The Effect of Aerobic Exercise on Tumor Hypoxia and Metabolism in a Murine Melanoma Model

*Mumina Sadullozoda, *Hannah Savage, *Keri L. Schadler

*CUNY Brooklyn College, *Department of Pediatrics Research, MD Anderson Cancer Center

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Introduction

Solid tumor vasculature is dysfunctional containing structural abnormalities, heterogeneous perfusion patterns, and hyper-permeability. These abnormalities make it a challenge to deliver systemic therapies effectively. In addition, poor vasculature function promotes a hypoxic tumor environment conducive to therapeutic resistance. One method to normalize tumor vasculature is aerobic exercise. Our lab has observed significant reductions in hypoxia after exercise but no change in vessel perfusion in a murine model of melanoma. This may be due to an alteration in tumor metabolic flux or oxygen consumption after exercise. Thus, this project aims to study the effect of exercise on tumor metabolism. We **hypothesized** that exercise reduces expression of genes associated with metabolism in tumors, contributing to decreased hypoxia within the tumor microenvironment.

Methods

YUMMER melanoma cell line was injected into fourteen C571Bl/6 male mice subcutaneously. When tumors became palpable, mice were exercised 45 min/day, 5 days/week, for 14 days at 12 meters/minute using a forced treadmill model to achieve moderate aerobic exercise training. Prior to mouse euthanasia, pimonidazole HCl was injected intravenously in tumor bearing mice and tissue was frozen for analysis. Hypoxia and metabolism related proteins and genes were measured by western blot and qPCR, respectively. Differentially expressed metabolites were mined in a public dataset (Rundqvist et al. 2020. eLife) using the limma package on R.

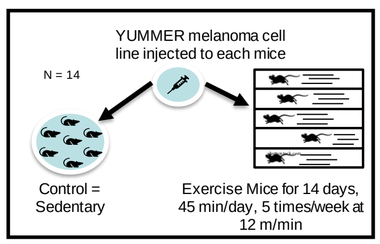

Fig. 1 Experimental design. Mice we randomized few days after the injection and divided into control and experimental groups.

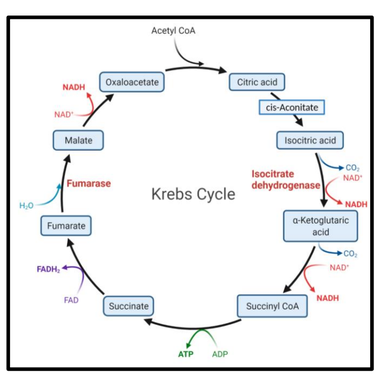

Fig. 2 Krebs cycle. Fumarate, malate, and α-ketoglutarate. were mined from a publicly available metabolomics dataset to identify differentially expressed metabolites in sedentary and exercised mouse plasma.

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Aerobic exercise decreased hypoxia but did not alter vessel perfusion

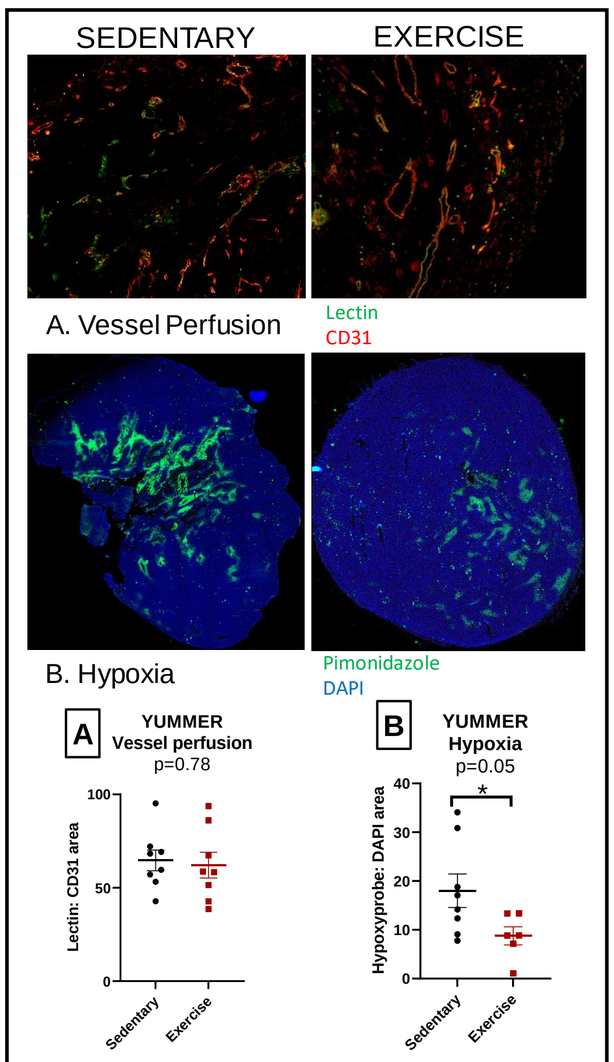

Fig. 3 YUMMER melanoma subcutaneous tumor. Tumor was stained with CD31 (A) to measure vessel perfusion. Mice were injected with pimonidazole prior to sacrifice (B) to measure hypoxia.

HIF1α protein and mRNA changes after exercise were not detected

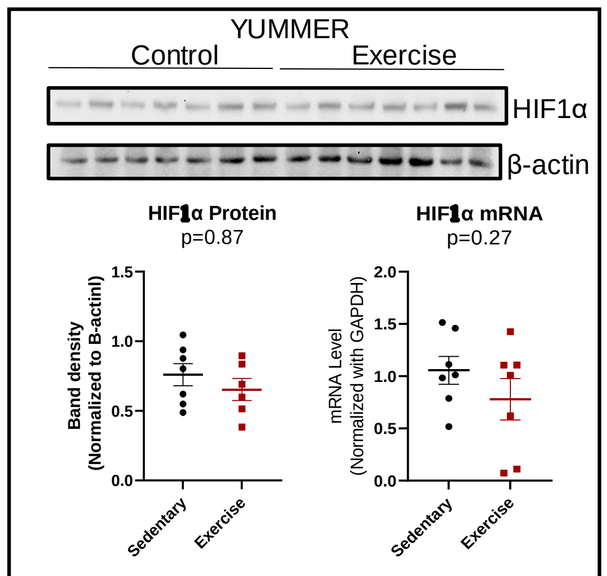

Fig. 4 YUMMER melanoma tumor bearing mice were exercised or sedentary. Tumor samples were collected upon euthanasia. Tumors were lysed and western blotting was performed for HIF1α protein normalized with β-actin. HIF1α gene was quantified using RT-qPCR.

HIF1α target genes demonstrated decreasing trends after exercise

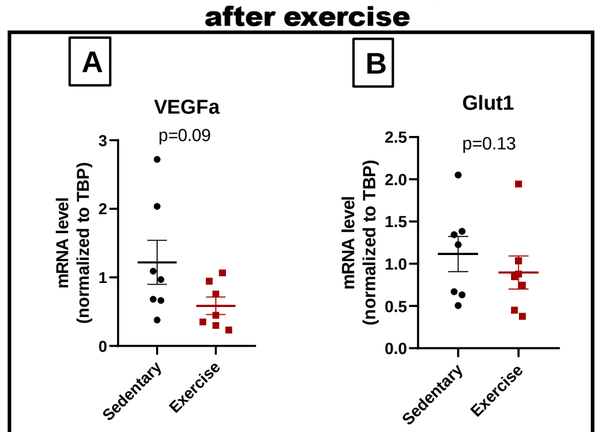

Fig. 5 qPCR data shows decreasing trend, but no significant changes in HIF1α target genes after exercise.

Krebs cycle genes

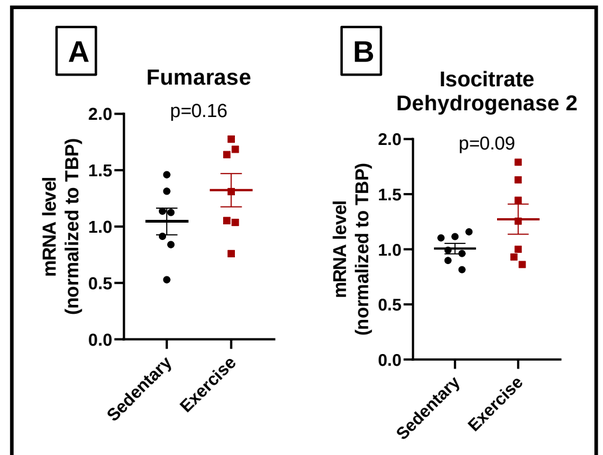

Fig. 6 Fumarase and isocitrate dehydrogenase 2 are Krebs cycle enzymes. Increasing trend but no significant changes were observed in Krebs cycle gene expression after exercise when measured by RT-PCR

OXPHOS Genes

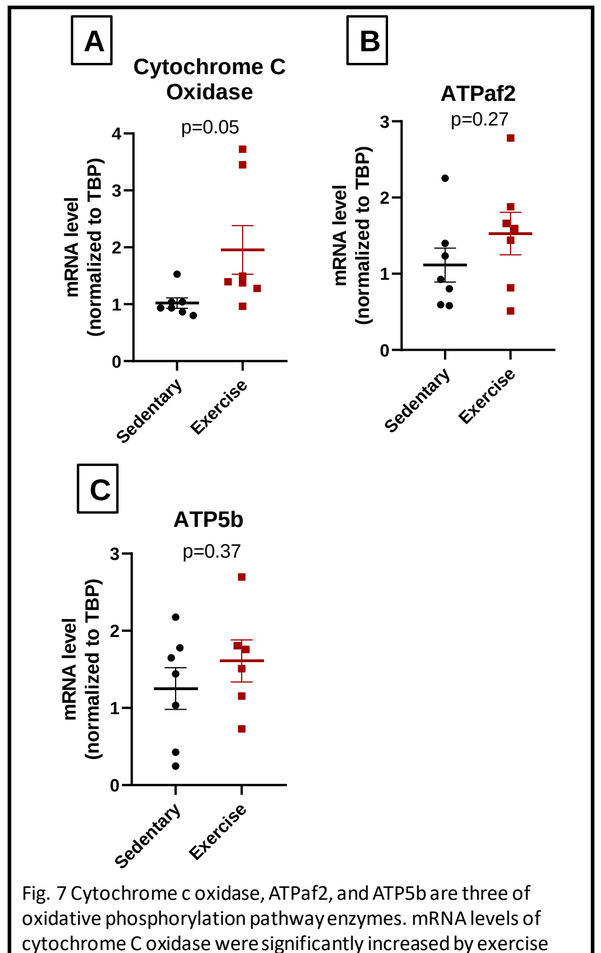

Fig. 7 Cytochrome c oxidase, ATPaf2, and ATP5b are three of oxidative phosphorylation pathway enzymes. mRNA levels of cytochrome C oxidase were significantly increased by exercise while ATPaf2 and ATP5b were not changed.

Krebs cycle metabolites fumarate, malate, α-ketoglutarate, aconitate, citrate, and isocitrate showed significant increase after exercise

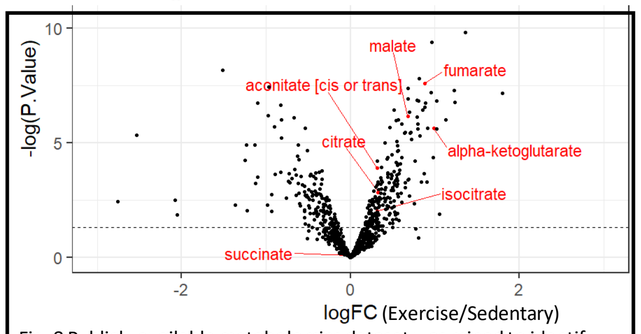

Fig. 8 Publicly available metabolomics dataset was mined to identify differentially expressed metabolites in sedentary and exercised mouse plasma. For differential expression analysis, exercise mouse plasma metabolites were compared to sedentary mouse plasma metabolites.

Results

- Exercise reduced tumor hypoxia, as indicated by pimonidazole staining. However, no changes in HIF1α mRNA or protein expression were detected in tumor homogenates. Decreasing trends were observed in mRNA levels of HIF1α target genes, VEGFa and Glut1.
- Genes associated with Krebs cycle function showed increasing trend in tumors from exercised mice (Idh2, p=0.09; and Fh1, p=0.16).
- No change in expression of ATP synthase related genes were observed (ATP5b, p=0.37; ATPaf2, p=0.27). However, expression of cytochrome c oxidase increased after exercise (Cox4i1, p=0.05).
- Consistent with our findings in tumor homogenates, Krebs cycle metabolites including citrate, α-ketoglutarate, fumarate, and malate increased in mouse plasma samples after exercise.

Conclusions

Our original hypothesis was not supported as we saw an increase in several metabolism related genes. These results suggest that aerobic exercise does modulate metabolism, however, it is unclear why exercise may increase metabolism in tumor tissue and how this impacts tumor hypoxia. Additional studies like seahorse assay to more directly determine metabolic flux and oxygen consumption are necessary to further elucidate the role of exercise in tumor metabolism.

Acknowledgements

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References

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