Center of Excellence for Pancreatic Cancer Research
Report for Year 2
June 24, 2020

Thrust 1: Cancer Detection

Electrochemical Techniques for Cancer Diagnosis and Treatment Monitoring

Jun Li and Duy Hua

Research Progress:

The Li and Hua groups have been collaborating in developing highly sensitive electrochemical techniques for detecting the activity profiles of multiple proteases for cancer diagnoses and treatment monitoring. So far, a set of 26 peptides have been designed, synthesized and characterized as substrates for selective electrochemical detection of the activity of cathepsin B, ADAM-10, ADAM-17, cathepsin D and MMP-9. The cleavage of these peptide substrate by the cognate proteases has been validated by HPLC and the proteolysis activity has been derived from the kinetic proteolysis curves based on activity-based assays including the commercial fluorogenic techniques and the proposed electrochemical method. Our initial electrochemical detection was based on a nanoelectronic technique, i.e. nanoelectrode arrays fabricated with vertically aligned carbon nanotubes (CNTs) embedded in the SiO_2 matrix. The peptide substrate was labelled with ferrocene (Fc) as an electroactive tag at the distal end and then covalently attached to the protruding CNT tips of ~10 nm in diameter and 200 nm long. The electrochemical signal was monitored during the proteolysis for ~30 – 70 minutes, which exhibits as an exponential decay. The inverse of the decay rate was found to indicate the activity of the target protease on this specific peptide substrate. Systematic investigation of the peptide length revealed that hexapeptide is the optimal length for the high selectivity and high electrochemical signal. We have demonstrated that we can detect down to 0.32 nM cathepsin B with this technique. Recently, we have further simplified the electronic chip by replacing the CNT nanoelectrode array with a 3x3 gold (Au) microelectrode array (MEA). With the proper surface functionalization, the Au MEAs match the similar performance as the CNT nanoelectrode array but are much easier to fabricate. This allowed us to attach 9 different peptides on the chip which are designed to simultaneously detect the activity of 3 cancerous proteases for profiling. We are currently working on developing the common physiological buffer for multiplex detection of three proteases, i.e. cathepsin B, ADAM-17 and MMP-9, in blood samples from cancer patients provided by our collaborator Dr. Priyanka Sharma from KUMC.

Publications in Refereed Journals:

Song, Y.; Fan, H.; Anderson, M. J.; Wright, J. G.; Hua, D. H.; Koehne, J.; Meyyappan, M.; Li, J., Electrochemical Activity Assay for Protease Analysis Using Carbon Nanofiber Nanoelectrode Arrays. *Anal. Chem.* **2019**.

Morgan J. Anderson, Yang Song, Huafang Fan, Jestin Gage Wright, Zhaoyang Ren, Duy H. Hua, Jessica Koehne, M. Meyyappan, and Jun Li. Simultaneous, Multiplex Quantification of Protease Activities Using a Gold Microelectrode Array. *Biosensors & Bioelectronics*, **2020**, 112330 (in press). https://doi.org/10.1016/j.bios.2020.112330

Conference Presentations:

Quantitative Electrochemical Analysis of Cathepsin B Activity Using Carbon Nanofiber Nanoelectrode Arrays with Optimized Peptide Peptide Substrate Length and Temperature, Yang Song, Huafang Fan, Morgan Anderson, Jeston G. Wright, Duy Hua, Jessia Koehne, M. Meyyappan and J. Li, 235th ECS Meeting, May 29th, 2019, Dallas, TX.

Quantitative Electrochemical Analysis of Protease Activity Using Carbon Nanofiber Nanoelectrode Arrays toward Cancer Diagnosis. Yang Song, Huafang Fan, Morgan J. Anderson, Jestin Gage Wright, Duy H. Hua, Jessica Koehne, Meyya Meyyappan, and Jun Li. 2019 Designing Molecules Workshop and Conference; August 15 – 17, 2019, Manhattan, KS.

Quantitative Electrochemical Analysis of Cathepsin B Activity Using Carbon Nanofiber Nanoelectrode Arrays with Optimized Peptide Substrate Length and Temperature, Yang Song, Huafang Fan, Morgan Anderson, Jestin G. Wright, Duy Hua, Jessica Koehne, M. Meyyappan and J. Li*, ACS Midwest Regional Meeting, Oct. 18, 2019, Wichita, KS.

Profiling Protease Activities Using a Multiplex Electrochemical Sensor Array, Jun Li, Yang Song, Huafang Fan, Morgan Anderson, Jestin G. Wright, Duy Hua, Jessica Koehne, and M. Meyyappan, invited session on "Nanomaterials and Nano-/micro- devices for Chemical/Biochemical Sensors" of The 15th IEEE Int'l Conference on Nano/Micro Engineered & Molecular Systems (IEEE-NEMS 2020), originally scheduled on Apr. 20-24, 2020, but was postponed to Sept. 2020 due to COVID-19.

Invention disclosure:

Gold microelectrode array for multiplex detection of the activity profile of protease biomarkers (Kansas State University and NASA Technology Transfer Program number: 1589249401, submitted in May 2020, in Progress). Inventors: Jun Li, Morgan Anderson, Yang Song, Jessica E. Koehne, Meyya Meyyappan, Huafang Fan, and Duy H. Hua.

Funding:

National Institutes of Health, National Cancer Institute. 1R01CA217657-01 (PI: Jun Li; co-PIs: Duy H. Hua and Priyanka Sharma), 8/1/2017 – 7/31/2021, $480,000 (for Hua); $1,371,309 (total budget). "Rapid protease profiling with a multiplex electronic method for detection of metastatic triple-negative breast cancer".

Johnson Cancer Center: Center of Excellence for Pancreatic Cancer. Stefan Bossmann (PI); Jun Li (PI of Thrust 1; Hua: co-PI); Hua: PI of Thrust 2; Jianzhong Yu: PI of Thrust 3. Funding period: 7/1/2018 – 6/30/2020; $10,000 for Year 1 and $5,000 for Year 2 to Jun Li.

Translational Research Institute for Space Health (TRISH), postdoctoral research fellowship to Dr. Morgan Anderson (Jun Li group), Oct. 1, 2019 – Sept. 2021.

Developing an Isoelectric Focusing Device for Cancer Diagnostics

Christopher T. Culbertson and Stefan H. Bossmann

Research Progress:

Matrix Metalloproteinases (MMPs) are extracellular matrix degrading enzymes broadly expressive in normal and pathological processes. Upregulation of MMP activities is commonly

observed in cancer metastasis where structural proteins are decomposed to reduce cell adhesion and facilitate tumor cell invasion. MMP activities are regulated via extracellular activations/inhibitions, making MMP activity determination only meaningful in the environment of a specific sample matrix. Although some methods, such as zymographic separation and fluorescence assays, have been reported to be capable of in vitro MMP activities quantification, none are applicable at clinical level. Our previous study demonstrated activity assays of multiple MMPs by loading MMP substrates onto magnetic nanoparticles and using these nanoparticles to react with MMP containing sample. Our goal is to incorporate these nanoparticles and isoelectric focusing(IEF) separation and integrate this platform on a microfluidic chip to realize online sample preparation and detection as a point of care device for clinical application.

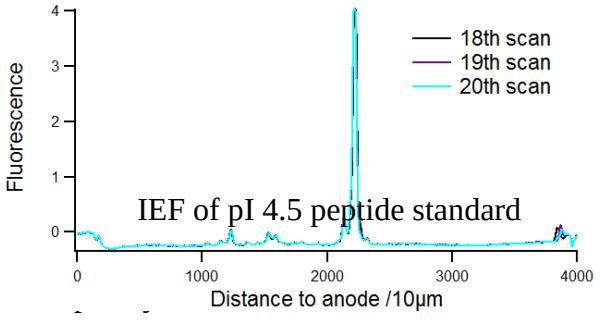
The bioanalytical nanoparticles will consist of a magnetic Fe/Fe_3O_4 core and covalent bonded fluorescently labelled MMP substrates. By incubating these nanoparticles with samples in the sample reservoir, a portion of the substrates will be cleaved and converted to shorter chains with different isoelectric points (pIs). After incubation, the nanoparticles will be magnetically transferred from the sample reservoir to the wash channel to wash off sample matrix and quench enzyme reactions. Reacted peptide substrates will be chemically detached from the mother nanoparticles in the IEF separation channel. Released peptide substrates of different pIs will be separated and detected using laser induced fluorescence. Eventually by the fluorescence signals of reacted and unreacted peptide substrates of different pIs, MMP activities can be determined.

Current development

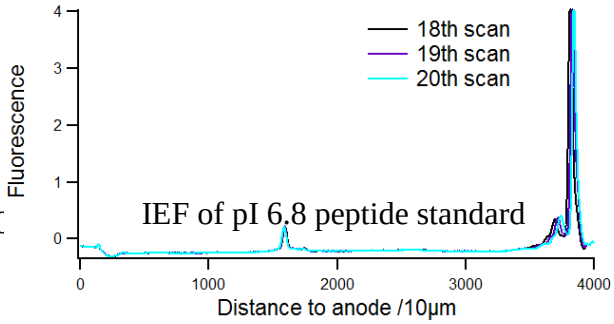
The development of MMP activity assay consist of two important components: substrate loaded magnetic nanoparticles and IEF separation microchip. For nanoparticles synthesis, successful enzyme digestions were demonstrated for MMP-7 against both synthesized peptide substrate and nanoparticles loaded with these substrates.

Synthesized MMP-7 substrate was digested and monitored with HPLC. As the digestion progressed, intact substrates got cleaved and converted to its cleaved version within 6 hours. This substrate was covalently bonded onto magnetic nanoparticles and digested under the same protocol, yielding similar digestion patterns. The next step for nanoparticle synthesis is to develop substrates for other MMP enzymes and load them onto nanoparticles.

For IEF microfluidic device, we have developed two generations of instrument and evaluated them based on the performance of standard pI markers.



Free substrate digestion with MMP-7



Nanoparticle digestion with MMP-7

Funding:

NSF, Emerging Frontiers in Research and Innovation (EFRI) 1933321; "EFRI CEE: Opening the Gates of Apoptosis in Cancer" Co-PI's: Dr. Christopher T. Culbertson, Dr. Bala Natarajan, Dr. Massoud Motamedi (UTMB), Dr. Michael Sheetz (UTMB), Dr. Larry Sowers (UTMB), Dr. Gracie Vargas (UTMB), $2,000,000.

Publications in Refereed Journals:

1. Culbertson, C. T.; Sibbitts, J.; Sellens, K.; Jia, S., Fabrication of glass microfluidic devices. *Methods Mol. Biol. (N. Y., NY, U. S.)* **2019,** *1906* (Microfluidic Electrophoresis), 1-12.

2. Kalubowilage, M.; Janik, K.; Bossmann, S. H., Magnetic nanomaterials for magnetically-aided drug delivery and hyperthermia. *Appl. Sci.* **2019,** *9* (14), 2927.

3. Covarrubias-Zambrano, O.; Yu, J.; Bossmann, S. H., Nano-Inspired Technologies for Peptide Delivery. *Curr. Protein Pept. Sci.* **2020,** *21* (4), 379-400.

Conference Presentations:

Johnson, C.; Zuercher, K.; Sibbitts, J.; Culbertson, C. T. In *Integrated heating element to maintain cell stability in high-throughput single-cell biological analysis*, American Chemical Society: 2020; pp CHED-0304.

Invention disclosure:

US 2019/0125443, Microfluidics-based Nanosensors and Devices, 16/349,700, Christopher T. Culbertson and Stefan H. Bossmann, published 11/12/2019.

Thrust 2: Drug Discovery for Pancreatic Cancer

Synthesis of Novel Drugs Using Chiral Bimetallic Nanoclusters

Duy H. Hua

Research Progress:

Members in Hua's laboratory have discovered a class of chiral bimetallic nanoclusters stabilized by chiral substituted polyvinylpyrrolidinones (CSPVP) and have applied them in catalytic asymmetric oxidation reactions. It was found that various complex biologically active molecules such as 1-*N*-acetyladamatanamine, 1-*N*-acetyl-3,5-dimethyladamantanamine, oxymetrine, 3-pivaloylestrone, and (-)-ambroxide underwent selective C-H bond oxidation to give 3-hydroxy-*N*-acetyladamatanamine, 1-*N*-acetyl-3,5-dimethyl-4β-hydroxy-adamantanamine, 13α-hydroxy-oxymetrine, 12β-hydroxy-3-pivalylestrone, and (-)-sclareolide, respectively. Molecules, 13α-hydroxy-oxymetrine and 12β-hydroxy-3-pivalylestrone including their synthetic derivatives likely possess anti-pancreatic cancer activities, since their precursor natural products have anticancer activities. Dr. Annelise Nguyen has agreed to study anti-pancreatic cancer activity of our synthetic molecules. 3,5-Dimethyladamantanamine or memantine is a drug for the treatment of Alzheimer's disease. Our novel synthetic analog, 1-*N*-acetyl-3,5-dimethyl-4β-hydroxy-adamantanamine, and its derivatives may possess similar or greater inhibitory activity of NMDA receptors, that may be

applied to the treatment of Alzheimer's disease. We are continuing our synthetic and biological effort in the discovery of novel biological active molecules focusing on anti-pancreatic cancer. We have also collaborated with Dr. Kun Yan Zhu at the Department of Entomology on the nano-delivery of dsDNA for enhancing RNA interference, which may be used for the enhancement of RNAi in pancreatic cancer. A manuscript has been submitted (see publication #8 below) and a second manuscript on the synthesis and design will be submitted soon.

Publications in Refereed Journals:

1. Yang Song, Huafang Fan, Morgan J. Anderson, Jestin Gage Wright, Duy H. Hua, Jessica Koehne, Meyya Meyyappan, and Jun Li. Electrochemical Activity Assay for Protease Analysis Using Carbon Nanofiber Nanoelectrode Arrays. *Analytical Chemistry*, **2019**, *91*, 3971-3979. DOI: 10.1021/acs.analchem.8b05189
2. Medha J. Gunaratna, Duy H. Hua, Bende Zou, Conrado Pascual, William Cao, Man Zhang, Sahani Weerasekara, Thi D. T. Nguyen, Kui Xiao, Xinmin Simon Xie. Synthesis of 1,4- and 1,4,4-substituted piperidines for the inhibition of neuronal T-type Ca^{2+} channels and mitigation of neuropathic pain in mice. *Arkivoc*, **2019**, part iii, 22-39. DOI: https://doi.org/10.24820/ark.5550190.p010.752.
3. Jianyu Lu, Serkan Koldas, Huafang Fan, John Desper, and Duy H. Hua. One-Pot Intramolecular Tandem Michael-Aldol Condensation Reaction in the Asymmetric Synthesis of Pentacyclic Terpenes. *Synthesis* **2019**, 51(21), 3964-3972. DOI: 10.1055/s-0039-1690521.
4. Man Zhang, Bende Zou, Medha J. Gunaratna, Sahani Weerasekara, Thi D. T. Nguyen, Serkan Koldas, William S. Cao, Christopher Lieu, Conrado Pascual, Xinmin Simon Xie, Duy H. Hua. Selective T-type Calcium Channel Inhibitors 1,3,4-Oxadiazoles for Anti-Seizures and Mitigation of Neuropathic Pain. *Heterocycles*, **2020**, *101*, 145-164. DOI: 10.3987/COM-19-S(F)5
5. Edruce Edouarzin, Connor Horn, Anuja Paduyal, Cunli Zheng, Jianyu Lu, Zongbo Tong, Guri Giaever, Marinella Gebbia, Corey Nislow, Raja Veerapandian, Duy H. Hua, and Govindsamy Vediyappan. Broad-Spectrum Antifungal Activities and Mechanisms of Drimane Sesquiterpenoids. *Microbial Cell*, **2020**, **7**(6):146-159, doi:10.15698/mic2020.06.719.
6. Medha Gunaratna Gamaralalage, Bo Hao, Man Zhang, Madoka Nakagomi, and Duy H. Hua. Synthesis of Probe Molecules, 6-(Dimethylamino)-2-phenylisoindolin-1-ones, For Acyl-CoA Synthetase. *Heterocycles*, **2020**, (accepted on 2/4/2020) in press. DOI: 10.3987/COM-20-S(K)1
7. Morgan J. Anderson, Yang Song, Huafang Fan, Jestin Gage Wright, Zhaoyang Ren, Duy H. Hua, Jessica Koehne, M. Meyyappan, and Jun Li. Simultaneous, Multiplex Quantification of Protease Activities Using a Gold Microelectrode Array. *Biosensors & Bioelectronics*, **2020**, 112330 (in press). https://doi.org/10.1016/j.bios.2020.112330
8. Anastasia M.W. Cooper, Huifang Song, Zhitao Yu, Marie Biondi, Jun Bai, Xuekai Shi, Zhaoyang Ren, Sahani M. Weerasekara, Duy H. Hua, Kristopher Silver, Jianzhen Zhang, Oili Feng, and Kun Yan Zhu. Comparison of strategies for enhancing RNA interference efficiency in the European corn borer, *Ostrinia nubilalis*. *Pest Management Science*, submit in June **2020**.

Presentations at Conferences:

1. Novel Therapeutic for Treatment of Alzheimer's Disease. Simon Xie, Duy H. Hua, Izumi Maezawa, Lee-Way Jin. The 2019 BIO International Convention Biotechnology Innovation Organization; at Philadelphia, PA, June 3 - 6, 2019.

2. Tricyclic pyrone CP2 improves long term memory in the TgF344 AD rat of an Alzheimer disease model. Bende Zou, William Cao, C. Pascual, Kelsey Erickson, Huafang Fan, Zongbo Tong, Andre van der Vlies, Izumi Maezawa, Lee-Way Jin, Duy H. Hua, Simon Xie. Neuroscience 2019, Society for Neuroscience, October 19 – 23, 2019, Chicago, IL.

3. Broad-Spectrum Antifungal Activity, Mechanism, and Synthesis of Drimane Sesquiterpenoids. Edruce Edouarzin, Connor Horn, Anuja Paudyal, Zongbo Tong, Cunli Zheng, Jianyu Lu, Xiaodong Huang, Guri Giaever, Marinella Gebbia, Corey Nislow, Duy H. Hua, and Govindsamy Vediyappan. 2019 Designing Molecules Workshop and Conference; August 15 – 17, 2019, Manhattan, KS.

4. Quantitative Electrochemical Analysis of Protease Activity Using Carbon Nanofiber Nanoelectrode Arrays toward Cancer Diagnosis. Yang Song, Huafang Fan, Morgan J. Anderson, Jestin Gage Wright, Duy H. Hua, Jessica Koehne, Meyya Meyyappan, and Jun Li. 2019 Designing Molecules Workshop and Conference; August 15 – 17, 2019, Manhattan, KS.

5. Quantitative Electrochemical Analysis of Cathepsin B Activity Using Carbon Nanofiber Nanoelectrode Arrays with Optimized Peptide Substrate Length and Temperature, Yang Song, Huafang Fan, Morgan Anderson, Jestin G. Wright, Duy Hua, Jessica Koehne, M. Meyyappan and J. Li*, ACS Midwest Regional Meeting, Oct. 18, 2019, Wichita, KS.

6. Quantitative Electrochemical Analysis of Cathepsin B Activity Using Carbon Nanofiber Nanoelectrode Arrays with Optimized Peptide Substrate Length and Temperature, Yang Song, Huafang Fan, Morgan Anderson, Jestin G. Wright, Duy Hua, Jessica Koehne, M. Meyyappan and J. Li*, 235th ECS Meeting, May 29th, 2019, Dallas, TX.

7. Pharmacokinetics and bioactivities of tricyclic pyrone molecules in Alzheimer's disease TgF344 rat model. Joshua Habiger, Bende Zou, Kelsey Erickson, Zongbo Tong, Huafang Fan, Williams Cao, Conrado Pascual, Izumi Maezawa, Lee-Way Jin, Xinmin Simon Xie, and Duy H. Hua. The 2020 K-INBRE Symposium, Hyatt Regency, Wichita, Kansas; January 18 – 19, 2020.

Invention Disclosure:

1. Chiral-substituted Poly-N-vinylpyrrolidinones and Complexes with Bimetallic Nanoclusters and Uses Thereof in Asymmetric Oxidation Reactions. Patent Cooperation Treaty (**PCT**) International Application. Inventor: Duy Hua; PCT Patent Application No.: PCT/US2020/016536; Filing Date: February 4, 2020.

Funding:

1. National Institutes of Health, SBIR (Small Business Innovation Research) Phase II grant, Aging Institute; grant number: 2R44 AG043203-03. Title: "Development of Patented Tricyclic Pyrone Molecules for the Treatment of Alzheimer's Disease". Total cost: $1,894,872. 3/1/2017 – 2/28/2021. For Hua: $344,282. PI: Xinmin Simon Xie; co-PIs: Duy Hua and Lee-Way Jin.

2. National Science Foundation, CHE 1662705 (Hua, single investigator), 7/1/2017 – 6/30/2020, $390,200. "Catalytic Asymmetric Oxidation Reactions by Chiral-supported Bimetallic Nanoclusters".

3. National Science Foundation, proposal number: 1826982. "MRI: Acquisition of a 400 MHz Nuclear Magnetic Resonance (NMR) Spectrometer to Support Research Projects from C-H Bond Oxidation to Engineered Molecular Materials". 8/1/2018 – 7/30/2020. PI; Duy H. Hua;

co-PIs: Stefan Bossmann, Dan Higgins, Jun Li, E. J. McLaurin and others. $419,518 (from NSF) and $179,794 (matching fund from various departments).

4. NIH General Medical Science, R01 GM128659-01 (Hua, single investigator). Title: "Catalytic asymmetric oxidation of alkenes and alkanes", 4/1/2019 – 2/28/2023; $1,199,260 (total; direct cost: $800,000 for Hua). An Administrative Supplement was awarded for $53,600 in September 2019 for the purchase of a HPLC system in Year 1.

Thrust 3: In Vitro Studies and MRI

Developing of a MRI-Guided Hyperthermia Device
Punit Prakash, Matthew Basel, Tej B. Shrestha, Stefan H.Bossmann

Research progress: Over the last year, we have worked collaboratively with Dr. Bossmann, Dr. Shrestha, and Dr. Basel to conduct pilot *in vivo* studies demonstrating the technical feasibility of delivering mild hyperthermia to a mouse model of pancreatic cancer under MRI guidance. In these studies, diffusion MRI was then used to assess changes within the tumors following delivery of hyperthermia. These preliminary data were a key part of an R01 proposal led by Prof. Bossmann submitted to the NCI in February 2020 (R01 CA255383, Developing Thermal Immunotherapy Against Pancreatic Adenocarcinomas). Under the joint mentorship of Dr. Shrestha, Electrical Engineering PhD student Faraz Chamani is developing an experimental platform for controlled delivery of heat to pancreatic cancer cells *in vitro* to be used for quantifying cell survival and stress protein release following heating at temperatures ranging between 40 – 60 °C. Findings from these studies will serve as preliminary data for grant applications, and be disseminated via publications and presentations.

Publication in Refereed Journal:

1. Faridi, P.; Bossmann, S. H.; Prakash, P., A simulation-based approach for optimization of a small-animal microwave applicator for pre-clinical MR-guided hyperthermia. *Biomedical Physics and Engineering Express,* Vol. 6, 015001, **2020** (DOI: 10.1088/2057-1976/ab36dd)

Conference Proceeding article

2. Faridi, P.; Shrestha, T. B.; Pyle, M.; Basel, M. T.; Bossmann, S. H.; Prakash, P.; Natarajan, B., Temperature estimation for MR-guide microwave hyperthermia using block-based compressed sensing. *Proceedings of the IEEE Annual Engineering in Medicine and Biology Conference (EMBC*), July **2020,** *accepted for publication (oral presentation)*.

Current Funding:

1. NIH/NCI, R01 CA218357, "Bronchoscope-guided microwave ablation of early-stage lung tumors." (2018 – 2022).
   PI: Prakash

2. NIH/NIBIB, R01 EB028848, "Treating primary aldosteronism-induced hypertension via microwave thermal therapy. " (2020 – 2023).
   PI: Prakash (co-PIs: Basel, Bossmann)

3. NSF IIP, “SBIR Phase II: Microwave ablation system for creating precision directed ablation zones.” (2020 – 2022)
   PI: Pfannenstiel; Prakash role: co-PI

4. Hologic, Inc., “Microwave ablation for treatment of uterine fibroids: technical feasibility assessment” (2018 – 2020)
   PI: Prakash

Development of Ultra-High-Field MRI Methods for Cancer Imaging Without Contrast Agents

Stefan H. Bossmann, Tej B. Shrestha, Matthew T. Basel, Punit Prakash, Jianzhong Yu

Rapid acquisition with relaxation enhancement (RARE)

Time is a critical factor in the preclinical and especially clinical application of MRI, which is constrained based on the key implication that higher resolution images often results in substantially longer imaging time. By utilizing rapid acquisition with **RARE** (**rapid acquisition with relaxation enhancement)**, we are able to circumvent the time cost often associated with the high-quality images as seen in Figure 1. Through the use of RARE imaging multiple lines of k space are filled with each pulse sequence simultaneously, resulting in rapid completion of entire images. This technique applies multiple evenly spaced RF pulses to order to turn back the dephasing protons and form an echo train. Each echo retains its independent phase encoding to produce independent lines of k space evenly spaced to avoid coherence pathway complications. This approach permits the recording of viable MRI images in less than 5 min.

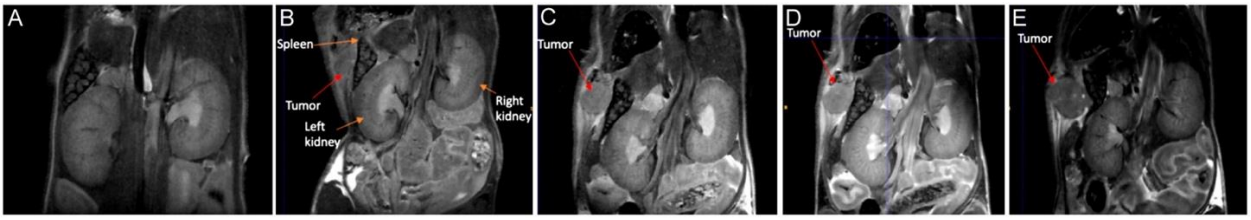


Figure 1. Coronal orientation of RARE images from C57BL/6 mouse with orthotopic pancreatic tumor from KPC cell lines. A; Healthy C57BL6 black mouse; B: day 12 after KPC injection; C: day 15, D: day 19, E: day 22

Obtaining multiple types of data through a single scan is beneficial in elaborating on many hidden features within tumor tissues without subjecting study participants to overly lengthy and redundant procedures. Echo planar imaging (EPI) utilizing a spin echo sequence allows for rapid imaging with little motion induced artifact.

Diffusion Weighted Imaging (DWI) and Diffusion Tensor Imaging (DTI)

This is due to the ability to acquire entire slices with a rapidly oscillating gradient. These images can be used to determine the diffusion tensor image (DTI), where the anisotropy of diffusion, or constraint of fluid movement due to tissue fibers, will alter the intensity of the signal while also providing directional information regarding the tissues causing the constraint. The tensor is derived from a matrix of values for each gradient and cell or tissue orientation. So long as six or more non-collinear gradients are used in combination with one “unweighted” image, the tensor can be determined. While lesser gradient variation can lead to decreased scan time, in order to improve the accuracy of measurements a minimum of 32 directions are used. The overall eigenvalues are then used to describe the “diffusion space” or ellipsoid generated from the now restricted flow of fluid, while the eigenvector denotes the overall direction of fluid movement.

Eigenvectors can be color coded to provide readily discernable directional information regarding the fibers within tissue structures and further connected generating tractography for determining connectivity within the systems. Diffusion tensor imaging relies upon the fractional anisotropy of a system. While unrestricted water is thought to freely move in all direction, restricted water will only move in constrained directions. This constraint is determined through the application of a series of gradients, where those in the same direction of the restriction will provide the primary eigenvector and generate the largest intensity within an image. The greater the intensity, the more freely protons will flow within the restricted direction, where a diminished intensity indicates minimal diffusion.

DWI images solid structures as bright signals and those with greater diffusion and flexibility as weaker signals. This allows more solid masses to be presented as brighter intensity within an image while those with high fluidity would appear dark. Based upon differences in Brownian motion utilized in DWI, it is often contradictory to apparent diffusion coefficient images. Where bright spots in a DWI indicate rigid tumors, these same masses would appear dark in ADC. Due to the unintuitive nature of DWI it is often useful to compare against the apparent diffusion coefficient maps where the restrictive diffusion is represented with decreased intensity and the more diffusive regions are bright. However, it fails to provide directional information and instead is merely a measurement on the magnitude of diffusion within each voxel.

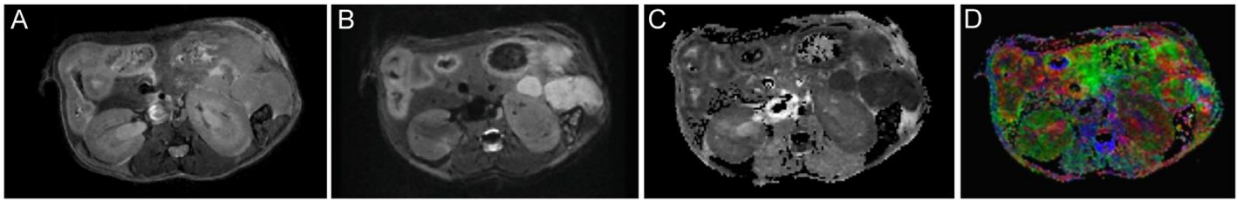


Figure 2. Investigation of desmoplasia using a variety of MRI techniques, which reveal different components of the shield around pancreatic cancer. Each image is taken from the same location. A to D are identical slices detailing different biophysical components of the tissues. A: Anatomical image detailing the tumor near the spleen (near the center of the mouse); B: Diffusion weighted image (DWI) detailing the random Brownian motion of water molecules within tissues; C: Apparent diffusion coefficient image demonstrating the intensity of diffusion of water within tissues; D: Diffusion tensor imaging (DTI) detailing the direction of diffusion of water within tissues.

DTI provides a spatially resolved map of the direction of diffusion of water within tissues yet comes at the greatest time expense (>30 min.). Thus, the combination between all images allows for the most thorough understanding regarding the behavior and physiological structures being initially examined through anatomical imaging rather than relying on what each image can offer individually.

The dissemination of this research and the quest for funding is an ongoing endeavor that was delayed by COVID-19.

Please note that there are ongoing endeavors, such as the NMR study of the nuclear delivery peptide WTAS (Om Prakash and Stefan H. Bossmann) that have been delayed by COVID-19 and then by the time required for installing new instrumentation in the Department of Biochemistry and Molecular Biophysics) and the ongoing protease expression profiling of more than 100 pancreatic cancer patients at the University of Kansas Cancer Center (Anup Kasi and Stefan H. Bossmann)