Gpr132 sensing of lactate mediates tumor–macrophage interplay to promote breast cancer metastasis

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Edited by Ruslan Medzhitov, Yale University School of Medicine, New Haven, CT, and approved December 13, 2016 (received for review August 23, 2016)

Macrophages are prominent immune cells in the tumor microenvironment that exert potent effects on cancer metastasis. However, the signals and receivers for the tumor–macrophage communication remain enigmatic. Here, we show that G protein-coupled receptor 132 (Gpr132) functions as a key macrophage sensor of the rising lactate in the acidic tumor milieu to mediate the reciprocal interaction between cancer cells and macrophages during breast cancer metastasis. Lactate activates macrophage Gpr132 to promote the alternatively activated macrophage (M2)-like phenotype, which, in turn, facilitates cancer cell adhesion, migration, and invasion. Consequently, Gpr132 deletion reduces M2 macrophages and impedes breast cancer lung metastasis in mice. Clinically, Gpr132 expression positively correlates with M2 macrophages, metastasis, and poor prognosis in patients with breast cancer. These findings uncover the lactate-Gpr132 axis as a driver of breast cancer metastasis by stimulating tumor–macrophage interplay, and reveal potential new therapeutic targets for breast cancer treatment.

macrophage | breast cancer | metastasis | Gpr132 | lactate

Breast cancer is the most frequently diagnosed nonskin type of malignancy, and the second leading cause of cancer-related death in women. The 5-y survival rate is 89% in patients who have primary breast cancer, whereas the medium survival of patients with metastatic breast cancer is only 1–2 y (1, 2). Metastasis is the primary cause of breast cancer-related deaths; however, the molecular mechanisms underlying this process are still poorly understood. It has been well established that the tumor microenvironment plays an important role in breast cancer metastasis (3–6). Tumor-associated macrophages (TAMs) make up the largest population of stromal cells that suppress antitumor immunity and foster tumor progression in mouse models of breast cancer (3, 6–8). TAMs also promote metastasis and correlate with poor prognosis in patients with breast cancer (7, 9). Conversely, TAM functions are also tightly regulated by tumor cells (10, 11). However, the mechanisms underlying this reciprocal regulation between cancer cells and macrophages during metastasis remain elusive.

Macrophages are heterogeneous immune cells that can exhibit distinct functions and phenotypes depending on different microenvironment signals (9, 12). They can be broadly divided into classically activated (M1) and alternatively activated (M2) macrophages, the latter of which generally display promalignancy activity (9, 12). In solid tumors, TAMs are usually biased toward M2 (9). Due to hypoxia and glycolytic cancer cell metabolism, the tumor environment is usually acidic, which affects tumor progression by acting on both cancer cells and stromal cells, including macrophages (10, 13, 14). A recent study shows that cancer cell-derived lactate can educate macrophages to functional TAMs, which, in turn, promotes tumor growth (14). Nonetheless, how lactate activation of TAMs affects cancer metastasis is poorly understood. Importantly, the molecular basis by which macrophages sense and respond to lactate is largely unknown.

G protein-coupled receptor 132 (Gpr132, also known as G2A) is a stress-inducible, seven-pass transmembrane receptor that actively modulates several cellular biological activities, such as cell cycle, proliferation, and immunity (15–17). Gpr132 is highly expressed in macrophages (18), and modulates macrophage activities in atherosclerosis (18, 19). However, the role of Gpr132 in TAM activation and cancer metastasis remains elusive. Considering that Gpr132 is a member of the pH-sensing G protein-coupled receptor family (13), we postulated that macrophage Gpr132 functions as both a sensor and a responder to the acidic tumor microenvironment to exacerbate breast cancer metastasis. Here, we identify that cancer cell-derived lactate is a Gpr132 ligand/activator that facilitates the macrophage M2 phenotype in a Gpr132-dependent manner. As a result, Gpr132 deletion impairs macrophage M2 activation and breast cancer metastasis in vitro and in vivo. These findings not only decipher the roles and mechanisms of Gpr132 in macrophage and breast cancer metastasis but also provide evidence for Gpr132 as a macrophage sensor/receptor for lactate. Collectively, our studies reveal a molecular basis for the vicious cycle between cancer cells and macrophages, and uncover Gpr132 as an exciting therapeutic target for breast cancer metastasis.

Results

Tumor-Derived Factors Activate M2-Like Macrophages via Gpr132. Macrophages in the tumor microenvironment can be educated by cancer cells (9, 10, 14). Gpr132 is a cell surface receptor highly expressed in macrophages (18) but largely absent from breast

Significance

Metastasis is a major cause of cancer mortality. However, the regulation of this complex process remains poorly understood. Due to low oxygen supply and enhanced sugar metabolism, cancer cells releases lactate to create an acidic environment. We show that a membrane receptor on macrophages called G protein-coupled receptor 132 (Gpr132) can sense and respond to this lactate signal from cancer cells. As a result, macrophages alter their functions, which, in turn, stimulates cancer metastasis to distant organs. Consequently, loss of Gpr132 in mice inhibits breast cancer metastasis; lower Gpr132 expression in patients with breast cancer correlates with better metastasis-free survival. These findings uncover knowledge and potentially novel treatment for cancer metastasis.

Author contributions: P.C. and Y.W. designed research; P.C., H.Z., M.J.K., and Q.C. performed research; H.X., M.J.K., A.S., and D.J.S. contributed new reagents/analytic tools; P.C., H.Z., and M.J.K. analyzed data; and P.C. and Y.W. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1614035114/-/DCSupplemental.

cancer cells (20). At the same time, Gpr132 is also highly sensitive to acidity (13), a hallmark of the cancer milieu. Thus, we hypothesized that Gpr132 might be the macrophage pH sensor that controls macrophage phenotype in response to the acidic tumor microenvironment. To examine whether cancer cell-derived acidic signals can modulate macrophage M2 activation and Gpr132 expression, we first measured the pH value in the conditioned medium/media (CM) from 10 equally seeded breast cancer cell lines, as well as B16F10 melanoma and RAW264.7 macrophage cell lines. We found that the pH values of breast cancer cell CM from EO771, EO771-LMB, 4T1.2, and SCP-6 cell lines were significantly lower than macrophage CM (Fig. 1*A*). Western blot, RT-quantitative PCR (qPCR), and flow cytometry analyses of RAW264.7 macrophage cell line and bone marrow-derived macrophages (BMDMs) showed that EO771 CM or 4T1.2 CM significantly enhanced the expression of Gpr132 and CD206 (mannose receptor, a M2 macrophage marker) (Fig. 1 *B–D* and Fig. S1*A*). Together, these results suggest that the acidic signals in EO771 CM and 4T1.2 CM may facilitate macrophage M2 activation via Gpr132.

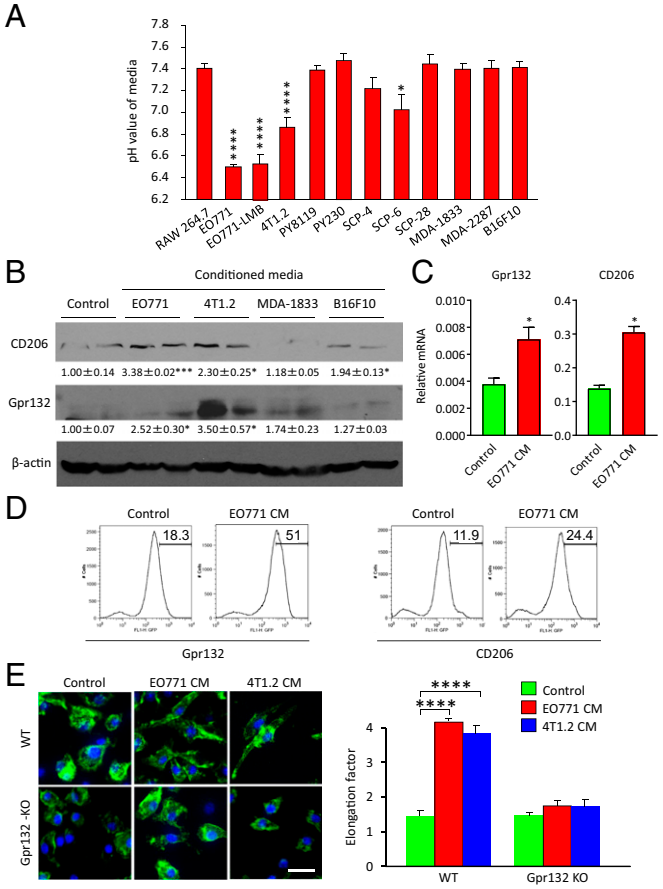

Fig. 1. Tumor-derived factors activate M2-like macrophages via Gpr132. (*A*) pH values of CM from cancer cells or macrophages ($n = 5–8$). *$P < 0.05$, ****$P < 0.001$ compared with RAW 264.7 macrophage CM. (*B*) Western blot for CD206 and Gpr132 in RAW 264.7 macrophages after treatment with the indicated cancer cell CM for 24 h. Actin was used as a loading control. The CD206/actin or Gpr132/actin ratio was quantified and is shown as fold changes compared with control ($n = 3$). *$P < 0.05$, ***$P < 0.005$ compared with control. (*C*) RT-qPCR analysis of Gpr132 and CD206 mRNA in BMDMs with or without EO771 CM treatment ($n = 3$). *$P < 0.05$. (*D*) Flow cytometry analysis of Gpr132 and CD206 in BMDMs with or without EO771 CM treatment. The experiments were repeated twice, and the representative results are shown. (*E*) Immunofluorescence staining for CD11b in WT and Gpr132-KO BMDMs after differentiation in the absence or presence of 30% (vol/vol) EO771 CM or 4T1.2 CM for 7 d. Elongated macrophage morphology indicates an M2-like phenotype. Nuclei were stained with DAPI. (*Left*) Representative images. (Scale bar, 25 μm.) (*Right*) Quantification of macrophage morphology as an elongation factor ($n = 2–4$). ****$P < 0.001$.

We differentiated bone marrow cells from wild-type (WT) or Gpr132 knockout (Gpr132-KO) mice into macrophages with or without 30% (vol/vol) EO771 CM or 4T1.2 CM for 7 d. BMDMs from WT mice, but not Gpr132-KO mice, when treated with EO771 CM or 4T1.2 CM, became elongated and stretched, a feature of M2-like TAMs (Fig. 1*E*). The change of cell morphology is an approach widely used to assess the phenotype of macrophages (10, 21–24). Specifically, Su et al. (10) showed that macrophages that are polarized to M2-like TAMs by cancer cell-derived lactate exhibit stretched and elongated morphology. Consistent with this observation, EO771 CM or 4T1.2 CM also enhanced the expression of M2 markers, such as arginase 1 (Arg-1) and CD206, in WT BMDMs, but not, or to a lesser extent, in Gpr132-KO BMDMs (Figs. S1*B* and S2*C*). These results indicate a key role of Gpr132 in macrophage M2 activation upon education by cancer cell acidic signals.

Tumor-Derived Lactate Stimulates Gpr132 to Promote Macrophage M2 Activation. Considering that Gpr132 is an acidic signal-sensing receptor and the reported Gpr132 ligands, such as 9-hydroxyoctadecadienoic acid (25), are small molecules, we fractionated EO771 CM by size (<3 kDa and >3 kDa), and then compared the pH value and M2 activation function in both fractions. We found that the <3-kDa fraction exhibited lower pH than the > 3-kDa fraction (Fig. S2*A*). Moreover, the <3-kDa fraction, but not the >3-kDa fraction, enhanced CD206 expression in WT macrophages, whereas neither fraction had an effect on Gpr132-KO macrophages (Fig. S2*C*). Fractionation of basal culture media did not change the pH value or CD206 expression in macrophages (Fig. S2 *A* and *B*). These results suggest that the small-molecule soluble factors in the <3-kDa fraction of EO771 CM may function as Gpr132 ligands/activators to promote the macrophage M2-like phenotype.

To test whether lipid factors were involved, we isolated lipids from the <3-kDa fraction of EO771 CM, and applied them to WT and Gpr132-KO macrophages. These lipids did not enhance M2 phenotype but, instead, exhibited slight inhibitory effects in both WT and Gpr132-KO macrophages (Fig. S3 *A–E*). These results exclude the potential role of CM lipids in stimulating M2 macrophages or activating Gpr132, suggesting other factors may be responsible, such as lactate, which is a potent tumor-derived factor inducing TAM polarization (14).

To determine whether lactate in the <3-kDa fraction of EO771 CM could bind to macrophage Gpr132, we performed coimmunoprecipitation with anti-Gpr132 in WT and Gpr132-KO BMDMs. Lactate pulled down by Gpr132 was quantified by liquid chromatography-mass spectrometry. The results showed that lactate was enriched by 7.1-fold in the eluent from WT macrophages compared with Gpr132-KO macrophages (Fig. 2*A*), suggesting that lactate is a potential ligand of Gpr132.

To determine whether Gpr132 is required for lactate signaling in macrophages, we performed a calcium mobilization assay. The results showed that Gpr132 deletion specifically compromised lactate-triggered, but not hydrochloric acid (HCl)-triggered, calcium mobilization (Fig. 2*B*). This finding not only further supports Gpr132 as a functional receptor for lactate but also reveals lactate, rather than simply low pH, as a key activation signal of Gpr132.

We next examined whether lactate was the main factor responsible for the Gpr132-mediated EO771/4T1.2 CM-induced M2 macrophage. First, we measured lactate levels in the CM of distinct cancer cell lines using a Vitros 250 chemistry analyzer (Johnson and Johnson). We found that the lactate level was significantly higher in the lower pH EO771 and 4T1.2 CM compared with CM of other breast cancer cell lines (Fig. S4*A*). Lactate was secreted from EO771 cells in a time-dependent manner (Fig. S4*B*), and distributed in the <3-kDa fraction (Fig. S4*C*). Second, we tested the effects of blocking lactate production from EO771 and 4T1.2 cells by oxamic acid, an inhibitor of lactate dehydrogenase (10). Oxamic acid treatment depleted lactate in the

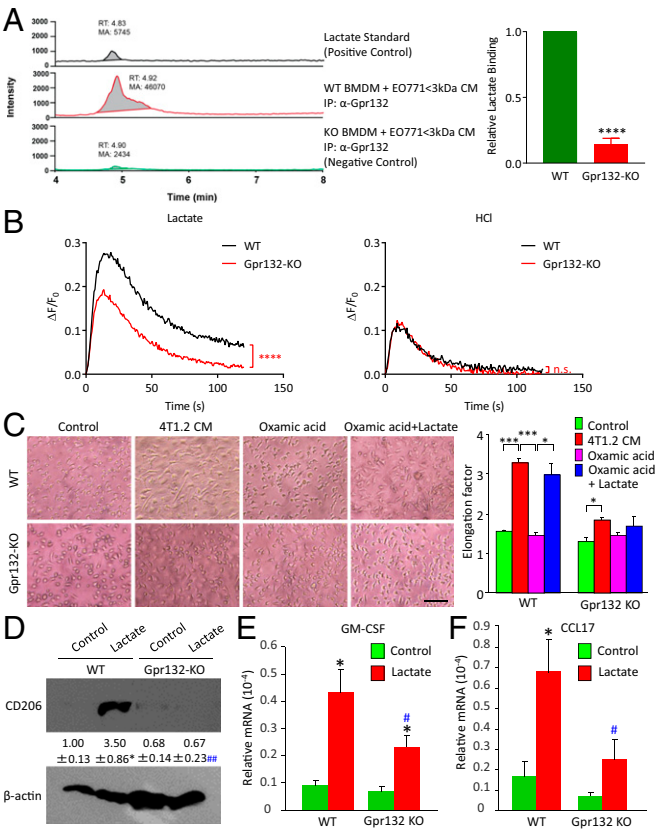

Fig. 2. Lactate is a Gpr132 ligand/activator that induces the macrophage M2 phenotype. (*A*) Lactate in the EO771 <3-kDa CM bound to macrophage Gpr132. Liquid chromatography-mass spectrometry (LC-MS) was used to quantify lactate in the eluent of Gpr132 coimmunoprecipitation (IP). (*Left*) Representative LC-MS chromatograms. MA, integrated peak mass area (area under curve); RT, retention time. (*Right*) Quantification of relative lactate binding ($n = 3$). ****$P < 0.001$ compared with WT. (*B*) Calcium mobilization triggered by lactate, but not HCl, was significantly impaired in Gpr132-KO BMDMs. WT or Gpr132-KO BMDMs were stimulated with 25 mM lactate (*Left*) or HCl (*Right*) ($n = 3$). (*C*) Macrophage morphology after cancer cell CM treatment. The 4T1.2 cells were cultured in the presence or absence of oxamic acid (90 mM) for 3 d. CM was harvested after the cells were cultured for another 24 h without oxamic acid. As a rescue treatment, CM of oxamic acid-treated 4T1.2 cells was supplemented with exogenous lactate. (*Left*) Representative images of WT and Gpr132-KO BMDMs treated with the indicated CM for 24 h. (Scale bar, 500 μm.) (*Right*) Quantification of macrophage morphology as an elongation factor ($n = 2$–3). *$P < 0.05$, ***$P < 0.005$. (*D*) Western blot for CD206 in WT and Gpr132-KO BMDMs after treatment with lactate (5 mM) for 24 h. Actin was used as a loading control. The CD206/actin ratio was quantified and is shown as fold changes compared with control ($n = 4$). RT-qPCR analysis of the expression of GM-CSF (*E*) and CCL17 (*F*) in WT or Gpr132-KO BMDMs in the presence or absence of lactate (25 mM) for 6 h ($n = 3$–4). In *D*–*F*, *$P < 0.05$ compared with vehicle control in the same genotype, ##$P < 0.05$ and #$P < 0.1$ compared with WT control under the same treatment condition.

CM (Fig. S4*D*), leading to a significant increase of pH (Fig. S4*E*), indicating that lactic acid was the main contributor of CM acidity. Importantly, oxamic acid treatment completely abolished the ability of the EO771 or 4T1.2 CM to induce M2-like morphology in WT BMDMs (Fig. 2*C* and Fig. S5*A*), which was restored by the addition of exogenous lactate (Fig. 2*C*). This finding indicates that lactate was the major mediator of the TAM-modulating activity in the cancer cell CM. Moreover, Gpr132-KO BMDMs were refractory to any of these treatments (Fig. 2*C* and Fig. S5*A*), further supporting the essential role of Gpr132 in sensing and responding to lactate.

To confirm the role of the lactate-Gpr132 axis in M2 macrophage activation, we treated WT and Gpr132-KO macrophages with exogenous lactate. Western blot and RT-qPCR analyses showed that lactate increased the expression of M2 markers in WT macrophages, including CD206, granulocyte macrophage colony-stimulating factor (GM-CSF), and C-C motif chemokine ligand 17 (CCL17), but these effects were absent or largely attenuated in Gpr132-KO macrophages (Fig. 2 *D*–*F* and Fig. S5*B*). Taken together, these results suggest that Gpr132 is a macrophage lactate receptor/sensor and cancer cell-derived lactate is a Gpr132 ligand/activator that stimulates macrophage M2 polarization.

Gpr132 Specifically Responds to Lactate to Activate M2 Macrophages. To examine whether Gpr132 is a specific receptor/sensor of lactate during M2 macrophage activation, we treated macrophages with interleukin-4 (IL-4), a notable and standard T helper 2 cytokine widely used to trigger macrophage M2 activation (7, 9, 26). We found that WT and Gpr132-KO macrophages responded to IL-4 equally well for the induction of M2 markers, including Arg-1, CCL17, CCL22, peroxisome proliferator-activated receptor gamma (PPAR-γ), and chitinase 3-like 3 and 4 (also known as YM-1 and YM-2, respectively) (Fig. S6 *A*–*F*). This finding suggests that IL-4 induction of M2 macrophages is independent of Gpr132. Moreover, lipopolysaccharide-induced M1 macrophage activation was also largely intact in Gpr132-KO macrophages (Fig. S7 *A*–*F*). These data indicate that Gpr132 is a specific macrophage receptor/sensor for lactate that specifically mediates lactate-induced M2 macrophage activation.

Lactate-Activated Macrophages Promote Breast Cancer Cell Adhesion, Migration, and Invasion via Gpr132 in Vitro. M2 macrophages have been shown to facilitate breast cancer metastasis via secreted factors (9). Thus, we investigated whether lactate-induced M2 macrophages promote breast cancer cell adhesion, migration, and invasion via paracrine mechanisms in a Gpr132-dependent manner. We first examined the effects of the CM from various pretreated macrophages on breast cancer cell adhesion. Compared with CM from untreated control macrophages, CM from 4T1.2 CM- and lactate-activated macrophages significantly increased the adherence of 4T1.2 cells to fibronectin (Fig. 3*A*), the most abundant extracellular matrix protein in breast cancer stroma (27). Interestingly, we found that these effects were abrogated by a Gpr132-blocking antibody, but not an IgG isotype control (Fig. 3*A*).

We next used Boyden chamber assays to examine the migration and invasion of breast cancer cells by plating them in uncoated or Matrigel-coated upper inserts, respectively, together with macrophages in the lower chambers. Compared with untreated WT control macrophages, 4T1.2 CM- and lactate-activated WT macrophages significantly enhanced the number of migrated cancer cells (Fig. 3*B*). Gpr132-KO macrophages led to decreased cancer cell migration under all treatment conditions, indicating that Gpr132 deletion in macrophages both attenuated the effects of endogenous lactate from the upper chamber cancer cell and exogenously added 4T1.2 CM and lactate (Fig. 3*B*). Moreover, Gpr132 deletion in macrophages not only diminished basal breast cancer cell invasion but also completely abrogated breast cancer cell invasion induced by lactate-activated macrophages (Fig. 3*C*).

To confirm further that lactate is a key factor in cancer cell CM that is responsible for M2 macrophage activation to promote cancer cell metastasis, we pretreated cancer cells with oxamic acid and then used their lactate-depleted CM (Fig. S4*D*) to culture macrophages used in Boyden chamber assays. The results showed that the effects of EO771 or 4T1.2 CM-activated WT macrophages on cancer cell migration and invasion were abolished by oxamic acid pretreatment (Fig. S8 *A* and *B*), and were restored by the addition of exogenous lactate in oxamic acid-pretreated cancer cell CM (Fig. S8 *A* and *B*). Once again, Gpr132-KO macrophages did not respond to these treatments (Fig. S8 *A* and *B*). Taken together, these data suggest that macrophage activation by cancer

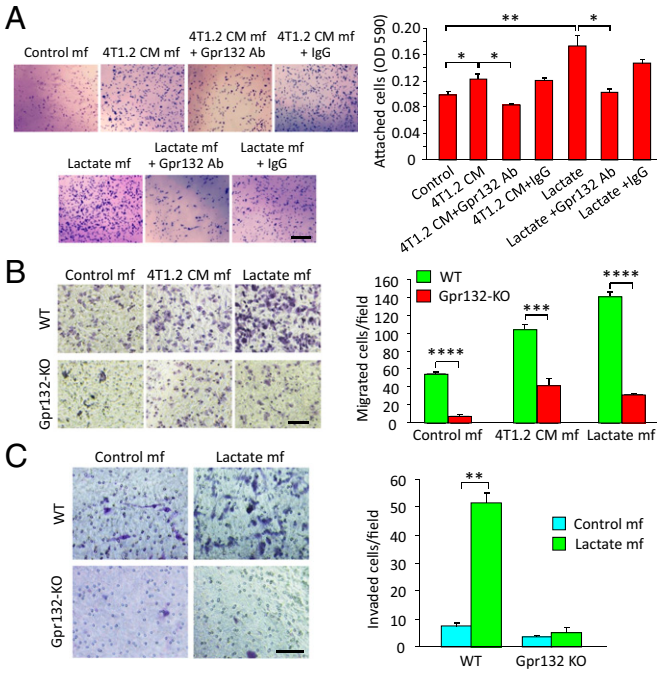

Fig. 3. Lactate-activated macrophage promotes cancer cell adhesion, migration, and invasion via Gpr132. (*A*) Adherence assays. The 4T1.2 cells were suspended in fibronectin (10 mg/mL)-precoated plates with CM from spleen-derived macrophages (mf) that were treated with 4T1.2 CM or lactate (5 mM) with or without Gpr132 antibody (6 mg/mL) or normal IgG (6 mg/mL). The adhered cells were stained with crystal violet, dissolved in 1% Triton X-100, and measured at OD_{590}. (*B* and *C*) Boyden chamber assay of cancer cell migration and invasion. The 4T1.2 cells (*B*) or EO771 cells (*C*) were plated on the upper chamber inserts with untreated (Control), and 4T1.2 CM-activated or lactate-activated spleen-derived WT or Gpr132-KO macrophages plated in the lower chambers. For the invasion assay, the inserts were precoated with 60 μL of Matrigel. After migration for 6 h (*B*) or invasion for 24 h (*C*), the migrated or invaded cells were stained with crystal violet and counted as cells per field of view under the microscope ($n = 3$–4). $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.005$, $^{****}P < 0.001$. (Scale bars, 500 μm.)

cell-derived lactate further promotes breast cancer cell metastasis via Gpr132 in vitro.

Gpr132 Deletion Impedes Breast Cancer Metastasis in Vivo. To investigate the in vivo significance of the lactate-Gpr132 axis in breast cancer metastasis, we first examined if primary tumors could influence Gpr132 expression in premetastatic sites, such as the lung. The results showed that Gpr132 expression was enhanced in the lung of EO771 tumor-bearing mice compared with tumor-free control mice (Fig. S8*C*). Next, we inoculated EO771 cells into the mammary fat pad of female WT and Gpr132-KO mice, and then examined spontaneous lung metastasis. Hematoxylin and eosin (H&E) staining showed that the number and size of EO771 lung metastases were significantly decreased in Gpr132-KO mice compared with WT mice (Fig. 4 *A*–*C*). Recently, a new breast cancer subline, EO771.LMB, has been established, which confers more aggressive lung metastasis without altering primary tumor growth compared with parental EO771 cells (28). We found that spontaneous lung metastasis from EO771-LMB cells was also diminished in Gpr132-KO mice compared with WT mice (Fig. 4 *D*–*G*). In addition to H&E staining-based metastatic foci measurements (Fig. 4 *D*–*F*), we have quantified lung metastatic tumor burden using an activatable, pH-responsive, fluorescence sensor called Probe 5c that has been demonstrated to "turn on" selectively in tumors but not in normal tissues (29), thus serving as a tumor indicator. The ratiometric Probe 5c activated much less in the lung metastases of Gpr132-KO mice than in the lung metastases of WT mice (Fig. 4*G*), which further suggests that Gpr132 deletion impedes breast cancer metastasis.

We next examined whether the reduced lung metastasis in Gpr132-KO mice was related to impaired M2 macrophages. Immunohistochemistry (IHC) and RT-qPCR showed that the expression of M2 macrophage markers, such as CD206, Arg-1, GM-CSF, and CCL22, was lower in the lung of EO771 tumor-bearing Gpr132-KO mice than in WT mice (Fig. 4 *H*–*K*). Together, these data suggest that disruption of the lactate-Gpr132 axis effectively blocks breast cancer metastasis in vivo via compromising M2 macrophage activation.

Gpr132 Correlates with Metastasis and M2 Macrophages in Human Breast Cancer. To assess the clinical significance of Gpr132 in breast cancer metastasis, we analyzed several datasets in PrognoScan. The results revealed that higher Gpr132 expression significantly correlated with lower metastasis-free and relapse-free survival (Fig. S9*A*). Moreover, linear regression analyses of RNA-sequencing

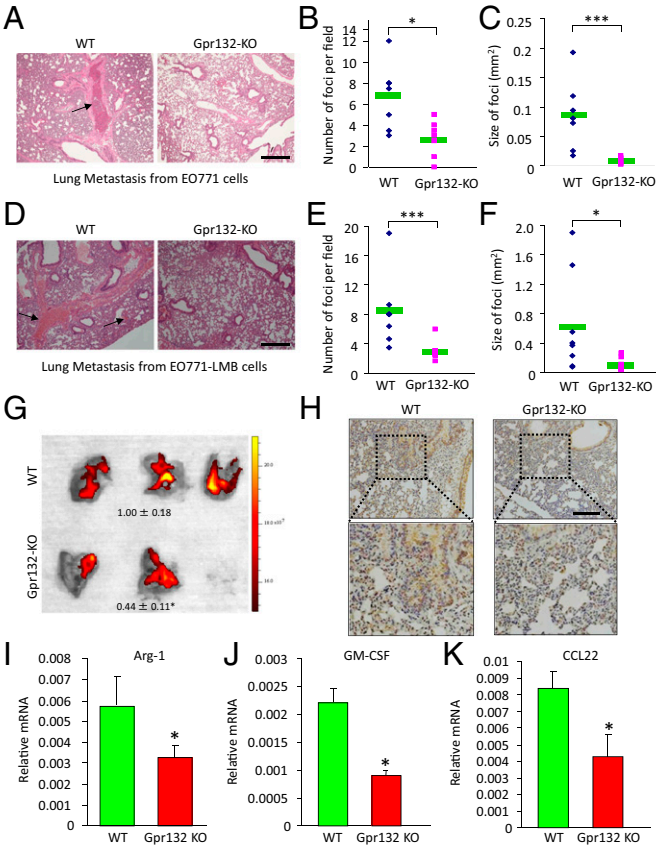

Fig. 4. Gpr132 deletion attenuates breast cancer lung metastasis by reducing M2 macrophages. (*A*–*G*) Lung metastases from breast cancer cells were decreased in Gpr132-KO mice. EO771 cells (*A*–*C*) or EO771-LMB cells (*D*–*G*) were transplanted into the mammary fat pad of WT and Gpr132-KO mice. Primary tumors were resected when they reached 1,500 mm^3 (*A*–*C*) or 650 mm^3 (*D*–*G*). After 25 d (*A*–*C*) or 16 d (*D*–*G*), lungs were harvested and subjected to H&E staining (*A* and *D*). Arrows indicate metastatic foci. The number (*B* and *E*) and size (*C* and *F*) of tumor nodules were quantified from the stained lung sections ($n = 7$–9). (*G*) Lung metastases were quantified using a fluorescent probe that selectively activates in tumors, but not normal tissues, by responding to low pH. In the EO771-LMB model, mice were injected i.v. with Probe 5c 24 h before lung dissection and image acquisition ($n = 2$–3). (*H*–*K*) M2 macrophages in lung metastasis were reduced in Gpr132-KO mice. IHC was performed for CD206 (*H*), as well as RT-qPCR for Arg-1 (*I*), GM-CSF (*J*), and CCL22 (*K*), in the lungs of WT or Gpr132-KO mice of the EO771-LMB model ($n = 4$). $^*P < 0.05$, $^{***}P < 0.005$. (Scale bars, 500 μm.)

data from The Cancer Genome Atlas breast invasive carcinoma database showed that higher Gpr132 expression in breast cancer also significantly correlated with higher expression of M2 macrophage markers, including CD163, CCL17, CCL22, C-C chemokine receptor type 2 (CCR2), toll-like receptor 1 (TLR1), TLR8, transglutaminase 2 (TGM2), and CD200R1 (Fig. S9*B*). These findings suggest that Gpr132 is clinically associated with breast cancer metastasis and M2 macrophage activation in patients with breast cancer, supporting Gpr132 as a valuable prognostic marker and therapeutic target.

Discussion

TAMs are generally biased toward the M2 phenotype and play a critical role in cancer metastasis (3, 9). However, precisely how TAMs are educated by cancer cells is still poorly defined. In this study, we have identified lactate-Gpr132 as a key signal and receiver pair that represents a critical mechanism for TAM polarization and breast cancer metastasis. We show that cancer cell-derived lactate activates macrophage Gpr132 to promote the M2 phenotype. In turn, lactate-activated macrophages enhance cancer cell adhesion, migration, and invasion in vitro and metastasis in vivo, forming a positive feedback loop (Fig. S9*C*). Importantly, we provide evidence that Gpr132 is a lactate receptor/sensor in macrophages that is essential for TAM education by cancer cells. Disruption of this lactate-Gpr132 axis abrogates TAM polarization and breast cancer lung metastasis in mice, and lower Gpr132 expression correlates with better survival in patients with breast cancer. Thus, our findings reveal tumor–macrophage interplay during cancer metastasis, and provide biological insights to tumor immunity and breast cancer intervention.

During tumor progression, the recruited macrophages are usually polarized toward M2 phenotypes by responding to cancer cell-secreted factors, such as macrophage-CSF and GM-CSF (9, 10, 30). Therefore, the specific receptors on macrophages are crucial for sensing these stimuli and TAM polarization. Indeed, inhibition of CSF 1 receptor (CSF-1R) on macrophages impairs M2 polarization and cancer progression (31). In this study, we show that (*i*) in vitro cancer cell CM not only increases Gpr132 expression in macrophages but also promotes macrophage M2 phenotype in a Gpr132-dependent manner and (*ii*) an in vivo primary mammary tumor not only augments the expression of Gpr132 in distant metastatic sites but also develops spontaneous lung metastasis in a Gpr132-dependent manner. As a result, loss of Gpr132 in the tumor environment abrogates both macrophage M2 activation and breast cancer lung metastasis in mice; a lower Gpr132 level correlates with less M2 TAMs and better prognosis with longer metastasis- and relapse-free survival in patients with breast cancer. These findings reveal Gpr132 as a key macrophage receptor for cancer cell signals that contribute to cancer cell education of TAMs. Our work reinforces the concept that macrophages are entrained by cancer cells, and expands the molecular understanding of the signals and receivers mediating TAM polarization.

Gpr132 has been implicated as a member of pH-sensing, G protein-coupled receptor family (13). We therefore screened the pH value in the CM of a panel of cancer cell lines, and examined their effects on M2 macrophage activation. Our data showed that CM of EO771 and 4T1.2 cells, which exhibited lower pH and higher levels of lactate than CM from other cells, stimulated macrophage M2 phenotype via Gpr132. We found that this Gpr132-dependent activity resided in the <3-kDa fraction of the CM and was largely attributed to lactate rather than lipids. Our results further demonstrated both physical and functional interaction of lactate with Gpr132: (*i*) Gpr132 coimmunoprecipitation significantly enriched lactate, indicating a physical binding (Fig. 2*A*); (*ii*) the calcium mobilization assay showed that Gpr132 was specifically activated by lactate, but not HCl, in WT, but not Gpr132-KO, macrophages, supporting Gpr132 as an essential mediator of lactate signaling (Fig. 2*B*); and (*iii*) multiple in vitro and in vivo functional assays illustrated the Gpr132 dependency of lactate regulation of TAMs (Figs. 1–4). These findings support lactate as a key cancer cell-derived ligand/activator for Gpr132 that triggers TAM polarization, whereas other reported Gpr132 ligands, such as 9-hydroxyoctadecadienoic acid (25), may be less important in this context. Moreover, Gpr81 has also been reported to be a lactate receptor that inhibits adipose lipolysis and promotes cancer cell survival (32–34). However, Gpr81 is specifically expressed in mesenchymal and epithelial lineages, such as adipocytes and cancer cells but is absent in macrophages. In contrast, Gpr132 is exclusively expressed in macrophage and other hematopoietic lineages but is absent in adipocytes or cancer cells. Our RT-qPCR analyses reveal that Gpr132 is predominantly expressed in the hematopoietic tissues and highly expressed in macrophages but is largely absent in other tissues or breast cancer cells (20); our IHC staining of human primary breast cancer samples also shows that GPR132 expression mainly originates from hematopoietic cells in the tumor environment, such as macrophages (20). This finding suggests that lactate engages different G protein-coupled receptors in distinct cell types to perform diverse functions. Therefore, our findings identify Gpr132 as a macrophage lactate receptor. This work opens an exciting path to future investigations on the functional roles of the lactate-Gpr132 axis in the cross-talk between metabolism and immunity.

Consistent with our findings, recent studies show that lactate is a pivotal cancer cell-secreted factor driving macrophage M2 polarization (10, 14). The notion that lactate, but not simply a pH drop, triggers macrophage M2 polarization is supported by recent findings that reacidification with lactic acid, but not HCl, in oxamic acid-pretreated cancer cell CM can rescue the effects on macrophages (10). In agreement, our results show that the pH reduction in EO771 CM was prevented after blocking lactate production using oxamic acid, confirming that rising lactate was the main cause of the acidic cancer environment (Fig. S4*E*). Our current work not only confirms previous findings that lactate is a key cancer signal that entrains TAMs but also identifies Gpr132 as a key lactate sensor/receiver on macrophages.

Lactate education of M2 macrophages involves the induction of Arg-1 and the hypoxia-inducible factor 1α (HIF1α)–vascular endothelial cell growth factor (VEGF) pathway (14). Because our results show that Gpr132 is a receptor/sensor of lactate, it is plausible that HIF1α and Arg-1 induction are also part of the downstream events of Gpr132. Indeed, Gpr132-KO macrophages (Fig. S3*A*) and lung metastasis in Gpr132-KO mice (Fig. 4*I*) showed lower Arg-1 expression. Future studies are required to delineate further the detailed downstream signals triggered by lactate activation of Gpr132.

The comigrating tumor cells and macrophages depend on each other for cancer metastasis (10, 35). Our findings show that lactate from cancer cells and Gpr132 on macrophages form a ligand-receptor/signal-receiver pair to activate M2 macrophages, which, in turn, stimulates cancer cell migration and invasion in a paracrine fashion, thereby inducing a positive feedback loop to promote metastasis (Fig. S9*C*). Activation of M2 macrophages may stimulate cancer metastasis via multiple cytokines, such as CCL17, CCL18, CCL22, IL-10, VEGF, and transforming growth factor β (TGF-β) (7, 36, 37). Thus, our study further extends our knowledge and highlights the importance of this vicious cycle in cancer metastasis. Indeed, our in vivo findings show that blockade of this vicious circle by Gpr132 deletion impairs breast cancer lung metastasis by reducing M2 macrophages; our analysis of breast cancer patient data reveals that Gpr132 expression positively correlates with M2 macrophages and poor prognosis. These findings uncover the remarkable clinical potential of Gpr132 as a breast cancer prognostic marker and therapeutic target.

It must be underlined that the specific function of Gpr132 may depend on the distinct microenvironments and ligands. For example, Gpr132 activation by hydroxyoctadecadienoic acids promotes inflammation (38); Gpr132 activation by lysophosphatidylcholine facilitates macrophage recruitment (39). Here, we found that Gpr132 activation by lactate in the tumor environment stimulates macrophage M2 phenotype and exacerbates cancer metastasis. Hence, the function and regulation of Gpr132 are context-dependent.

In summary, our work has uncovered lactate as a ligand/activator of Gpr132 that exerts a key function in macrophages during cancer metastasis. In addition, our identification of Gpr132 as a macrophage lactate sensor/receptor deepens our molecular understanding of how lactate educates TAMs. Furthermore, our elucidation of the roles of the lactate-Gpr132 axis in both macrophages and cancer cells reveals another important mechanism underlying the positive feedback loop between cancer cells and macrophages that is essential for breast cancer metastasis. Finally, our patient data analysis and our genetic Gpr132 blockade provide exciting evidence for Gpr132 inhibition as a therapeutic strategy for the prevention and treatment of breast cancer metastasis.

Materials and Methods

Gpr132-KO mice on a C57BL/6J background were purchased from The Jackson Laboratory (40). All protocols for mouse experiments were approved by the Institutional Animal Care and Use Committee of The University of Texas Southwestern Medical Center.

ACKNOWLEDGMENTS. We thank Dr. Zhaohui Wang [The University of Texas (UT) Southwestern Medical Center] for assistance with concentrating cancer cell CM, Dr. Melanie Cobb and Kathleen Tucker (UT Southwestern Medical Center) for assistance with the calcium mobilization assay, and Dr. Robin L. Anderson (University of Melbourne) for providing the EO771-LMB cell line. Y.W. is a Virginia Murchison Linthicum Scholar in Medical Research and Lawrence Raisz Professor in Bone Cell Metabolism. This work was supported, in part, by the Cancer Prevention Research Institute of Texas (Grant RP130145 to Y.W. and Grant R1212 to D.J.S.), Department of Defense (Grant W81XWH-13-1-0318 to Y.W.), NIH (Grant R01DK089113 to Y.W.), Mary Kay Foundation (Grant 073.14 to Y.W.), March of Dimes (Grant 6-FY13-137 to Y.W.), Welch Foundation (Grant I-1751 to Y.W. and Grant I-1855 to D.J.S.), UT Southwestern Endowed Scholar Startup Fund (Y.W.), and National Cancer Institute Cancer Center Support Grant 5P30CA142543.

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