Lactate-mediated Metabolic Cooperation between Human Stromal and Breast Cancer Cells

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Introduction: During tumor development and progression, the epithelial cells undergo metabolic reprogramming leading to defects in oxidative phosphorylation, lactate production/extrusion, increased expression of glucose transporters and enhanced glycolysis [1]. Lactate is extruded from tumor cells to prevent intracellular acidification as a result of continued enhanced glycolysis [2-3]. The resulting acidification of the tumor microenvironment favors cancer cell invasion and metastasis [4]. It has been suggested that the extracellular lactate can be taken up and metabolized to pyruvate by aerobic tumor cells [5-6]. Additionally, it has been shown that tumor-associated fibroblasts express the monocarboxylate transporters MCT1 and MCT2 [7]. Since MCTs facilitate the efflux of lactate, pyruvate, and butyrate across plasma membranes [8-9], a metabolic cooperation between the lactate-expelling tumor cells and the cancer-associated stromal cells may play an important role in maintaining metastatic/proliferative potency of the tumor cells. In the current study, we investigated if stromal fibroblasts are able to take up and metabolize lactate.

Materials and Methods: Human bone marrow-derived mesenchymal stem cells (hMSCs, 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 hMSCs to tumor cell-conditioned medium produced by MDA-MB-231 cells [10-11]. To study lactate metabolism, hMSCs and CAFs were incubated with cell culture media supplemented with 10 mM ^{13}C-3-lactate (Isotec, Sigma-Aldrich, St. Louis, MO) for 4 h. Cells were harvested and extracted using perchloric acid (PCA), lyophilized and dissolved in 10 mM 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) in D_2O for ^{13}C MRS, based on previously published methods [12-13]. ^{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 MR spectra were 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 of hMSCs or CAFs were plated per well 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) or (ii) 0.05 µCi ^{14}C-3-lactate (Perkin Elmer, Waltham, MA) plus 10 mM CHC (alpha-Cyano-4-hydroxy-cinnamic acid, dissolved in DMSO, Sigma, St.Louis, MO) 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. Cellular migration in response to lactate was quantified using the Transwell migration assay [10]. Briefly, cell culture media with no, 5 mM, or 15 mM lactate was placed in the bottom chamber of 24-well, notched tissue culture plates (BD Biosciences, Franklin Lakes, NJ) and cell culture inserts containing $2.5x10^4$ hMSCs or CAFs were placed in the upper chamber. After 18 h, hMSCs or CAFs that had migrated across the 8 µm, uncoated polythene membrane were stained with crystal violet and counted manually using an inverted microscope.

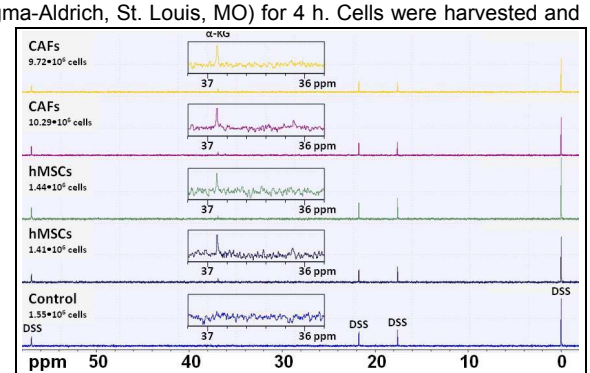

Fig. 1: Zoomed regions of ^{13}C MR spectra obtained from PCA extracts of one control hMSC sample and 2 cell samples each of hMSCs and CAFs exposed to 10 mM ^{13}C-3-lactate for 4 h.

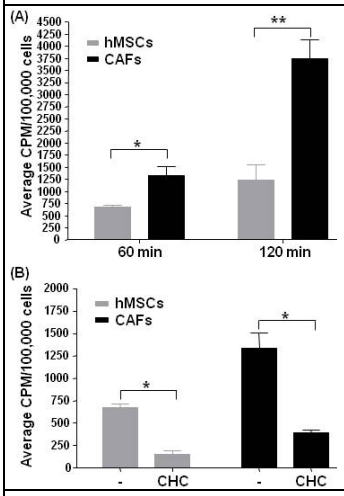

Fig. 2: **(A)** CAFs exhibit higher uptake of ^{14}C-lactate than hMSCs at 60 min and 120 min (*p<0.05 *p ≤ 0.007, 2-tailed, unpaired, unequal variance t-Test, n = 3). **(B)** Inhibition of ^{14}C-lactate uptake by CHC indicates that ^{14}C-lactate transport occurs via MCTs (*p<0.005).

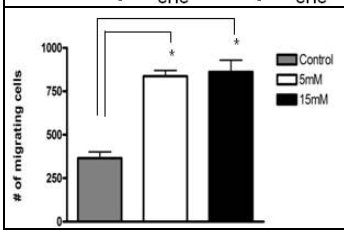

Fig. 3: Lactate stimulates hMSC migration (*p<0.05, unpaired t-Test, n = 4).

Results: Preliminary, metabolic ^{13}C MRS indicate that lactate is taken up and metabolized by hMSCs and CAFs (**Fig. 1**). In both, hMSCs and CAFs, a signal at 36.9 ppm, assigned to ^{13}C-labeled α-ketoglutarate (C-3) based on the chemical shift [14] and the fate of the ^{13}C-3 label of lactate [15], is visible for cells incubated with ^{13}C-3-lactate. Additional signals visible in the full ^{13}C MR spectra were from the standard DSS and HCO_3^-. In hMSCs and CAFs, the lactate uptake is linear for up to 2 h at 37 °C and, on a per cell basis, CAFs took up significantly higher amounts of lactate than hMSCs at all time points measured (**Fig. 2A**). Inhibition by CHC indicated that this uptake was mediated by monocarboxylate transporters (**Fig. 2B**). We found that in the presence of 5 mM or 15 mM lactate the migration of hMSCs (2.5-fold more) was significantly higher than in the control (**Fig. 3**). There was no significant difference in the number of migrating cells towards 5 mM or 15 mM lactate.

Discussion: The uptake and metabolism of lactate in stromal cells demonstrate that stromal cells can utilize lactate produced by tumor cells. Previous studies using immunohistochemical techniques have suggested such metabolic cooperation between stromal fibroblasts and tumor cells [7]. To our knowledge this is the first *in vitro* model system demonstrating that stromal cells can utilize lactate produced by tumor cells. In future, varying incubation periods with ^{13}C-lactate will allow us to investigate in detail the metabolic fate of lactate in stromal cells. Based on the reaction mechanisms, it is likely that lactate is converted to α-ketoglutarate via the TCA cycle. However, it will be important to determine whether ^{13}C-lactate is converted to ^{13}C-α-ketoglutarate exclusively via the TCA cycle or whether this conversion may also occur in the cytoplasmic space via other metabolic conversion pathways.

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