Metabolic Profiling of Pancreatic Cancer for Early Detection and Determining Therapeutic Efficacy

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Making Cancer History

Background

Pancreatic cancer is asymptomatic in nature and usually is not detected until metastasis has occurred. At Stage I, the five-year survival rate is 34% but drops dramatically to 3% at Stage IV.[1,2] Techniques can be used to analyze and compare metabolites as cancer tissue and normal tissue metabolize glucose differently.[3] Targeting glutaminolysis, the breakdown of glutamine, through glutaminase inhibition can disrupt cancer metabolism and tumor progression.[4] Therefore, analyzing the metabolic profile of pancreatic cancer can lead to early detection and therapeutic efficacy.

Objective

Through a blinded study, a metabolomic analysis of tumors from mouse models, utilizing nuclear magnetic resonance (NMR) spectroscopy, can be used to detect the presence of pancreatic cancer. This analysis can also determine the efficacy of using glutaminase-inhibition based treatment (V9301) to slow the progression of pancreatic cancer.

Relation to Cancer Prevention

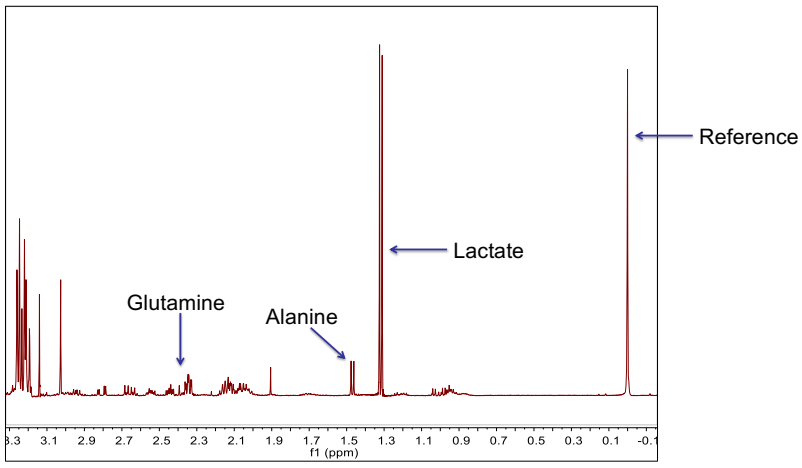
This project contributes to the field of cancer prevention by studying alternative methods to detecting pancreatic cancer in its early stages. Ultimately, these novel methods, if used early enough, can detect pancreatic cancer before it occurs or prevent cancer from metastasizing and reaching later stages when they are usually deadly.

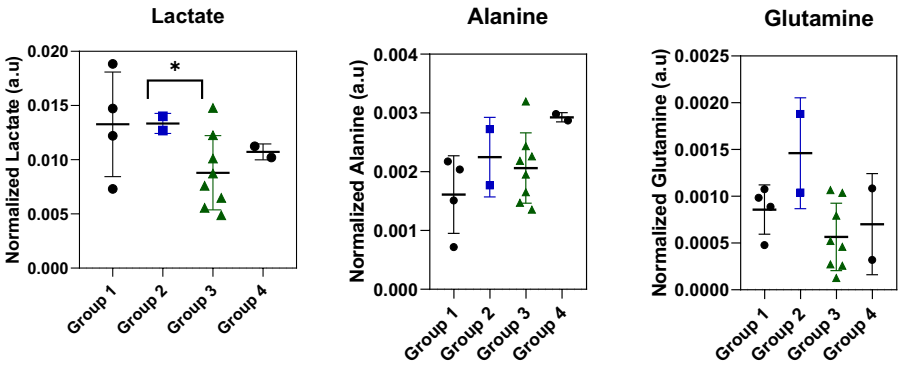
Methods

1. Specific pancreatic cancer tumors were collected from collaborators in the department
2. Four groups of pancreatic mice with KRAS, P53, and SMAD mutations were employed
   - One group was treated with 12.5 mg/kg of V9301 twice per day for 4 weeks (low dosage treatment)
   - Another group was treated with 75 mg/kg single bolus dose for 4 hours begore being sacrificed (high dosage treatment
   - A third and fourth group were control mice with pancreatic cancer that received a sham vehicle treatment (low and high dosage respectively)
3. Frozen tumor samples are collected from these models and are pulverized using a biopulverizer
4. The samples were lysed using lysing ceramic beads in a 2:1 methanol–water solution to extract the metabolites
5. The solution was then evaporated using a rotovapor and lyophilizer and the remaining solvent was dissolved in D_2O with a DSS reference
6. Nuclear magnetic resonance (NMR) spectroscopy (1D-proton) was performed on a 500 MHz Bruker Advance III HD NMR spectrometer
7. Data was analyzed for the specific metabolites and metabolic profiles in the tumor samples

Results

Representative ^{1}H Spectrum





The levels of lactate, alanine, and glutamine were the main metabolites analyzed from the NMR spectroscopy. Lactate is a metabolite that is high in cancer cells, while alanine levels are low. Glutamine is an amino acid that causes tumor growth. Therefore, a glutaminase inhibitor reduces the levels of glutamine, which limits tumor progression. The results show changes in the levels of these three metabolites based on the various four groups of pancreatic mice:

- Group 1: Contains a high amount of lactate, but lower amounts of alanine and glutamine
- Group 2: Contains a high amount of lactate and glutamine, and a medium amount of alanine
- Group 3: Contains a low amount of lactate, a medium amount of alanine, and a low amount of glutamine
- Group 4: Contains a medium amount of lactate, a high amount of alanine, and a low amount of glutamine

Conclusion

The results strongly indicate Group 1 and 2 in the study are the control groups. Group 1 most likely consisted of the mice that received the low dosage of the sham vehicle treatment, while Group 2 the high dosage. Group 3 is predicted to be the group that received the high single bolus dosage of V9301 and Group 4 with the low dosage. This study provides evidence that comparing metabolites successfully detects the progression of cancer and determines the efficacy of novel treatments, such as a glutaminase-inhibitor based treatment.

Responsible Conduct of Research

This study was approved by the University of Texas MD Anderson Cancer Center. Dr. Pratip Bhattacharya, the MD Anderson PI, submitted a research protocol and obtained research approval for this project. We considered the ethical needs and protections for animal welfare and safe laboratory practices.

Acknowledgements

This work was supported by NIH/NCI R25CA056452 (Trinity Brigham)
Trinity Brigham was supported by a training grant from the National Cancer Institute (R25CA056452, Dr Shine Chang, Principle Investigator)
Special thank you to the Cancer Prevention Research Training Program, José Enriquez, and Dr. Pratip Bhattacharya

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