Downregulating Glutamine Uptake in an Effort to Minimize Pancreatic Cancer-Induced-Cachexia

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Introduction

Cancer-Induced-Cachexia (CIC), or wasting syndrome, accounts for up to 20% of deaths in cancer patients[1]. Even if a cachectic patient has a high caloric intake, their fat and muscle stores are depleted to nourish quickly-proliferating tumor cells. CIC is the most severe and common in cases of pancreatic cancer. Cachexia is associated with poor prognosis, treatment tolerance, and quality of life in cancer patients[1]. Additionally, cachexia significantly negatively impacts the outcomes of patients in palliative and post-operative pancreatic cancer care[1].

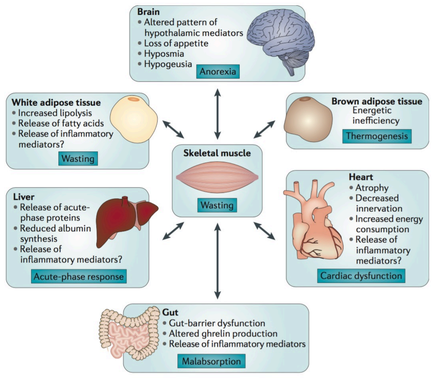

Figure 1: Cancer-induced-cachexia has widespread effects of on the body. Adapted from: Argiles, J. M.; et al. *Nature,* 2014.

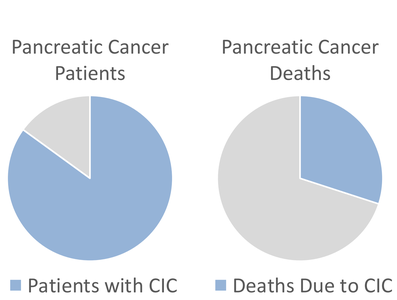

Figure 2: Shows the prevalence of Cancer-Induced Cachexia in pancreatic cancer cases

Based on metabolic changes in glutamine/glutamate we identified in xenograft models of cachexia and human plasma results, we downregulated the glutamine transporter SLC1A5 in human pancreatic cancer cells to understand the impact on CIC. We hypothesized that decreasing glutamine uptake would decrease the degree of pancreatic CIC in mouse models. If so, selectively downregulating SLC1A5 may be a potential therapy to prevent CIC in pancreatic cancer patients.

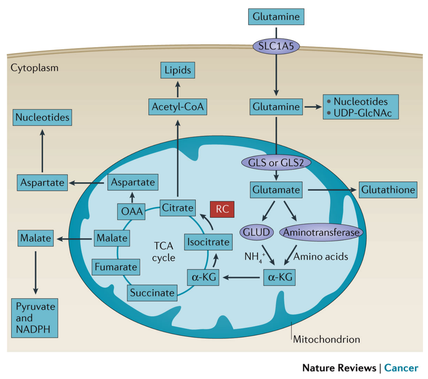

Figure 2: Fates of glutamine metabolism in cancer cells, indicating which part of the pathway will be inhibited in this project. Adapted from Altman B. J., et al., *Nature* 2016:

Methods and Materials

1)Hs_SLC1A5-5'-CCGGGCCTGAGTTGATACAAGTGAACTCGAGTTCACTTGTATCAACTCAGGCTTTTTG-3'
2)Hs_SLC1A5-5'-CCGGGCAGTCCTTGGACTTCGTAAACTCGAGTTTACGAAGTCCAAGGACTGCTTTTTG-3'

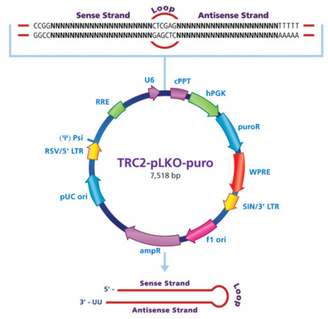

Figure 3: A schematic of the pLKO-puro viral vector used to transfect the cells. Sequence 1 proved most effective in silencing SLC1A5 in non-cachexia inducing Panc1 cells. Sequence 2 proved most effective for silencing SLC1A5 in cachexia-inducing Pa04C cells[2,3]. Because the vector encodes for puromycin resistance, addition of puromycin was used to select for transfected cells.

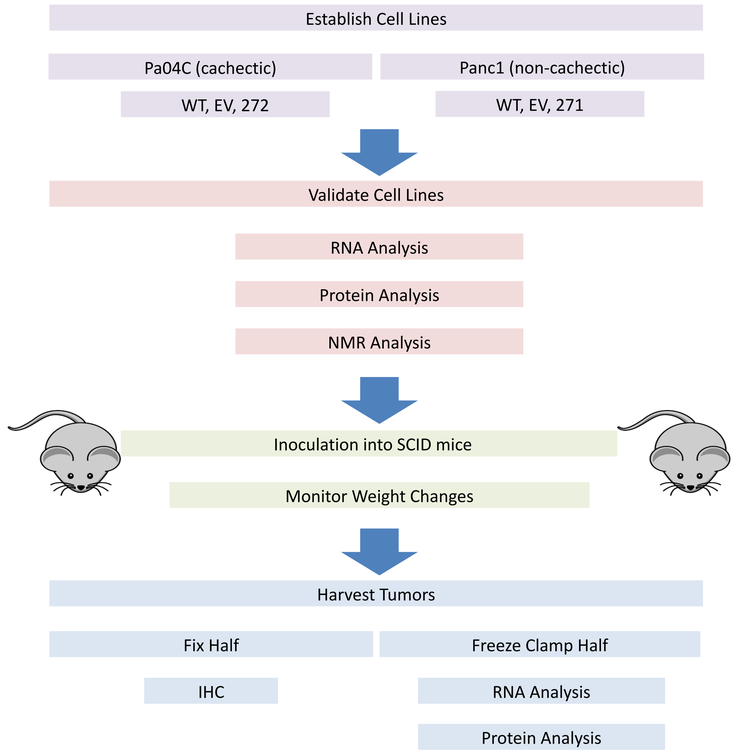

Figure 4: Illustrates the chronology of the project and research aims. SCID mice have recently been inoculated with Pa04C WT, EV and SLC1A5 knockdown cells, and their weight is being monitored.

Results

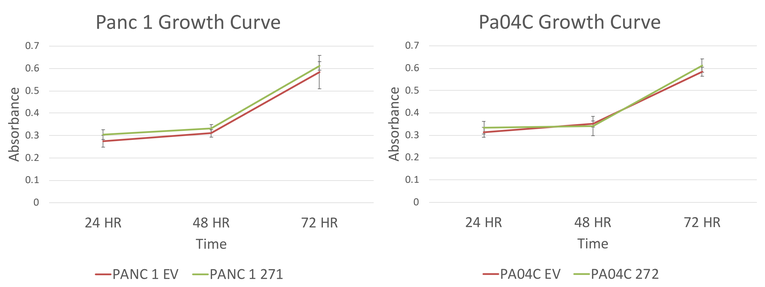

Figure 6: CCK assays were performed at three timepoints to delineate the growth curves for the constructed cell lines. Notably, the Panc1 and Pa04C EV and SLC1A5 downregulated cells grow at similar rates.

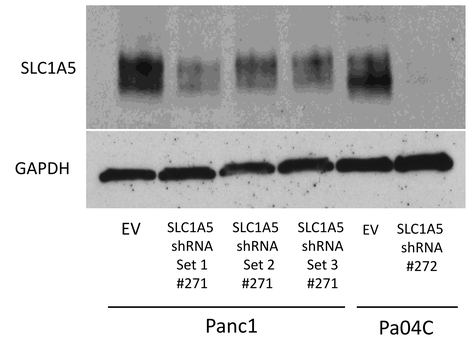

Figure 5: The western blot indicates changes in protein expression comparing empty vector lines to their respective SLC1A5 downregulated cells. SLC1A5 downregulation is more effective in Pa04C cells than in Panc1 cells.

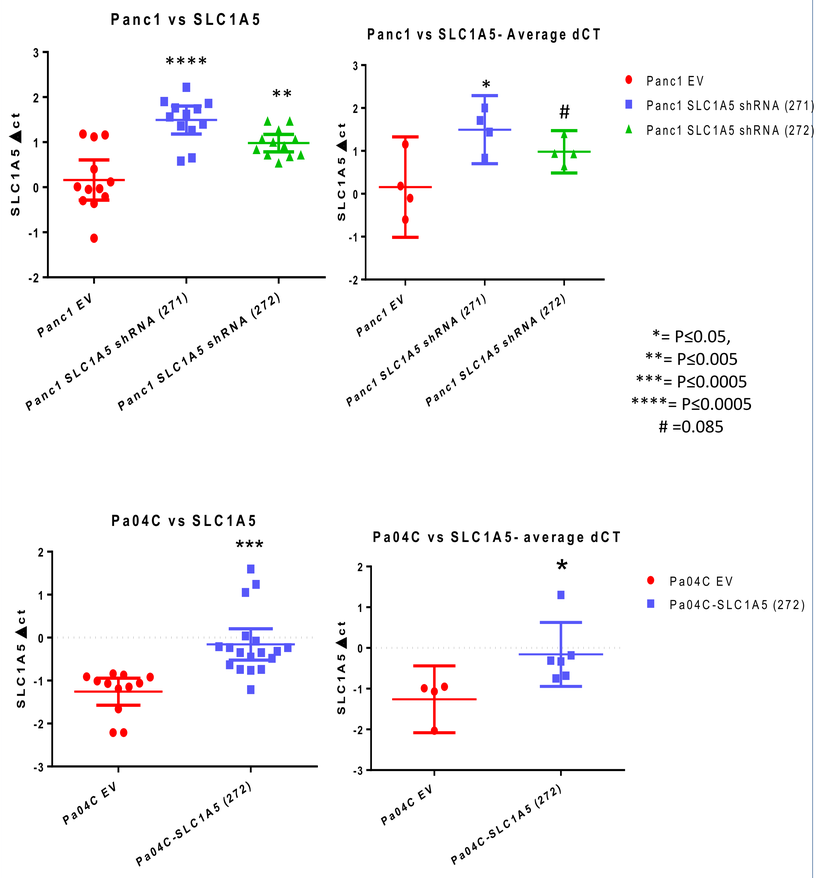

Figure 7: rtPCR analysis was done on the 3 independent cell sets to determine the downregulation of SLC1A5 in the transduced cell lines. Approximately 55% SLC1A5 downregulation was detected in Pa04C cells and 45% dowregulation in Panc1 cells. The partial knockdown is due to the incomplete penetrance of the vector.

Conclusions and Future Directions

Here, we have focused on reducing glutamine uptake, but future directions will include the downregulation of glutaminase (GLS) 1 and 2, the enzymes that convert glutamine to glutamate for further metabolic use. Combinatorial downregulation of both SLC1A5, GLS1and GLS2 may prove to be even more effective for modifying pancreatic cancer glutamine metabolism to reduce cachexia.

References:
1. Yakovenko A, Cameron M, Trevino JG. Molecular therapeutic strategies targeting pancreatic cancer induced cachexia. *World J Gastrointest Surg*. 2018;10(9):95-106; 2. Jones S, Zhang X, Parsons DW, et al. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. *Science (80)*. 2008; 3. Winnard PT, Jr., Bharti SK, Penet MF, Marik R, Mironchik Y, Wildes F, Maitra A, Bhujwalla ZM. Detection of Pancreatic Cancer-Induced Cachexia Using a Fluorescent Myoblast Reporter System and Analysis of Metabolite Abundance. Cancer research. 2016;76(6):1441-50.

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