Lactate and Pyruvate as Mediators of Metabolic Cooperation between Stromal and Breast Cancer Cells

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Introduction: The metabolic reprogramming of epithelial cells during tumor development and progression results in defective oxidative phosphorylation, lactate production/extrusion, increased expression of glucose transporters and enhanced glycolysis[1]. The extrusion of cellular lactate from tumor cells prevents intracellular acidification due to continued enhanced glycolysis[2, 3], while acidifying the tumor microenvironment, and thus, favoring cancer cell invasion and metastasis[4]. While it has been suggested that aerobic tumor cells take up and metabolize extracellular lactate to pyruvate[5, 6], we have previously shown that cancer-associated fibroblasts (CAFs) take up and metabolize lactate via the Krebs cyle[7]. Also, it has been shown that CAFs express the monocarboxylate transporters MCT1 and MCT2[8], which are part of a group of transporters facilitating the efflux of lactate, pyruvate, and butyrate across plasma membranes[9, 10]. In the current study, we further investigated the metabolic cooperation between lactate-expelling MDA-MB-231 breast cancer cells and CAFs which may play an important role in maintaining metastatic/proliferative potency of the tumor cells.

Materials and Methods: Human bone marrow-derived mesenchymal stem cells (MSCs, Lonza Walkersville, Inc. Walkersville, MD) were cultured in α-MEM with 10% fetal bovine serum and 1% penicillin-streptomycin and maintained below passage 8. Cancer-associated fibroblasts (CAFs) were induced by the exposure of MSCs to tumor cell-conditioned medium produced by MDA-MB-231 cells[11, 12]. MDA-MB-231 cells were cultured in DMEM plus 10% fetal calf serum. To study lactate and pyruvate metabolism, hMSCs, CAFs, and MDA-MB-231 cells were incubated with cell culture media supplemented with ^{13}C-3-lactate or ^{13}C-3-pyruvate (Isotec, Sigma-Aldrich, St. Louis, MO) as indicated. Conditioned media were collected; cells harvested and extracted using perchloric acid (PCA), as done previously[13, 14]. Cell extracts were lyophilized and dissolved in 10 mM 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) in D_2O for ^{13}C MRS, while media had added DSS (1:10 dilution). ^{13}C MRS was performed on a 600 MHz Bruker Avance III MR spectrometer with a ^{13}C/^{1}H cryoprobe. 1D ^{1}H-decoupled ^{13}C MRS was acquired using a 30° flip angle, 1536 averages, 39063 Hz spectral width, 1.7 s acquisition time, 134144 number of points, and 2 s relaxation delay. After applying 1 Hz exponential line broadening, the free induction decays (FIDs) were Fourier transformed, phased, and the reference standard DSS set to 0 ppm. To measure lactate uptake rates, $5x10^4$ cells/well of hMSCs or CAFs were plated into 24-well plates and after 18 h allowing for cell attachment, the culture media was exchanged for 200 µL of uptake buffer (10 mM HEPES, 5 mM KCl, 150 mM NaCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, pH 7.5) containing (i) 0.05 µCi ^{14}C-U-lactate (uniformly labeled) for 1 h or 2 h at 37 °C, 5% CO_2. Cells were washed thrice in uptake buffer, lysed for 15 min with 1% SDS in uptake buffer. The uptake of ^{14}C-labeled lactate in the cell lysates quantified using a scintillation counter. To measure pyruvate uptake, MDA-MB-231 cells were seeded into the bottom wells of a Boyden chamber plate, co-incubated for 72 h with media alone or CAF-containing inserts, and subsequently assayed for ^{14}C-pyruvate uptake as previously indicated for ^{14}C-lactate uptake in CAFs. Cancer cell proliferation in response to pyruvate and glucose availability was also determined.

Figure 2: (A) ^{14}C-Lac uptake. ^{13}C MRS of (B) cell extracts from cells incubated with ^{13}C-3-Lac and (C) of MSC-conditioned medium from cells incubated with ^{13}C-3-Lac.

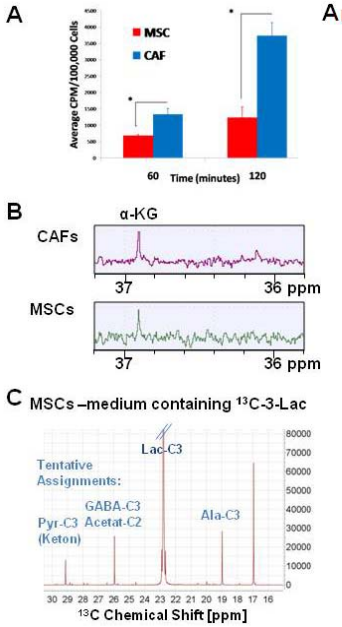
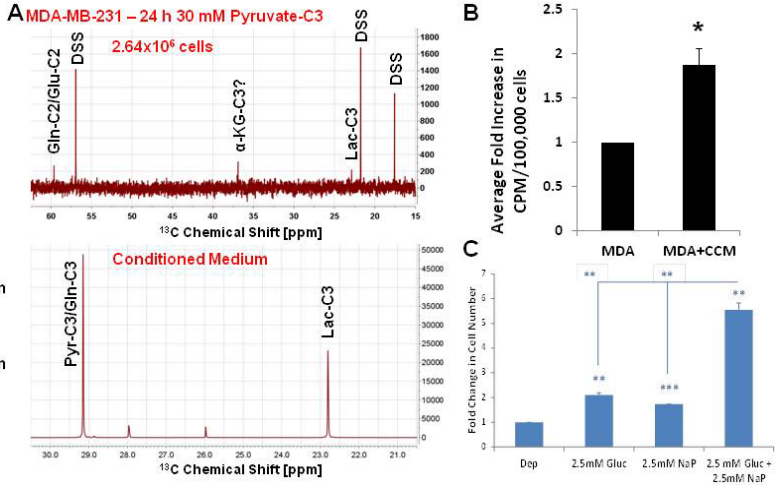


Figure 1: (**A**) ^{13}C MR spectra of an MDA-MB-231 cell extract and their conditioned medium after incubation with ^{13}C-3-pyruvate. (**B**) ^{14}C-pyruvate uptake in MDA-MB-231 cells (MDA) alone and in the presence of CAFs (MDA+CCM). (**C**) MDA-MB-231 cell proliferation changes in response to availability of the nutrients glucose (Gluc) and pyruvate (NaP).



Results/Discusssion: As shown in **Fig. 1A**, CAFs display significantly higher ^{14}C-lactate uptake than their precursors (**n=3, two independent experiments**).

Spectroscopic analyses indicate that ^{13}C-lactate is taken up and further metabolized via the Krebs cycle in MSCs and CAFs (**Fig. 1B**), suggesting that stromal cells metabolize lactate oxidatively. Analyses of MSC-conditioned medium indicate that lactate-metabolizing MSCs secrete pyruvate (**Fig. 1C**). MDA-MB-231 cells convert supplied ^{13}C-3-pyruvate in the medium to ^{13}C-3-lactate, which is predominantly secreted into the medium (**Fig. 2A**). The uptake of pyruvate in MDA-MB-231 cells (MDA) increases in the presence of CAFs (MDA+CCM) (**Fig. 2B**, **n=1**). Also, cell proliferation of MDA-MB-231 cells exposed to culture media either completely depleted of glucose and pyruvate (Dep) or containing indicated amounts of glucose (Gluc) and/or pyruvate (NaP) for 6 days was measured (**Fig. 2C**). These data indicate that the added NaP synergistically increases MDA-MB-231 cell proliferation when Gluc is limited.

Conclusion: These data support that tumor-derived lactate may be used by CAFs to (1) fuel their energetic needs and (2) supply neighboring tumors with valuable energetic and biosynthetic precursors, such as pyruvate, thereby supporting tumor growth.

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