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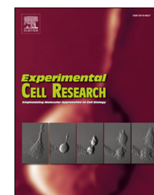

Research Article

Modulation of the uptake of critical nutrients by breast cancer cells by lactate: Impact on cell survival, proliferation and migration

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ABSTRACT

This work aimed to characterize the uptake of folate and glucose by breast cancer cells and to study the effect of lactate upon the transport of these nutrients and upon cell viability, proliferation and migration capacity.

Data obtained showed that: a) MCF7 cells uptake ^{3}H-folic acid (^{3}H-FA) at physiological but not at acidic pH; b) T47D cells accumulate ^{3}H-FA and ^{14}C-5-methyltetrahydrofolate (^{14}C-5-MTHF) more efficiently at acidic than at physiological pH; c) ^{3}H-deoxyglucose (^{3}H-DG) uptake by T47D cells is sodium-independent, inhibited by cytochalasin B (CYT B) and stimulated by insulin.

Regarding the effect of lactate, in T47D cells, acute (26 min) and chronic (24 h) exposure to lactic acid (LA) stimulated ^{3}H-FA uptake. Acute exposure to LA also stimulated ^{3}H-DG uptake and chronic exposure to LA significantly stimulated T47D cell migratory capacity.

In conclusion, the transport of folates is strikingly different in two phenotypically similar breast cancer cell lines: MCF7 and T47D cells. Additionally, lactate seems to act as a signaling molecule which increases the uptake of nutrients and promotes the migration capacity of T47D cells.
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1. Introduction

Breast cancer is the second most common cancer in the world and the most common cancer among women, both in developed and developing regions of the world [1]. Although the survival is improving, it is expected that the burden of this disease will increase globally [2].

Tumor cells are characterized by accelerated cell growth and proliferation and thus they are strictly dependent on the uptake and metabolism of nutrients. Indeed, the reprogramming of energy metabolism is recognized as a cancer hallmark [3].

Folates constitute a family of structurally related compounds which include folic acid (FA; pteroylmonoglutamic acid) and reduced folates of which 5-methyltetrahydrofolate (5-MTHF) is the most abundant plasma folate form [4].

The transport of folates into cells is mediated by highly specific transporters, such as, the reduced folate carrier-1 (RFC1), the proton-coupled folate transporter (PCFT) and folate receptors (FR) [5]. Once inside the cells, folates facilitate the transfer of one-carbon units in cellular processes critical for the regulation of gene expression and for cell division [6,7].

Glucose is the primary source of energy for mammalian cells. There are two types of glucose transporters: the sodium-dependent glucose co-transporters (SGLTs; gene symbol *SLC5A*) and the facilitative glucose transporters (GLUTs; gene symbol *SLC2A*).

Actively proliferating cancer cells convert glucose into lactic acid (LA) even when oxygen is present, a process known as the Warburg effect [8]. This *aerobic fermentation of glucose* seems to be an inefficient bioenergetic process when compared with oxidative phosphorylation, but it is thought to be compensated by the much higher rates of glucose uptake and glycolysis observed in tumor cells when compared to normal cells [9].

Lactate is produced by anaerobic glycolysis, and for much of the last century it was largely considered as a waste product of metabolism [10]. However, not only lactate released from cells is taken up by other tissues (mainly the liver) where it is used to

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synthesize glucose (via gluconeogenesis) that is released back into blood for consumption by extra-hepatic tissues (the Cori cycle) [11], but also, released lactate can be directly used as a fuel for some tissues, such as muscle and brain. This seems to be also the case with the majority of solid tumors which contain two subpopulations of cells: hypoxic tumor cells, which produce lactate, and oxygenated tumor cells, which use lactate [10]. Indeed, tumor hypoxia may occur in a disorganized manner throughout tumor tissue, arising not only from distance from blood supply, but also from inefficient tumor vessel orientation, disturbed red blood cell homeostasis (resulting for example in altered red cell flux and increased blood viscosity) and also from an increased demand of oxygen that exceeds the threshold of supply [12]. According to this model, tumors are *metabolic symbionts* by which hypoxic and oxygenated tumor cells mutually regulate their access to energy metabolites [13].

The transfer of lactate across the plasma membrane is mediated by monocarboxylate transporters (MCTs; gene symbol *SLC16A*), which co-transport the lactate anion together with one proton (H^+), or by sodium-coupled MCTs (SMCTs) [14].

Despite the importance of folates in tumor progression and the use of many well-known antifolate drugs (e.g., methotrexate) for breast cancer chemotherapy, folate uptake in breast cancer cells is still largely unexplored. The same occurs with glucose, since there are few studies on glucose uptake and its regulation by these tumor cells.

In this work we hypothesize that lactate may regulate breast cancer cell biology regulating nutrient uptake and potentially cell viability, proliferation and migratory capacity.

2. Materials and methods

2.1. Materials

^{3}H-DG (Deoxy-D-glucose, 2-[1,2-^{3}H(N)]; specific activity 60.0 Ci/mmol), ^{3}H-FA (folic acid, [3′,5′,7,9-^{3}H] sodium salt; specific activity 56.8 Ci/mmol) (American Radiolabeled Chemicals Inc., St. Louis, MO, USA), ^{14}C-5-MTHF (5-[^{14}C]-methyl-tetrahydrofolic acid barium salt; specific activity 55.0 mCi/mmol), [^{3}H]thymidine ([methyl-^{3}H]thymidine; specific activity 79.0 Ci/mmol) (GE Healthcare GmbH, Freiburg, Germany). 2-deoxy-D-glucose, 5-MTHF (5-methyltetrahydrofolate), antibiotic/antimycotic solution (100 U ml^{-1} penicillin, 100 μg ml^{-1} streptomycin and 0.25 μg ml^{-1} amphotericin B), choline chloride, cytochalasin B (CYT B, from *Drechslera dematioidea)*, DMEM (Dulbecco's modified Eagle's medium), folic acid (FA), HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid), human recombinant insulin, L(+)-lactic acid (LA), lithium chloride, MES (2-[*N*-morpholino]ethanesulfonic acid), MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide), MTX (methotrexate), phloridzin, PTX (pemetrexed), SITS (4-acetamido-4′-isothiocyano-2,2′-disulfonic acid stilbene), sodium L-lactate (SL), sodium propionate (SPROP), TPP (thiamine pyrophosphate), trypsin–EDTA solution (Sigma, St. Louis, MO, USA). FBS (fetal bovine serum), MEM (Minimum Essential Medium) (Gibco, Life Technologies Corporation, CA, USA). DMSO (dimethylsulfoxide), Triton X-100, ethanol, DNAse I (deoxyribonuclease) (Invitrogen Corporation, CA, USA). D-glucose, sodium bicarbonate (Merck, Dramstad, Germany) and Tripure® (Roche Diagnostics, Basel Switzerland).

The compounds to be tested were dissolved in water, ethanol, DMSO, sodium bicarbonate, 0.01 M HCl or 0.1 M NaOH. The concentration of these solvents was 1% in the buffer or 0.1% in the culture media for acute or chronic treatments, respectively. Controls for test compounds were run in the presence of the respective solvent.

2.2. Cell culture

MCF7 cell line was obtained from the American Type Culture Collection (ATCC 37-HTB, Rockville, Md., USA) and used between passage number 27 and 40. T47D cell line was kindly donated by Prof. Lígia Rodrigues (Dept. Biological Engineering, University of Minho, Braga, Portugal) and used between passage number 30 and 100. MCF7 and T47D cells were cultured in MEM and DMEM, respectively, both media supplemented with 10% inactivated FBS and 1% antibiotic/antimycotic solution. Cells were incubated at 37 °C in a humidified atmosphere of 5% CO_2-95% air. Culture medium was changed every 2–3 days and the culture was split every 7 days. For transport experiments, determination of cell viability or migration capacity, cells were seeded on 24-well plastic cell culture dishes (2 cm^2; Ø 16 mm; TPP®) and were used after 2–5 days in culture (90–100% confluence). For proliferation assays, cells were seeded on 24-well plastic cell culture dishes and were used after 1–3 days in culture (70–80% confluence). For RNA extraction, cells were seeded on 21 cm^2 plastic culture dishes and were used after 4 days in culture (90–100% confluence).

2.3. Transport studies

^{3}H-FA and ^{14}C-5-MTHF transport experiments were performed in buffer with the following composition (in mM): 12.5 HEPES-NaOH, 12.5 MES, 5 KCl, 140 NaCl and 5 D(+)-glucose, pH 5.0, 5.5 or 7.5 (except in the pH-dependence studies, where buffer was adjusted to a range of pH 5 to 8).

^{3}H-DG transport experiments were performed in a glucose-free buffer with the following composition (in mM): 12.5 HEPES-NaOH, 12.5 MES, 5 KCl, 140 NaCl, pH 7.4 (except in the pH-dependence studies, where buffer was adjusted to a range of pH values from 5 to 8).

^{3}H-DG was used as model of glucose since deoxyglucose is an unmetabolizable D-glucose analogue which is transported efficiently by GLUTs, but it is poorly transported by SGLT1 [15].

Initially, the culture medium was aspirated and the cells were washed with 0.3 ml buffer at 37 °C. Then the cell monolayers were pre-incubated for 20 min in buffer at 37 °C. Uptake was initiated by the addition of 0.2 ml buffer at 37 °C containing a total amount of 20 nM ^{3}H-FA, 5 μM ^{14}C-5-MTHF or 1 μM ^{3}H-DG, except in the saturation experiments, as indicated. Incubation was stopped after 6 min (except in the time-course experiments) by removing the incubation medium, placing the cells on ice and rinsing them with 0.5 ml ice-cold buffer. Cells were then solubilized with 0.3 ml 0.1% (v/v) Triton X-100 (in 5 mM Tris–HCl, pH 7.4), and placed at room temperature overnight. Radioactivity in the cells was measured by liquid scintillometry.

2.4. Acute and chronic effects of selected compounds

The concentrations of compounds to be tested both acutely and chronically were chosen based on previous work of our group [16–18]. Lactate concentrations used were chosen based on the knowledge that lactate levels in human tumors (e.g., cervix cancer) may range from 4 mM up to 40 mM, with an average level of 10 mM [19].

The acute effect of the selected compounds on ^{3}H-FA and ^{3}H-DG uptake was tested by pre-incubating (20 min) and incubating (6 min) cells with ^{3}H-FA or ^{3}H-DG in the presence of the compounds to be tested, at pH 5.0 or 7.4, respectively.

The chronic effect of the compounds on the uptake of the same substrates was tested by cultivating cell cultures (90–95% confluence) in FBS-free culture medium containing the compounds to be tested or the respective solvent. The transport experiments were performed after 24 h and they were identical to the

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