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HMGB1-activated fibroblasts promote breast cancer cells metastasis via RAGE/aerobic glycolysis

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Highly expressed high mobility group box-1 protein (HMGB1) promotes tumor metastasis. Whether HMGB1 participates in breast cancer cell activation of fibroblasts is unknown. The culture medium of 6 breast cancer cell lines with different migration potential, and with HMGB1 overexpression or knockdown was used to induce fibroblast activation, and collagen and α-SMA expression were measured. We evaluated the migration potential of MDA-MB-231 cells with fibroblasts treated with 3-PO (3-(3-pyridinyl)-1-(4-pyridinyl)-2-propen-1-one) inhibitor, anti-HMGB1 treatment, or RAGE (receptor for advanced glycation end products) knockdown. A lung metastasis murine model was used to evaluate whether the RAGE-knockdown fibroblasts mitigates MDA-MB-231 metastasis. Breast cancer cells that are highly migratory and have a high invasive potential, had higher HMGB1 expression and induced greater fibroblast activation strongly than cells with poorer motility. hrHMGB1 and the supernatants of HMGB1-overexpressed MCF-7 cells promoted fibroblast activation, but loss-HMGB1 of MDA-MB-231 abolished potential. Moreover, a novel mechanism was identified by which HMGB1 facilitated fibroblast activation by RAGE/aerobic glycolysis. Consistently, fibroblasts enhanced MDA-MB-231 metastasis, but the enhancement was reversed by 3-PO inhibition, anti-HMGB1 treatment, or RAGE knockdown *in vitro* and *in vivo*. We identified that HMGB1 secreted by breast cancer cells promotes fibroblast activation via RAGE/aerobic glycolysis, and activated fibroblasts enhance breast cancer cell metastasis through increased lactate.

Key words: breast cancer cells, fibroblast, high mobility group box-1 protein, RAGE, aerobic glycolysis

The surrounding microenvironment is a well-recognized prometastatic factor for cancer cells [1, 2]. Paracancerous fibroblasts are the most dominant cells in the stroma of breast cancer, which secrete extracellular matrix (ECM) to induce cancer cell growth and metastasis, and act as an important paracrine source that regulates cancer hallmark [3]. On the other hand, paracancerous fibroblast behavior also is influenced by breast cancer cells [4].

High-mobility group box protein 1 (HMGB1) is abundantly expressed in the nucleus and has an important role in chromatin organization [5]. HMGB1 also plays a role as a cytokine with multiple effects when it is secreted into the extracellular space [6]. A report has found that an increase in HMGB1 level in the blood of early-stage breast cancer patients who received neoadjuvant chemotherapy shown a better overall survival [7]. But another research reported that HMGB1 and its receptor RAGE were multiple risk-associated factors in breast cancer progression [8]. However, whether breast cancer cells that secreted HMGB1 influence fibroblast behavior is unknown.

Aerobic glycolysis (Warburg effect) is an important mechanism for the development of lung fibrosis [9]. The Warburg effect is characterized by increased glucose intake as well as declined endogenous adenosine triphosphate (ATP) because of incompletely glycolysis, thus numerous intermediate metabolites and lactate are produced in fibroblasts for activation and ECM production [10]. Especially, lactate accumulation provides an environment with a lower pH value that accelerates the motility of cancer cells, leading to tumor metastasis [11]. However, whether paracancerous fibroblasts exhibit aerobic glycolysis, and whether the metabolic shift influences breast cancer cell metastasis is unclear.

This study was to identify whether secretion of HMGB1 from breast cancer cells influences fibroblasts activation and whether aerobic glycolysis is a participant in this process.

Patients and methods

Cell culture. Breast cancer cell lines (MCF-7, T-470, BT474, MDA-MB-231, ZR-75-30, and BT549) and fibro-

blast (human embryonic lung fibroblast, HFL-1) used in our study were all purchased from ATCC (Manassas, VA). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) or RPMI-1640 medium, in which 10% fetal bovine serum (FBS) (Life Technologies/Invitrogen, Carlsbad, CA) was supplemented. The cells were incubated in 5% CO_2 at 37 °C. The breast cancer cell lines were validated by Short Tandem Repeat (STR) profiling. Cells were used for experiments after 3 cell passages.

Human breast cancer tumor samples, immunohistochemistry, and scoring. Institutional Human Subject Research Review Committee of Nanchang University approved all procedures. The study was approved by the Ganzhou People's Hospital Ethics Committee and written consent was obtained from all participants.

All patients received radical resection of breast tumor dissection at Ganzhou People's Hospital, the Affiliated Ganzhou Hospital of Nanchang University between June 2017 and December 2018. Tumors were confirmed histopathologically and staged according to TNM classification by UICC [12]. The paraffin sections of tumor samples were collected from the pathology department for routine immunohistochemistry (IHC) staining for anti-HMGB1 (Abcam, U.S.A.). The expression levels of HMGB1 in the tissue were scored according to the percentage of HMGB1-positive breast cancer cells in each whole area of tissue and their staining intensity as previously described [13, 14]. Specifically, percentages ≤1%, 2–25%, 26–50%, 51–75%, and ≥75% were scored as 0, 1, 2, 3, and 4, respectively; non-significant brown, light brown, moderate brown, and deep brown staining intensities were scored as 0, 1, 2, and 3, respectively. Combined scoring was then calculated and graded as negative (–; 0–1), weak (+; 2–4), moderate (++; 5–8), and strong (+++; 9–12).

Lentivirus plasmids and siRNA. Lentivirus containing pCMV-HMGB1-EGFP-puro plasmid was purchased from Cyagen (Shanghai, China) and used to infect MCF-7 cells to generate polyclonal cells with stable overexpression and secretion of HMGB1. siRNA-HMGB1 (siHMGB1) or siRNA-RAGE (siRAGE) (Ribobio, China) were respectively transduced into MDA-MB-231 or HFL-1 to knockdown HMGB1 or protein levels by Lipofectamine 2000 (Invitrogen, USA).

Western blotting analysis. Western blotting studies referred to the previous publication [15]. The primary antibodies included against HMGB1 (Abcam, ab18256), collagen (Abcam, ab34710), α-SMA (Proteintech, 23081-1-AP), RAGE (Abcam, ab3611), and β-actin (CST, 3700). Secondary antibodies were IRDye800 (LI-COR Biosciences, Lincoln, NE, 929-32211) and IRDye680 (LI-COR Biosciences, Lincoln, NE, NO.925-68070). Signal intensities were collected and analyzed by Odyssey Infrared Image System (LI-COR Biosciences).

Enzyme-linked immunosorbent assay (ELISA). The cell supernatants were harvested and HMGB1 concentration was measured by ELISA (eBioscience, San Diego, USA) according to the kit instructions.

Invasion assays. All invasion assays referred to the previous publication [16]. In the top compartment of a Transwell chamber (Millipore, USA) were placed 5×10^4 MDA-MB-231 cells. The Transwell chambers were put in 24-well plates with cultured fibroblasts which were treated as follows. 1) Control group: lower chamber without fibroblasts. 2) Lactate group: lactate (10 mM) was added in the lower chamber [9]. 3) Control-fibroblasts: fibroblasts (1×10^5) were placed in the lower chamber. 4) 3-PO-fibroblasts: fibroblasts were placed in the lower chamber with 3-PO (3-(3-pyridinyl)-1-(4-pyridinyl)-2-propen-1-one, 10 mM, Selleck). 5) IgG-fibroblasts: fibroblasts were placed in the lower chamber with IgG (20 μg/ml, R&D Systems). 6) Anti-HMGB1-fibroblasts: fibroblasts were placed in the lower chamber with anti-HMGB1 (20 μg/ml, R&D Systems). 7) siNC-fibroblasts: fibroblasts were placed in the lower chamber and transfected with siNC for 48 h. 8) siRAGE-fibroblasts: fibroblasts were placed in the lower chamber and transfected with siRAGE for 48 h. Then, crystal violet was used for staining, and an inverted microscope (Olympus, Tokyo, Japan) with 20× objective lens was used for photographing.

Analysis of glucose uptake and lactate production. Glucose uptake levels of fibroblasts were measured by the uptake of ^{3}H-2-deoxyglucose [17]. A lactate assay kit (Jiancheng Bioengineering Institute) was used for measuring lactate accumulation levels in the culture medium.

Animal model. Animal studies were approved by the Institutional Animal Care and Use Committee of the Standing Committee on Animals at the Southern Medical University. Female NOD/SCID mice with 15–20 g and 8–10 weeks of age were purchase from Laboratory Animal Center, Nanchang University, Nanchang, China. Mice were housed in standard cages with filtered air, 12 h light-dark cycle, *ad libitum* access to food and autoclaved water. The mice were randomly placed into 2 groups (6 mice/group) and injected with cells (2×10^6, half MDA-MB-231 cells and half fibroblasts) via the tail vein. Groups setting were as follows: siNC (MDA-MB-231 cells and fibroblasts with transfected with siNC were suspended) and siRAGE (MDA-MB-231 cells and fibroblasts with transfected with siRAGE were suspended). The mice were sacrificed after 6 weeks and their lungs were collected. Tissue was prepared in slides and HE stained. Metastatic nodules were counted for analysis of metastasis.

Statistical analysis. All experiments in vitro were performed in triplicate at a minimum. Results were presented as mean ± standard deviation by using a software program (SPSS version 13.0). All statistical tests were 2-sided. Student's t-test was used to compare continuous variables, $p<0.05$.

Results

The metastatic potential of breast cancer cells varies with the secretion of HMGB1. It was recently reported that HMGB1 is increased in malignancies, and participates in

tumor growth and metastatic potential [18, 19]. To determine if the secretion of HMGB1 enhances breast cancer cells' ability to metastasize, we measured HMGB1 expression and compared different metastatic potentials in 6 breast cancer cell lines (MCF-7, T-470, BT474, MDA-MB-231, ZR-75-30, and BT549). Compared with MCF-7, T-470, and BT474 cells, MDA-MB-231, ZR-75-30, and BT549 cells showed higher HMGB1 expression as well as the stronger ability of motility, migration, and invasion than in those which exhibited poorer motility (all, $p<0.01$, Figure 1A). HMGB1 secretion was also in line with above results (all, $p<0.01$, Figure 1B). We further examined the levels of HMGB1 in human breast cancer tumors collected from 43 patients with different clinical and pathological characteristics (Table 1) and found ACE2 was highly expressed in distant metastatic cancer samples than that in ductal (Figure 1C). These data indicate that high expression and secretion of HMGB1 was associated with migratory and invasive breast cancer cells.

HMGB1 secreted by breast cancer cells promotes fibroblast activation. Fibroblasts were treated with the supernatants from incubated breast cancer cells for 48 h. Collagen and α-SMA protein expressions were higher in fibroblasts treated with the supernatants from MDA-MB-231, ZR-75-30, and BT549 cells, than those from MCF-7, T-470, and BT474 cells (all, $p<0.01$, Figure 2A).

Based on the finding that highly migratory and invasive breast cancer cells exhibit greater HMGB1 expression and secretion, and these was more stroma fibrosis in breast cancer sample with higher HMGB1 expression (Figure 1C),

Table 1. Clinical and pathological characteristics of breast cancer patients.

	ductal cancer	invasive	p-value
Number	20	23	–
Age	51.93±8.35	62.08±5.48	
Tumor stage			
T1,T2	20	10	<0.001
T3,T4	0	13	
Lymph node metastasis			
No	20	9	<0.001
Yes	0	14	
Distant metastasis			
No	20	9	<0.001
Yes	0	14	
Stage grouping			
I,II	20	6	<0.001
III,IV	0	17	
ER(+)			
No	8	8	0.724
Yes	12	15	

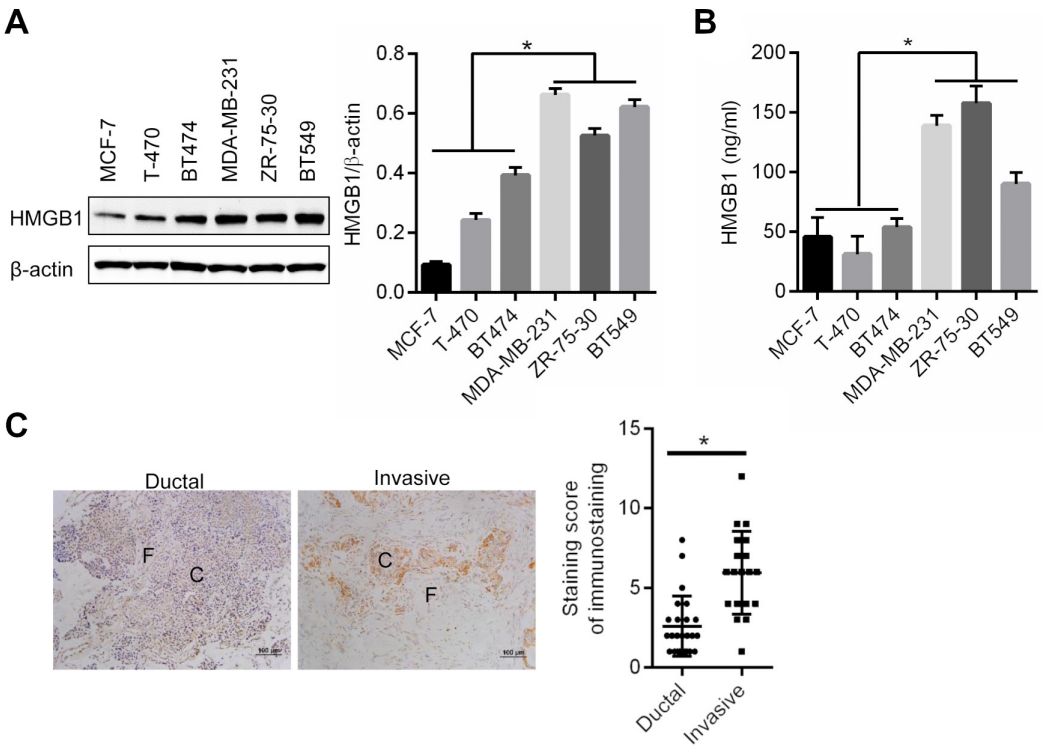

Figure 1. The expression of HMGB1 in breast cancer. A) Detecting HMGB1 protein expression levels by western blotting in breast cancer cell lines with different metastatic potential. B) Secretory HMGB1 in supernatants were detected by ELISA. C) HMGB1 protein expression levels in the surgical tissue section from the patient. Abbreviations: F-stroma fibrosis; C-cancer cells. Scale bars: 100 μm. *$p<0.05$

we hypothesized that HMGB1 secreted by breast cancer cells promotes fibroblast activation. To test this hypothesis, we infected MCF-7 cells with lenti-HMGB1 or lenti-NC, and transfected MDA-MB-231 cells with siHMGB1. The infection or transfection efficiency was confirmed by western blotting (Figures 2B, 2C) and ELISA (Figures 2D, 2E). The overexpression of HMGB1 in MCF-7 cells obviously enhanced fibroblast collagen and α-SMA protein expression (Figure 2F). In contrast, interfering HMGB1 expression in MDA-MB-231 cells produced a marked decrease of fibroblasts collagen and α-SMA protein expression (Figure 2F). Here, we concluded that HMGB1 secreted by breast cancer cells promotes the activation of fibroblasts.

HMGB1 induces fibroblasts activation through RAGE/glycolysis. RAGE has been reported to be the receptor for HMGB1 [20]. We found that increased expression of collagen and α-SMA protein was arrested in RAGE knockdown fibroblasts induced by HMGB1 (Figures 3A, 3D). These indicated that HMGB1 induces fibroblasts activation through RAGE.

Recently, investigators revealed the role of glycolysis in regulating fibroblast activation [9]. To identify whether the HMGB1/RAGE signaling pathway affects glycolysis in fibroblasts, we measured the level of glucose uptake and lactate production in fibroblasts. Unsurprisingly, HMGB1 induced fibroblasts showed a higher level of glucose uptake and lactate production compared with those of the control group

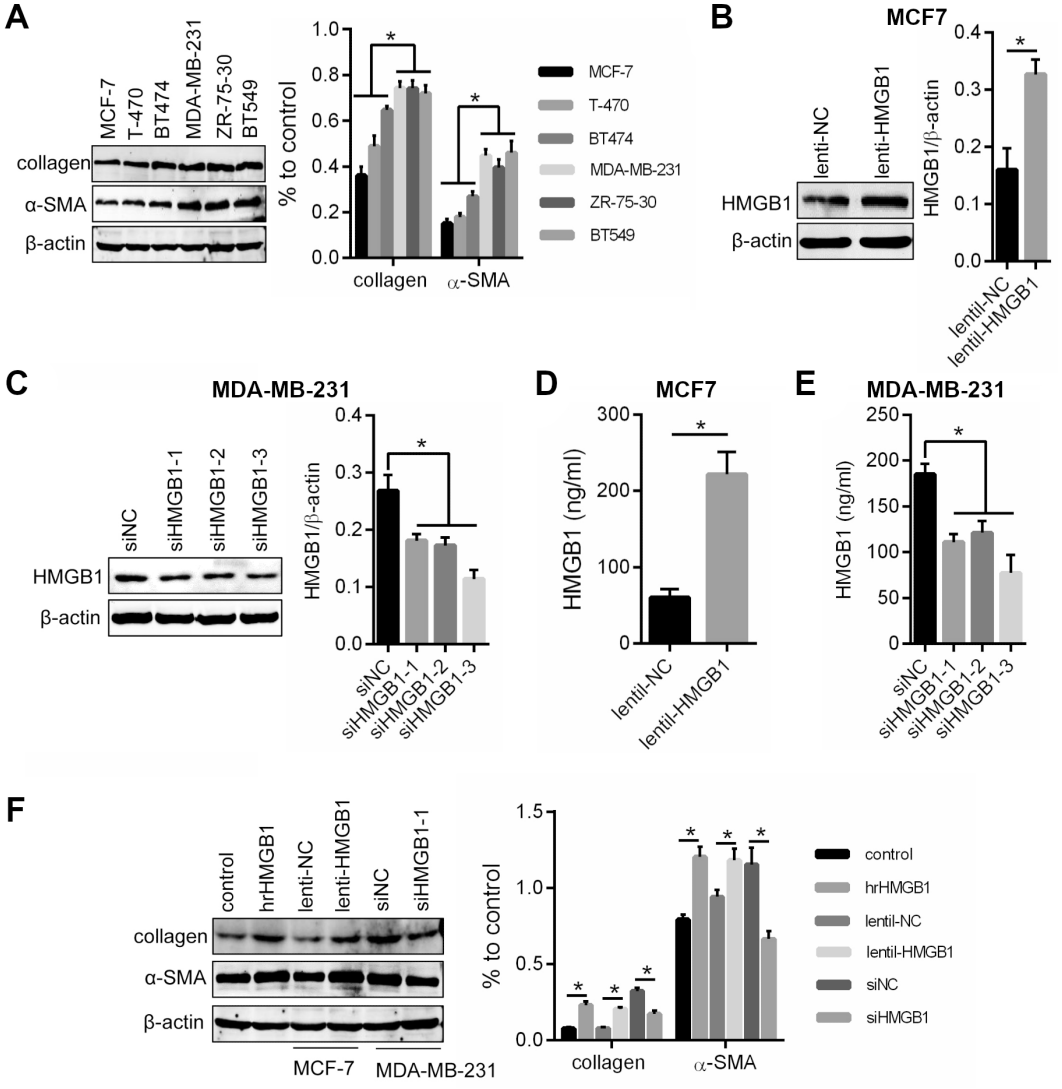

Figure 2. HMGB1 secreted by breast cancer cells promotes the activation of fibroblasts. A) Collagen and α-SMA protein expression levels in fibroblasts treated with supernatants from breast cancer cells. B) The expression levels of HMGB1 in the cytoplasm and supernatants of MCF-7 cells transfected with lenti-HMGB1 or lenti-NC by western blotting (B) and ELISA (D). MDA-MB-231 cells were transfected with siRNA, and then the expression of HMGB1 in cytoplasm and supernatants were detected by C) ELISA and E) western blotting, respectively. F) Fibroblasts were treated with hrHMGB1 and the supernatants of MCF-7 cells (lenti-NC or lenti-HMGB1) and MDA-MB-231 cells (siNC or siHMGB1) for 48 h. Collagen and α-SMA protein expression levels in fibroblasts by western blotting. *p<0.05

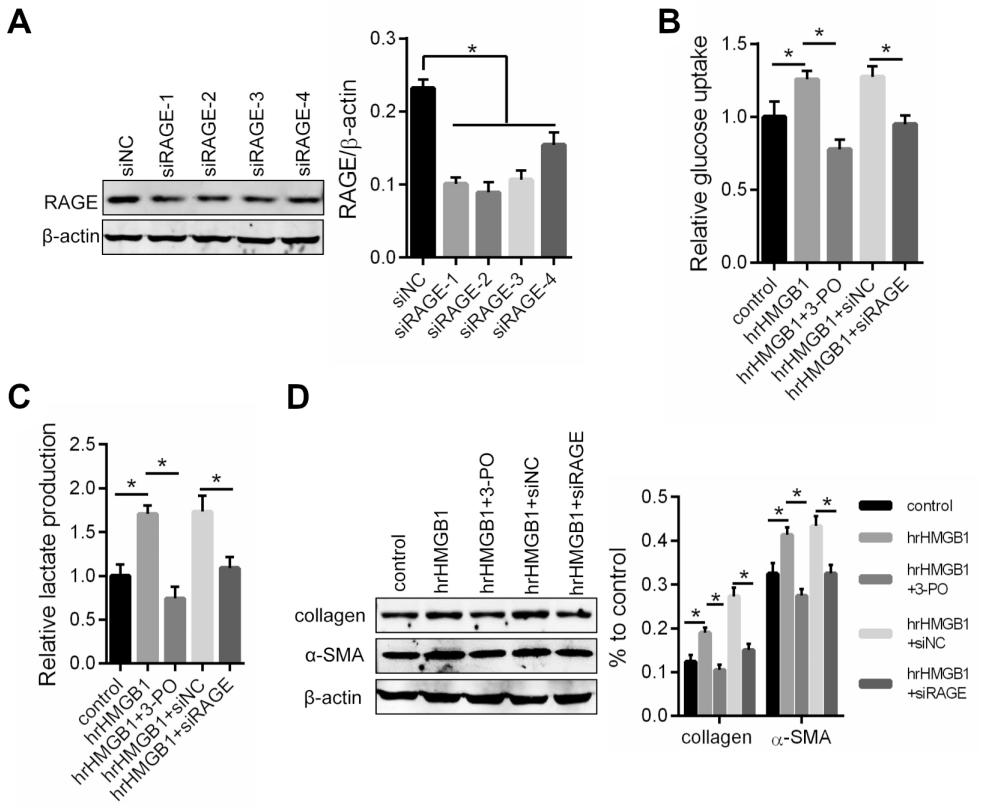

Figure 3. HMGB1 induces glycolysis and activation of fibroblasts by RAGE. A) Fibroblasts were transfected siRNA, and the expression of RAGE was examined by western blotting. B) Glucose uptake and C) lactate production in fibroblasts treated with hrHMGB1, 3-PO, or siRNA. D) Western blots showing collagen and α-SMA protein expression levels in fibroblasts treated with hrHMGB1, 3-PO, or siRNA. *p<0.05

(Figures 3B, 3C). In 3-PO, glycolysis inhibitor, pretreated fibroblasts, HMGB1 induced higher glucose uptake and lactate production, and the expression of collagen and α-SMA were significantly reduced (Figures 3B–3D), and these findings were observed in RAGE-knockdown fibroblasts (Figures 3B–3D). These results suggest that HMGB1 induces fibroblasts activation through RAGE/glycolysis.

HMGB1-activated fibroblasts promote breast cancer cells metastasis. Previous research has indicated that cancer cells can alter paracancerous fibroblasts to provide a metastatic-supportive microenvironment [21, 22]. To ascertain the effect of HMGB1 and anaerobic glycolysis on fibroblasts and breast cancer cell metastasis, fibroblasts and MDA-MB-231 cells were co-cultured in the upper and lower compartments of Transwell chambers, respectively (Figure 4A). We found more MDA-MB-231 cells invaded through the membrane of the Transwell chamber in co-culture with fibroblasts or the low compartment of the Transwell chamber with lactate alone than in the control group (low compartment of the Transwell chamber without fibroblast). In contrast, fewer MDA-MB-231 cells invasive through the membrane of Transwell chamber in co-culture with 3-PO or anti-HMGB1 treated, or RAGE-knockdown fibroblasts.

To determine whether fibroblasts promote the metastatic potential of breast cancer cells in mice model, we injected mice via the tail vein with MDA-MB-231 cells and fibroblasts with or without downregulated RAGE expression. Mice were sacrificed 42 days after injection. Fewer metastatic lung nodules were founded in mice injected with MDA-MB-231 cells and fibroblasts with downregulated RAGE expression than that in the siNC group (Figure 4B). These demonstrated that HMGB1-activated fibroblasts promote breast cancer cell metastasis by RAGE/anaerobic glycolysis.

Discussion

In this study, we demonstrated that the expression and secretion of HMGB1 were higher in breast cancer cell lines with the high ability of motility, migration, and invasive potential. Moreover, HMGB1 promotes fibroblast activation through its receptor-RAGE to upregulate aerobic glycolysis. And also, lactate production of fibroblasts induced by HMGB1 facilitated breast cancer cell metastasis.

In breast cancer, HMGB1 contributes to cancer progression and chemotherapy resistance [23]. A study has found that nuclear HMGB1 can maintain telomere homeostasis,

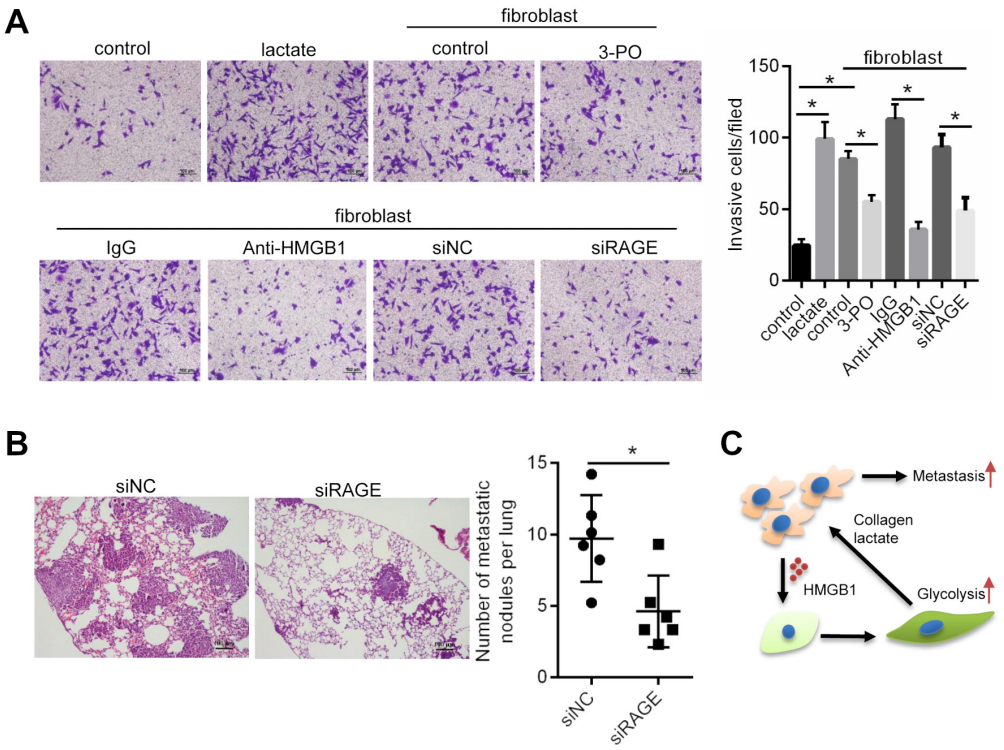

Figure 4. HMGB1-activated fibroblasts promote breast cancer cells metastasis. A) Transwell invasion assays with MDA-MB-231 cells treated with the supernatants of fibroblasts. B) H&E staining of lung tissue sections from mice injected with the suspended fluid of MDA-MB-231 breast cancer cells or fibroblasts with (without) RAGE. Scale bars: 100 μm. Lung metastatic foci were counted in 5 randomly selected fields in each group. C) Schematic model for the HMGB1-activated fibroblasts promoting breast cancer cell metastasis. *p<0.05.

and repair damaged DNA in human breast cancer cells [24]. HMGB1 acts as an extracellular signaling molecule during inflammation, cell differentiation, and tumor ability of migration and metastasis [25–27]. We demonstrated that the expression and secretion of HMGB1 were higher in breast cancer cells that are highly migratory and have a high invasive potential than in those with poorer motility. Moreover, we found that HMGB1 secreted from breast cancer cells induced fibroblast activation. Lee et al. [28] and Wu et al. [29] also reported that HMGB1 participates in fibroblast activation. Taken together, these results suggest that HMGB1 secreted by breast cancer cells acts as a fibroblast activating factor.

Like Ge et al. [30], we found that HMGB1-induced activation was weakened in RAGE knockdown fibroblasts, and the increased aerobic glycolysis, which is a metabolic shift of activated fibroblasts, was suppressed. Therefore, we guessed that elevated aerobic glycolysis is essential for HMGB1-induced fibroblast activation. To verify the hypothesis, we inhibited aerobic glycolysis. Interestingly, HMGB1-induced fibroblasts activation was strongly inhibited. Consistently, the same results were observed in RAGE knockdown fibroblasts. Some recent studies report that RAGE is involved in regulating the expression of HIF-1α [31], a key factor in glycolysis [17, 32]. These results revealed the role of HMGB1/RAGE-induced anaerobic glycolysis in the regulation of fibroblast activation.

Increased anaerobic glycolysis is known to regulate the growth of cancer cells, and a high lactate environment facilitates cancer cell motility, eventually leading to metastasis. Thus, fibroblasts and MDA-MB-231 cells were co-cultured to determine whether fibroblasts enhance breast cancer metastasis by lactate production. We identified increased metastasis of the MDA-MB-231 cells. However, if the anaerobic glycolysis of fibroblasts was reduced, the increased metastasis of the MDA-MB-231 cells was reversed. Additionally, there was no increase of MDA-MB-231 cells' metastasis when co-cultured with RAGE knockdown fibroblasts. Taken together, these results illustrated that lactate from fibroblasts facilitates MDA-MB-231 cells' metastasis.

In conclusion, our study demonstrated that HMGB1-mediated anaerobic glycolysis of fibroblasts is required for breast cancer cell activation of fibroblasts, and the fibroblast enhancement of breast cancer cell metastasis. The study also identified a new HMGB1-mediated anaerobic glycolysis pathway involving RAGE and fibroblasts. Furthermore, the pathway suggested that inhibiting HMGB1 expression may treat the subtypes of breast cancer with elevated HMGB1 levels. Further mechanism studies by which HMGB1

enhancement modulates the process of anaerobic glycolysis in breast cancer cells are expected.

References

[1] TANG T, YANG Z, ZHU Q, WU Y, SUN K et al. Up-regulation of miR-210 induced by a hypoxic microenvironment promotes breast cancer stem cells metastasis, proliferation, and self-renewal by targeting E-cadherin. FASEB J 2018; fj201801013R. https://doi.org/10.1096/fj.201801013R

[2] MALIK DE, DAVID RM, GOODERHAM NJ. Mechanistic evidence that benzo[a]pyrene promotes an inflammatory microenvironment that drives the metastatic potential of human mammary cells. Arch Toxicol 2018; 92: 3223–3239. https://doi.org/10.1007/s00204-018-2291-z

[3] ORIMO A, GUPTA PB, SGROI DC, ARENZANA-SEISDEDOS F, DELAUNAY T et al. Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. Cell 2005; 121: 335–348. https://doi.org/10.1016/j.cell.2005.02.034

[4] HEMALATHA SK, SENGODAN SK, NADHAN R, DEV J, SUSHAMA RR et al. Brcal Defective Breast Cancer Cells Induce in vitro Transformation of Cancer Associated Fibroblasts (CAFs) to Metastasis Associated Fibroblasts (MAF). Sci Rep 2018; 8: 13903. https://doi.org/10.1038/s41598-018-32370-w

[5] TANIGUCHI N, YOSHIDA K, ITO T, TSUDA M, MISHIMA Y et al. Stage-specific secretion of HMGB1 in cartilage regulates endochondral ossification. Mol Cell Biol 2007; 27: 5650–5663. https://doi.org/10.1128/MCB.00130-07

[6] MARTINOTTI S, PATRONE M, RANZATO E. Emerging roles for HMGB1 protein in immunity, inflammation, and cancer. Immunotargets Ther 2015; 4: 101–109. https://doi.org/10.2147/ITT.S58064

[7] EXNER R, SACHET M, ARNOLD T, ZINN-ZINNENBURG M, MICHLMAYR A et al. Prognostic value of HMGB1 in early breast cancer patients under neoadjuvant chemotherapy. Cancer Med 2016; 5: 2350–2358. https://doi.org/10.1002/cam4.827

[8] YUE L, ZHANG Q, HE L, ZHANG M, DONG J et al. Genetic predisposition of six well-defined polymorphisms in HMGB1/RAGE pathway to breast cancer in a large Han Chinese population. J Cell Mol Med 2016; 20: 1966–1973. https://doi.org/10.1111/jcmm.12888

[9] KOTTMANN RM, KULKARNI AA, SMOLNYCKI KA, LYDA E, DAHANAYAKE T et al. Lactic acid is elevated in idiopathic pulmonary fibrosis and induces myofibroblast differentiation via pH-dependent activation of transforming growth factor-beta. Am J Respir Crit Care Med 2012; 186: 740–751. https://doi.org/10.1164/rccm.201201-0084OC

[10] GATENBY RA, GILLIES RJ. Why do cancers have high aerobic glycolysis? Nat Rev Cancer 2004; 4: 891–899. https://doi.org/10.1038/nrc1478

[11] MAFTOUH M, AVAN A, SCIARRILLO R, GRANCHI C, LEON LG et al. Synergistic interaction of novel lactate dehydrogenase inhibitors with gemcitabine against pancreatic cancer cells in hypoxia. Br J Cancer 2014; 110: 172–182. https://doi.org/10.1038/bjc.2013.681

[12] GREENE FL, SOBIN LH. A worldwide approach to the TNM staging system: collaborative efforts of the AJCC and UICC. J Surg Oncol 2009; 99: 269–272. https://doi.org/10.1002/jso.21237

[13] ZHANG Z, LIU X, FENG B, LIU N, WU Q et al. STIM1, a direct target of microRNA-185, promotes tumor metastasis and is associated with poor prognosis in colorectal cancer. Oncogene 2016; 35: 6043. https://doi.org/10.1038/onc.2016.140

[14] YU C, TANG W, WANG Y, SHEN Q, WANG B et al. Downregulation of ACE2/Ang-(1-7)/Mas axis promotes breast cancer metastasis by enhancing store-operated calcium entry. Cancer Lett 2016; 376: 268–277. https://doi.org/10.1016/j.canlet.2016.04.006

[15] MENG Y, YU CH, LI W, LI T, LUO W et al. Angiotensin-converting enzyme 2/angiotensin-(1-7)/Mas axis protects against lung fibrosis by inhibiting the MAPK/NF-kappaB pathway. Am J Respir Cell Mol Biol 2014; 50: 723–736. https://doi.org/10.1165/rcmb.2012-0451OC

[16] ZHANG Z, LIU X, FENG B, LIU N, WU Q et al. STIM1, a direct target of microRNA-185, promotes tumor metastasis and is associated with poor prognosis in colorectal cancer. Oncogene 2015; 34: 4808–4820. https://doi.org/10.1038/onc.2014.404

[17] NAGAO A, KOBAYASHI M, KOYASU S, CHOW C, HARADA H. HIF-1-Dependent Reprogramming of Glucose Metabolic Pathway of Cancer Cells and Its Therapeutic Significance. Int J Mol Sci 2019; 20: 238. https://doi.org/10.3390/ijms20020238

[18] TODOROVA J, PASHEVA E. High mobility group B1 protein interacts with its receptor RAGE in tumor cells but not in normal tissues. Oncol Lett 2012; 3: 214–218. https://doi.org/10.3892/ol.2011.459

[19] NI P, ZHANG Y, LIU Y, LIN X, SU X et al. HMGB1 silence could promote MCF-7 cell apoptosis and inhibit invasion and metastasis. Int J Clin Exp Pathol 2015; 8: 15940–15946.

[20] LIU C, KONG X, WU X, WANG X, GUAN H et al. Alleviation of A disintegrin and metalloprotease 10 (ADAM10) on thromboangiitis obliterans involves the HMGB1/RAGE/NF-kappaB pathway. Biochem Biophys Res Commun 2018; 505: 282–289. https://doi.org/10.1016/j.bbrc.2018.09.002

[21] YEON JH, JEONG HE, SEO H, CHO S, KIM K et al. Cancer-derived exosomes trigger endothelial to mesenchymal transition followed by the induction of cancer-associated fibroblasts. Acta Biomater 2018; 76: 146–153. https://doi.org/10.1016/j.actbio.2018.07.001

[22] MORGAN MM, LIVINGSTON MK, WARRICK JW, STANEK EM, ALARID ET et al. Mammary fibroblasts reduce apoptosis and speed estrogen-induced hyperplasia in an organotypic MCF7-derived duct model. Sci Rep 2018; 8: 7139. https://doi.org/10.1038/s41598-018-25461-1

[23] AMORNSUPAK K, INSAWANG T, THUWAJIT P, OCHAROENRAT P, ECCLES SA et al. Cancer-associated fibroblasts induce high mobility group box 1 and contribute to resistance to doxorubicin in breast cancer cells. BMC Cancer 2014; 14: 955. https://doi.org/10.1186/1471-2407-14-955

[24] KE S, ZHOU F, YANG H, WEI Y, GONG J et al. Downregulation of high mobility group box 1 modulates telomere homeostasis and increases the radiosensitivity of human breast cancer cells. Int J Oncol 2015; 46: 1051–1058. https://doi.org/10.3892/ijo.2014.2793

[25] HOSAKOTE YM, BRASIER AR, CASOLA A, GAROFALO RP, KUROSKY A. Respiratory Syncytial Virus Infection Triggers Epithelial HMGB1 Release as a Damage-Associated Molecular Pattern Promoting a Monocytic Inflammatory Response. J Virol 2016; 90: 9618–9631. https://doi.org/10.1128/JVI.01279-16

[26] ZABINI D, CRNKOVIC S, XU H, TSCHERNER M, GHANIM B et al. High-mobility group box-1 induces vascular remodelling processes via c-Jun activation. J Cell Mol Med 2015; 19: 1151–1161. https://doi.org/10.1111/jcmm.12519

[27] CHITANUWAT A, LAOSRISIN N, DHANESUAN N. Role of HMGB1 in proliferation and migration of human gingival and periodontal ligament fibroblasts. J Oral Sci 2013; 55: 45–50. https://doi.org/10.2334/josnusd.55.45

[28] LEE WJ, SONG SY, ROH H, AHN HM, NA Y et al. Profibrogenic effect of high-mobility group box protein-1 in human dermal fibroblasts and its excess in keloid tissues. Sci Rep 2018; 8: 8434. https://doi.org/10.1038/s41598-018-26501-6

[29] WU RN, YU TY, ZHOU JC, LI M, GAO HK et al. Targeting HMGB1 ameliorates cardiac fibrosis through restoring TLR2-mediated autophagy suppression in myocardial fibroblasts. Int J Cardiol 2018; 267: 156–162. https://doi.org/10.1016/j.ijcard.2018.04.103

[30] GE X, ARRIAZU E, MAGDALENO F, ANTOINE DJ, DELA CR et al. High mobility group box-1 drives fibrosis progression signaling via the receptor for advanced glycation end-products in mice. Hepatology 2018; 68: 2380–2404. https://doi.org/10.1002/hep.30093

[31] HAN X, MA W, ZHU Y, SUN X, LIU N. Advanced glycation end products enhance macrophage polarization to the M1 phenotype via the HIF-1alpha/PDK4 pathway. Mol Cell Endocrinol 2020; 514: 110878. https://doi.org/10.1016/j.mce.2020.110878

[32] COURTNAY R, NGO DC, MALIK N, VERVERIS K, TORTORELLA SM et al. Cancer metabolism and the Warburg effect: the role of HIF-1 and PI3K. Mol Biol Rep 2015; 42: 841–851. https://doi.org/10.1007/s11033-015-3858-x