

Supplemental Material

Targeting lactate-fueled respiration as a new antitumor strategy

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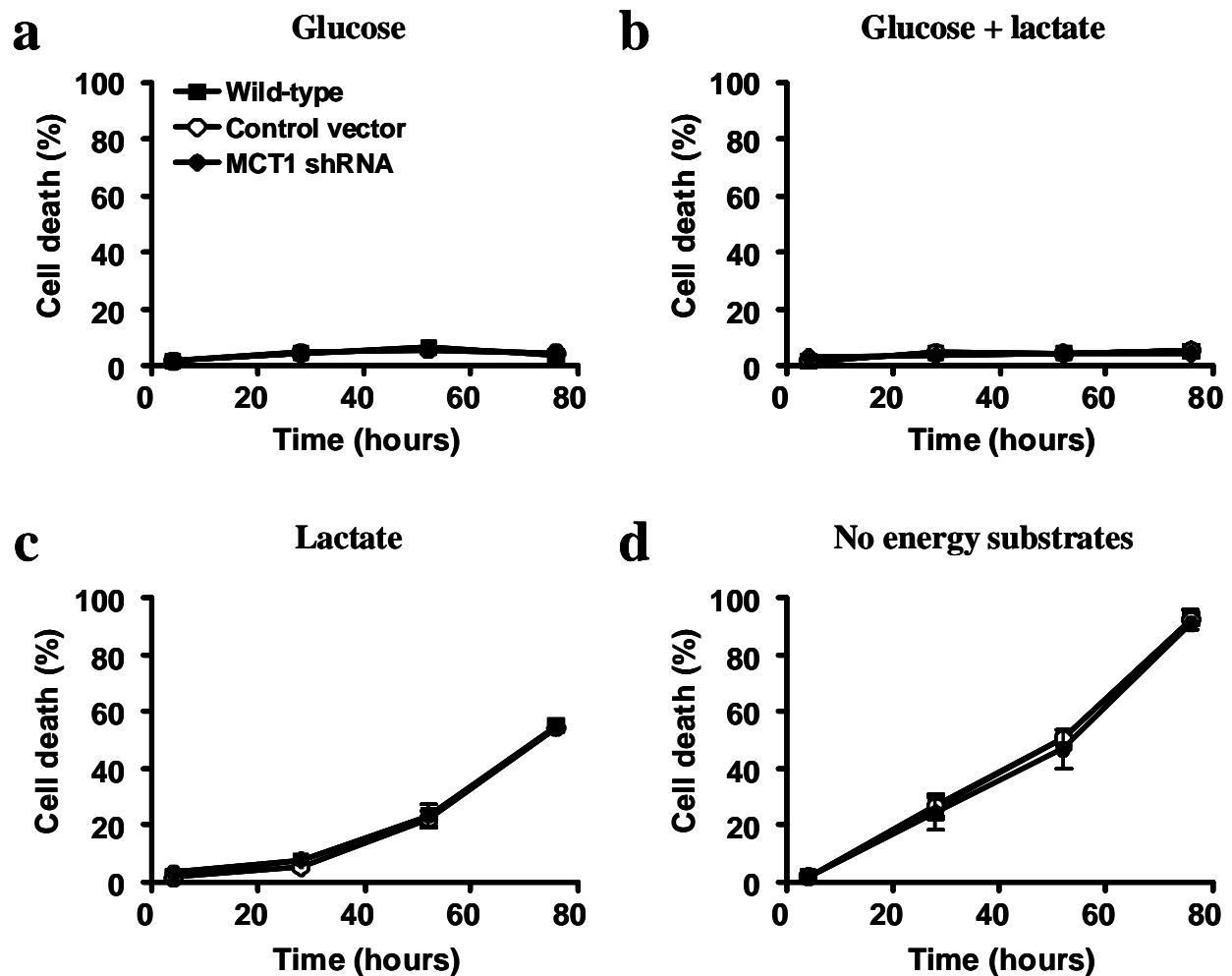
Supplemental Results and Discussion

Neither lactate nor MCT1 modulate glucose starvation of Warburg-phenotype tumor cells. In the presence of glucose, *MCT1* gene silencing (RT-PCR confirmed an 85.51 ± 0.33 % transcript extinction) did not affect survival of glycolytic WiDr tumor cells cultured in the absence (Supplemental Figure 1A), or in the presence of exogenous lactate (Supplemental Figure 1B). Moreover, when lactate was the sole potential source of energy, WiDr cells died at a rate independent of *MCT1* expression (Supplemental Figure 1C), but delayed compared to full nutrient starvation (Supplemental Figure 1D). This latter observation provides evidence for a possible *MCT1*-independent lactate recycling path in glycolytic WiDr tumor cells.

MCT1 inhibition does not induce overt toxicity. Possibility of systemic toxicity to *MCT1* inhibition deserves outmost attention. In this study, we used chronic delivery of CHC, one of the most potent commercially available *MCT1* inhibitor, as a model treatment. CHC possesses the important advantage to be a reversible inhibitor (1), which should limit the duration of any toxic event. Occurrence of transient lethargy in healthy animals treated by CHC is a good illustration of that property (2). It is however of note that CHC also inhibits the mitochondrial pyruvate carrier (MPC) (3) and the anion exchanger AE-1 (4). Identification and evaluation of specific *MCT1* inhibitors are thus warranted to translate our treatment to the clinics.

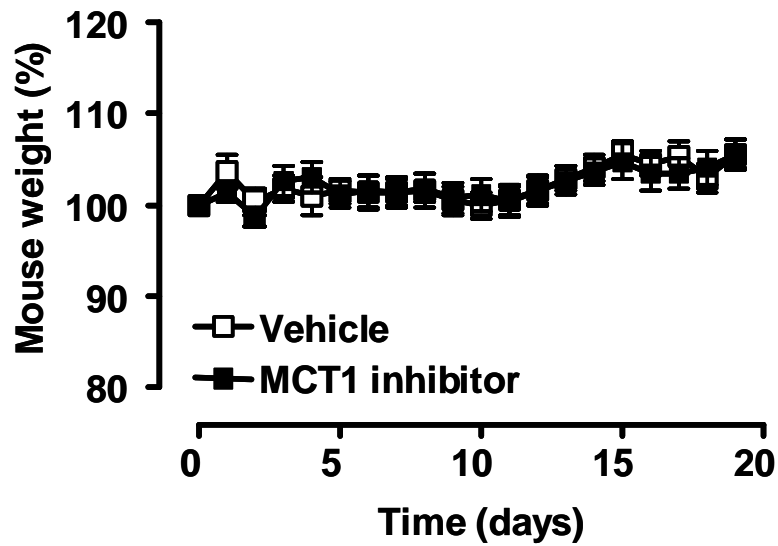
MCT1 is found in the great majority of tissues, with high expression in heart (5), skeletal muscle (6), brain (7), and erythrocytes (8). In heart and skeletal muscles, *MCT1* transports lactic acid and ketone bodies into cells for oxidation as respiratory fuels (6). It should however be noted that in the rare condition of cryptic exercise intolerance (CEI) due to ubiquitous *MCT1* deficiency in humans (9), resting patients present no symptoms of pathology; severe chest pain and muscle cramps only occur after intensive exercise and resume upon completion. Similarly, we observed no apparent symptoms after *MCT1* inhibition by CHC for 20 days in mice, and no weight loss that could have signed rhabdomyolysis (Supplemental Figure 2). In addition to high level *MCT1* expression, myocytes co-express two functionally distinct *MCTs* with high lactate affinity ($K_m = 2.5-3$ mM), one of them being totally insensitive to clinically relevant doses of *MCT1* inhibitors (10, 11). Because these transporters control preferred paths for lactate uptake (12), *MCT1* inhibition does not block lactate transport in myocytes (11) and, as in CEI patients, should not cause cardiotoxicity to cancer patients at rest. In erythrocytes of CEI patients, reduced lactate uptake was also asymptomatic (13).

Supplemental Figures



Supplemental Figure 1

Glucose starvation-induced death of Warburg-phenotype tumor cells is not influenced by lactate or MCT1 expression. (A-D) Cell death was determined over time with a NucleoCounter when culturing wild-type, vector-transfected, and MCT1-silenced WiDr cells in the presence of glucose only (A), glucose + lactate (B), lactate only (C), or in the absence of both energy substrates (D). Error bars represent the SEM and are sometimes smaller than symbols. No statistical difference was detected (two-way ANOVA, $n = 3$).

**Supplemental Figure 2**

MCT1 inhibition does not induce mouse weight loss. Rj:NMRI mice that received daily intraperitoneal injections of CHC (25 μmol in 200 μl) or vehicle (200 μl) were weighted every day. Error bars represent the SEM. No statistical difference was detected (two-way ANOVA, $n = 6$).

Supplemental References

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