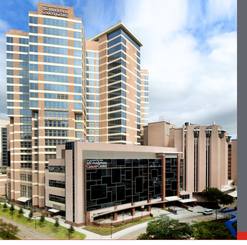

Distinction Between Pancreatitis and Early-Stage Pancreatic Cancer with HP-MRI

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Objective

Currently, there are no high specificity tools for the distinction between pancreatitis and early-stage pancreatic cancer; therefore, the primary objective of this project is to develop and optimize a method for detecting metabolic differences between pancreatitis and pancreatic cancer through hyperpolarized magnetic resonance imaging (HP-MRI). This technique can increase the sensitivity of conventional magnetic resonance by over 10,000-fold, meaning the signal can be enhanced and focused on, allowing for the metabolism occurring in the tissue to be seen in vivo. Through HP-MRI, a non-invasive investigation of the metabolic flux in pancreatitis and pancreatic cancer can occur in real-time. This allows physicians to distinguish between patients presenting with pancreatitis and early-stage pancreatic cancer, improving patient survival and treatment outcomes.

Methods

Hyperpolarization experiments were performed with 1-^{13}C-labeled pyruvate containing 15mM trityl radical (OX63) using a commercial DNP polarizer (HyperSense, Oxford Instruments, UK) at a 3.35T magnetic field and a temperature of 1.4 Kelvin. Using a Bruker BioSpec 7T imaging scanner, which utilized a dual tuned (^{1}H) volume coil and (^{13}C) surface coil (Doty Scientific, SC), the ^{13}C-spectra was obtained. For these studies, four different mice models were used: Wild Type (WT), P48CreERT2 (control), P48CreERT2:LSLKras (KC) and P48CreERT2:LSLKras:LSLP53 (KPC). The cerulein induction system was implemented to regulate pancreatitis in control and KC models as shown in *Figure 1*. Alternatively, tamoxifen was employed in the induction of pancreatic cancer in KC and KPC models. Pancreatitis models were imaged at two time points, pre-induction and 10 weeks, where the area under the curve was then collected for each metabolite allowing for the comparison of the pyruvate-to-lactate ratios. To further demonstrate how the phenotype for pancreatitis reacts to the induction system, the histology as indicated in *Figure 2* was provided to us by one of our collaborators.

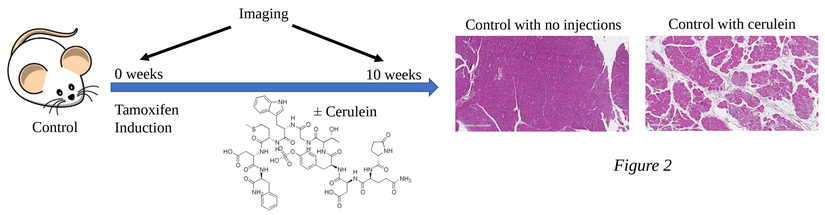

Figure 1

Figure 2

Hyperpolarized ^{13}C Metabolic MRS/MRI

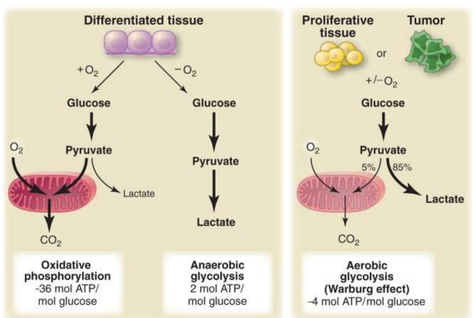

Figure 3

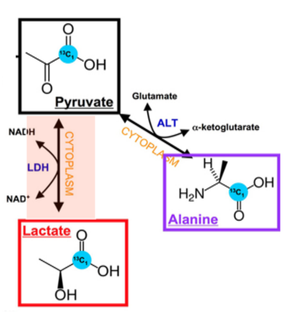

Figure 4

In order to understand how cancer works, as discovered by Otto Warburg, *Figure 3* discusses the metabolizing of sugar. In normal cells, there are two paths that can be taken, one being when O_2 is present and the other in which O_2 is not present. When O_2 is present, Pyruvate is going into the mitochondria for the TCA cycle. When O_2 is not present, however, most of the pyruvate is converted into lactic acid. In cancer cells, whether or not O_2 is present does not matter as most of the pyruvate is converted into lactic acid, known as the Warburg Effect. With an effort to analyze the pyruvate-to-lactate ratio, the HP-MRI process can be applied allowing for the signal of pyruvic acid to be increased. As highlighted in *Figure 4*, knowing that pyruvic acid goes to lactate acid through the enzyme LDH, the actual chemical transformation can be seen in real-time. Knowing this, the data can be observed and focused on as the process is unfolding in the tumor.

Preliminary Data

Average ratio at time point	Control model (n)	KC model (n)
1 (14 weeks)	0.23672 (7)	0.248053 (3)
2 (21 weeks)	0.144264 (7)	0.258622 (3)
3 (28 weeks)	0.209469 (6)	0.258443 (4)

Table 1

Previous research was performed in our lab using a spontaneous pancreatic cancer mouse model (KC). As displayed above in *Table 1*, in comparison to the control mouse model, the KC model had a significantly higher pyruvate-to-lactate ratio when evaluated using HP-MRI. To further observe the differences in the ratio, we look to assess the specificity of the method by investigating its ability to affectively distinguish between pancreatitis and pancreatic cancer. *Figure 5* shows the anatomical MRI image of mice where the blue box highlights the area of the pancreas.

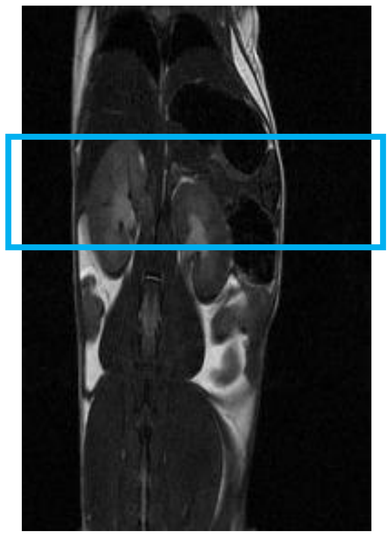

Figure 5

Results

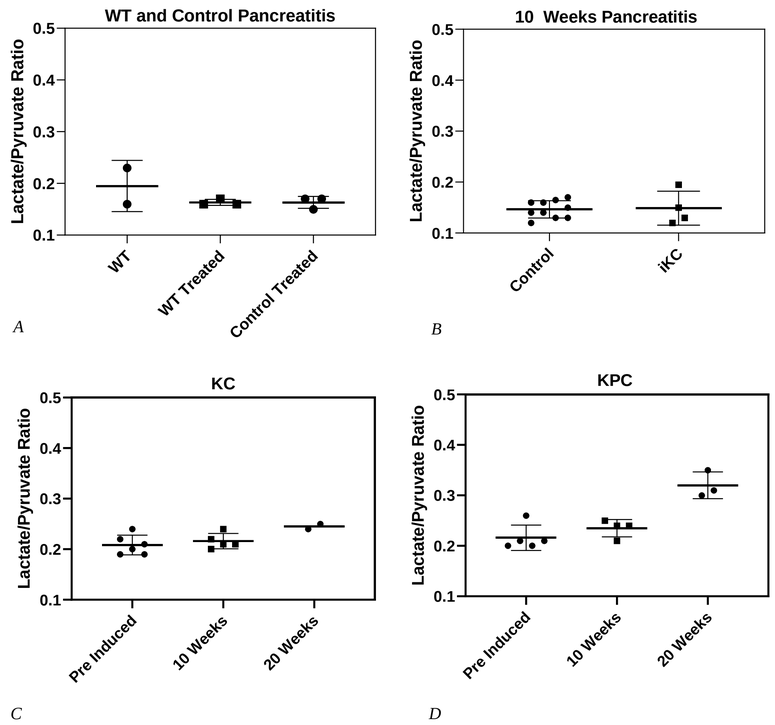

Figure 6

Figure 6. HP-MRI 13C-spectra comparing pyruvate-to-lactate ratio. [A] Comparison between WT and control mice shows no difference between any treated or untreated groups. **[B]** Comparison between control and KC mice 10 weeks after induction with cerulein shows no difference in conversion ratio. **[C]** KC mice imaged at timepoints pre-induction, 10 weeks, and 20 weeks demonstrated increased pyruvate-to-lactate ratio as cancer developed. **[D]** KPC mice imaged at the same timepoints as KC but demonstrated more aggressive growth, which is well corroborated with the increased conversion ratios. Especially at 20 weeks, KPC had higher conversion than KC.

In *Figure 6A*, groups observed include, WT, WT treated with cerulein, and control treated with cerulein. Data acquired show no considerable change in the pyruvate-to-lactate ratio between all three groups. Furthermore, as shown in *Figure 6B*, control and KC mouse models had no statistically different pyruvate-to-lactate ratios at 10 weeks post pancreatitis induction. KC models have a mutation in the Kras gene that will not develop into cancer in the time period of pre-induced to 10 weeks. However, the cerulein induction might result in the formation of lesions as the Warburg Effect begins to take place. Thus, in comparison to the control model, some KC ratios tend to be slightly higher as the conversion is around 0.20. *Figures 6C* and 6*D* focus on the pancreatic cancer mouse models, KC and KPC. During the 30-40 weeks post induction, KC models can progress to PanIN which may later develop to pancreatic cancer. On the other hand, the more aggressive KPC model, which contains both the Kras and p53 gene mutation, can take a remarkably shorter amount of time to develop into cancer. Using HP-MRI, we are able to observe the increased pyruvate-to-lactate ratio in both models as the cancer developed, as well as the differences between the two pancreatic cancer models at later time points.

Conclusion

Using HP-MRI technique to compare the pyruvate-to-lactate ratio in both WT and inducible mouse models, we validated the method's high specificity in distinguishing between pancreatitis and pancreatic cancer. In stark contrast to the KC and KPC models which developed increasing pyruvate-to-lactate ratios as the cancer progressed, pancreatitis models had the same pyruvate-to-lactate ratio as the control and WT models. This validates the use of pyruvate-to-lactate ratio acquired through HP-MRI as a way to accurately distinguish between pancreatitis and pancreatic cancer.

Future Research

Further research on pancreatitis can be made that allows for the identification of populations at high risk of acquiring pancreatic cancer. As pancreatitis models are more prone to evolve into pancreatic cancer, pancreatitis can be used as a pre-curser. HP-MRI is a real-time imaging modality that can non-invasively follow metabolic fluxes in vivo. Clinical translation of this technique could enable physicians to detect pancreatic cancer at a much earlier stage, resulting in the improvement of the patients' survival and overall outcome. In addition, a newly installed clinical DNP polarizer will enable further research of the pyruvate-to-lactate ratio, including the initiation of a clinical trial for early detection of pancreatic cancer.

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Responsible Conduct of Research

For this research project, Dr. Bhattacharya submitted a research protocol and obtained research approval from partner institutions.