Extracellular lactate promotes osteosarcoma progression via its effect on mitochondrial dynamics

Shambhavi Bhagwat[1, 2], Matthew L. Goodwin[1],
[1]Washington University, School of Medicine, MO
s.bhagwat@wustl.edu

Introduction: Osteosarcoma (OS) is a primary malignant tumor most commonly found in long bones such as the femur, tibia, or humerus. OS progression, like other cancers, can be influenced by metabolites present in the tumor microenvironment (TME). Clinically, OS is ^{18}FDG-PET positive (based on glucose-uptake). However, recent in silico studies have shown that glucose has a very limited influence on growth of OS cells, while lactate can be utilized as a fuel source. In addition, OS tumors induce higher rates of glycolysis in the surrounding stromal cells, resulting in an excess in production and export of lactate via monocarboxylate transporters (MCTs). MCTs are responsible for shuttling lactate between producing and consuming cells throughout the body. Targeting this lactate shuttling in tumors has recently become a promising focus for anti-cancer therapy. However, the importance of lactate in OS is yet to be determined. This study focuses on determining the role of lactate transport in OS progression. Here, we show that extracellular lactate may promote OS progression via it's effect on mitochondrial dynamics.

Material and Methods: Pedigree-established osteosarcoma cell lines were used to determine the effect of varying lactate concentration and MCT KD on OS cell viability/proliferation as well on mitochondrial morphology. Cell viability and proliferation was analyzed using the CyQuant Direct Cell proliferation kit. Genes were knocked down by siRNA transfections, using RNAiMAX (Invitrogen) and gene expression was determined via qPCR analysis. Conditions included combinations of low (0.1mM), normal (1mM) and high (5mM) [lactate] and low (1mM), normal (6mM) and high (20mM) Glucose. All data are presented as the means ± SD where appropriate. We performed statistical analysis using Student's t-test or three-way ANOVA with post hoc analysis as appropriate; p value less than 0.05 was considered statistically significant.

Results: First, we determined whether OS cells could survive and proliferate in the absence of glucose and presence of lactate as the only source of carbon. Cells did not proliferate in low or normal [lactate] alone, however proliferation was significantly higher in cells that were cultured in high [lactate] than in normal concentration (Fig. 1). We then determined extracellular lactate affected the expression of mitochondrial fusion and fission related genes. We found that when OS cells were cultured in high [lactate] (10mM), expression of fusion related gene OPA1 was significantly higher than in low [lactate] (Fig. 1A). However, when these cells were cultured in sufficient concentration of glucose (6mM), there was no significant change in gene expression indicating that this effect is only observed when glucose is no longer the predominant nutrient source (Fig. 2B). We observed that expression of OPA1 decreased significantly when MCT4 was knocked down, indicating that MCT4 could be the primary importer of lactate (Fig. 2C).

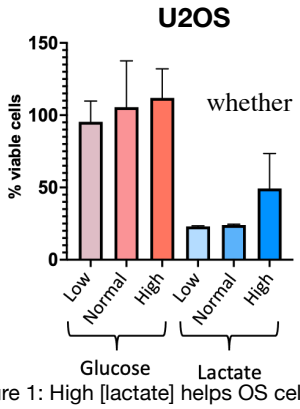

Figure 1: High [lactate] helps OS cells remain viable in the absence of glucose

Conclusion: In conclusion, our data suggest that in the absence/limited availability of glucose, OS cells can take up lactate from the surrounding medium and metabolize it for their energy requirement. Inhibition of lactate uptake by blocking lactate transport using MCT inhibitors slowed both metabolism and proliferation. Targeting lactate transport can be a promising therapeutic strategy for OS.

Significance: This study determines how lactate from the TME directly affects OS cell proliferation. The data acquired by the end of the study will be beneficial in developing novel therapies. Success in this study could lead to a clinical trial.

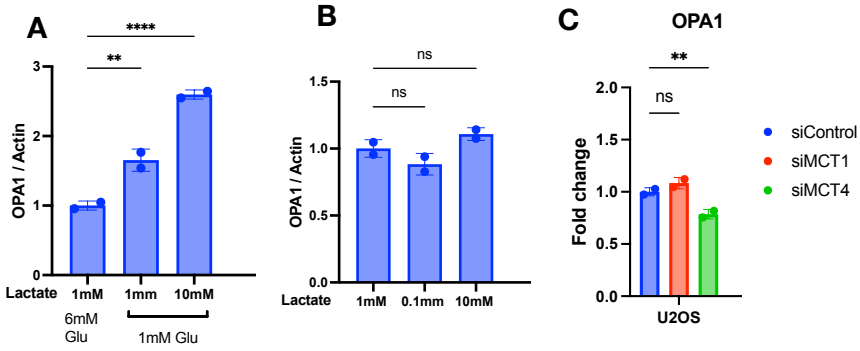

Figure 2: Lactate plays a role in expression of mitochondrial protein OPA1.