate the correlation between changes in ketogenesis, β-OHB levels, and disease progression. Finally, additional pathways influenced by β-hydroxybutyrylation were not identified, and therefore, it could not exclude the involvement of other pathways in β-OHB-induced invasion and metastasis.

Conclusion

In conclusion, the findings exhibited in this study unequivocally demonstrate that upregulated ketogenesis fosters TNBC metastasis. Mechanistically, upregulated ketogenesis triggers the up-regulation of the ketone body β-hydroxybutyrate, thereby promoting β-hydroxybutyrylation of CAST at K43, subsequently activating CAPN and promoting FAK phosphorylation and EMT. The study also identifies a new metabolic target for TNBC intervention, highlighting how endogenous ketone bodies drive TNBC cell invasion and metastasis, thus offering a foundation for targeted metabolic therapies.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12944-024-02364-x.

Supplementary Material 1

Supplementary Material 2: Figure S1 CAST β-hydroxybutyrylation activates CAPN to promote FAK phosphorylation and EMT. The WB results were quantitatively analyzed (A). Immunofluorescence was used to detect CAST and CAPN in tumor specimens from the patients with the highest 25% and lowest 25% of HMGCS2 expression (B).

Supplementary Material 3: Supplementary Figure S2 (Graphical Abstract) Illustration of the prometastatic effect of ketogenesis in TNBC. Increased ketogenesis fosters TNBC metastasis by promoting the up-regulation of β-OHB, a key ketone body. Mechanistically, β-OHB facilitates Kbhb of CAST, an endogenous CAPN inhibitor, at K43, strengthening the interaction with CAPN and subsequently promoting FAK phosphorylation and EMT.

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Author contributions

Haoran Jiang: conceptualization, data curation, formal analysis, visualization, writing – original draft; Yuan Zeng: conceptualization, data curation, formal analysis; Xiaoye Yuan: data curation, formal analysis; Liwen Chen: data curation, formal analysis; Xuni Xu: formal analysis; Xue Jiang: formal analysis; Quan Li: supervision, validation; Gang Li: conceptualization, supervision, writing – review & editing; Han Yang: conceptualization, funding acquisition, supervision, writing – review & editing;

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Data availability

Data will be made available on request.

Declarations

Ethical approval

All experiments received approval from the Clinical Trial Ethics Committee of the First Affiliated Hospital of Wenzhou Medical University (KY2024-R080) and were conducted in accordance with the Helsinki Declaration principles. Animal experiments were conducted with the approval of the Animal Ethics Committee of the First Affiliated Hospital of Wenzhou Medical University in accordance with the Regulations on the Management of Experimental Animals.

Competing interests

The authors declare no competing interests.

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