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Tumor stroma interaction is mediated by monocarboxylate metabolism

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ABSTRACT

Human breast tumors contain significant amounts of stromal cells. There exists strong evidence that these stromal cells support cancer development and progression by altering various pathways (e.g. downregulation of tumor suppressor genes or autocrine signaling loops). Here, we suggest that stromal carcinoma-associated fibroblasts (CAFs), shown to be generated from bone marrow-derived mesenchymal stem cells, may (i) recycle tumor-derived lactate for their own energetic requirements, thereby sparing glucose for neighboring glycolytic tumor cells, and (ii) subsequently secrete surplus energetically and biosynthetically valuable metabolites of lactate oxidation, such as pyruvate, to support tumor growth. Lactate, taken up by stromal CAFs, is converted to pyruvate, which is then utilized by CAFs for energy needs as well as excreted and shared with tumor cells. We have interrogated lactate oxidation in CAFs to determine what metabolites may be secreted, and how they may affect the metabolism and growth of MDA-MB-231 breast cancer cells. We found that CAFs secrete pyruvate as a metabolite of lactate oxidation. Further, we show that pyruvate is converted to lactate to promote glycolysis in MDA-MB-231 cells and helps to control elevated ROS levels in these tumor cells. Finally, we found that inhibiting or interfering with ROS management, using the naturally occurring flavonoid phloretin (found in apple tree leaves), adds to the cytotoxicity of the conventional chemotherapeutic agent doxorubicin. Our work demonstrates that a lactate-pyruvate, reciprocally-supportive metabolic relationship may be operative within the tumor microenvironment (TME) to support tumor growth, and may be a useful drug target.

1. Introduction

The Warburg effect, which describes the dependence of tumors on glucose and constitutive aerobic glycolysis, results in the accumulation of cellular lactate as a metabolic byproduct [1,2]. The excess lactate is evacuated from the tumor cell to prevent intracellular acidosis and subsequent apoptosis [3–5]. To facilitate the trafficking of lactate and other monocarboxylic acids, tumor cells and normal tissue express a family of proton-coupled monocarboxylate transporters (MCTs) [6,7]. Specifically, it has been shown that lactate, secreted via low-affinity MCT4 in white, fast-twitch glycolytic myocytes, is taken up through high-affinity MCT1 in neighboring red, slow-twitch oxidative muscle fibers, wherein the metabolite is used to drive energy production [7]. These studies were first evidence of the existence of lactate shuttles and have collectively characterized MCT1 and MCT4 proteins as respective net importers and exporters of lactate [8]. A compelling finding of these investigations is that MCT4/MCT1-mediated lactate shuttles allow oxidative muscles to utilize lactate preferentially over glucose, thereby sparing it for the more glycolytic myocytes [7,8]. Subsequent studies demonstrated the existence of such cell-cell lactate shuttles in astrocytes and neurons [9] and in sperm [10]. Investigations of intercellular metabolic complementarity in pathological systems have suggested that such lactate shuttles may be operative in tumors. Indeed, a recent report indicates that glycolytic triple negative basal breast adenocarci-

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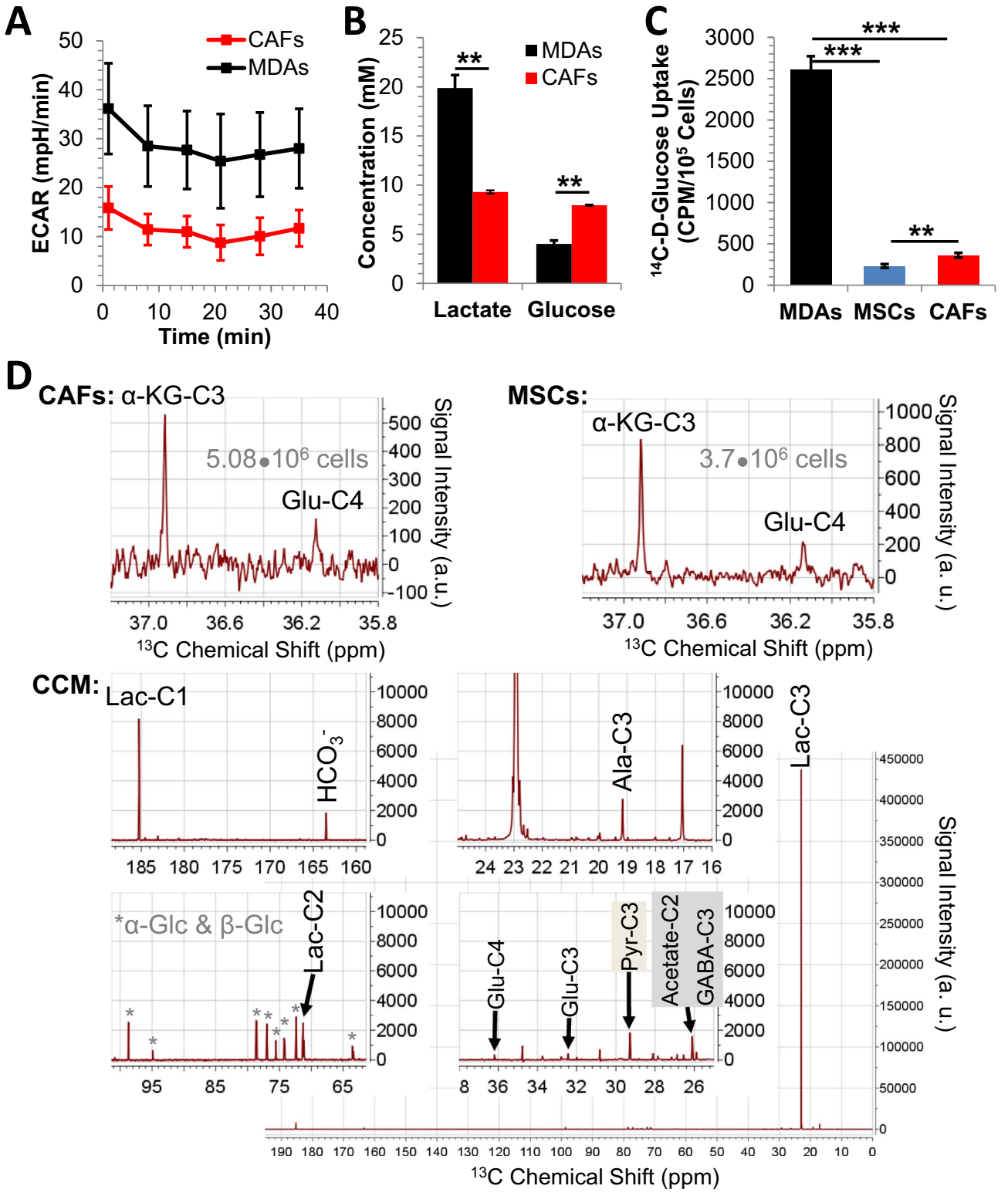

Fig. 1. Glycolytic flux and lactate metabolism. **(A)** The extracellular acidification rate (ECAR; mean ± SD) of MDA-MB-231 cells and CAFs, measured by Seahorse analyzer, reveals that MDA-MB-231 cells are more glycolytic than CAFs. The extracellular acidification rate ECAR measures proton excretion (representing cellular glycolysis) over time in units mpH/min where 1 mpH=4.3 pmole excreted H^+. **(B)** Extracellular glucose consumption and lactate production of MDA-MB-231 cells and stromal cells confirm the higher glycolytic activity of MDA-MB-231 cells compared to CAFs observed by Seahorse Analyzer analysis. Data are displayed as mean ± SD (n=3). **(C)** The glucose uptake is significantly higher in MDA-MB-231 cells (MDAs) than in MSCs or CAFs, in good agreement with the higher aerobic glycolysis observed in the cancer cells. Data are displayed as mean ± SD (n=4). **(D)** CAFs take up and metabolize lactate as well as secrete lactate oxidation metabolites, as shown by ^{13}C MR spectroscopy on cell extracts and CCM. Signal assignments are: α-KG – α-ketoglutarate, Glu – glutamate; Ala – alanine; Lac – lactate; Pyr – pyruvate; α-Glc & β-Glc – α-glucose and β-glucose; "-C" followed by number – position of ^{13}C labeling as a result of metabolic conversion of exogenous 10 mM 3-^{13}C-L-lactate. ** $p < 0.005$, *** $p < 0.0005$ by two-tailed, unpaired, unequal variance Student's T-test; Abbreviations: MDAs – MDA-MB-231 cells; MSCs: human mesenchymal stem cells; CAFs: cancer-associated fibroblasts; CCM – CAF-conditioned medium.