AWARD NUMBER: W81XWH-18-1-0620

TITLE: Modeling the Effects of Stroma on Clear Cell Renal Cell Carcinoma

PRINCIPAL INVESTIGATOR: Leif Oxburgh

CONTRACTING ORGANIZATION: MaineHealth
PORTLAND ME 04102-3134

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Fort Detrick, Maryland 21702-5012

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Modeling the Effects of Stroma on Clear Cell Renal Cell

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W81XWH-18-1-0620

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6. AUTHOR(S)
Leif Oxburgh

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5e. TASK NUMBER

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U.S. Army Medical Research and Materiel Command
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13. SUPPLEMENTARY NOTES

14. ABSTRACT
The year 1 project period was focused on: 1. Obtaining regulatory compliance approvals from DoD, 2. Generating baseline data required for modeling clear cell renal cell carcinoma (ccRCC), 3. Isolating primary ccRCC cells from patient samples. The regulatory approvals were issued within the first 3 months of the award, giving us 9 months to work on human tumor samples. We have successfully measured the rigidity of tumor samples and modified our silk scaffolding material to match. Two sequential window acquisition of all theoretical fragment ion spectra (SWATH) mass spectrometry experiments were run; first a pilot to define parameters and identify problems, and then an experimental run on 7 tumors and neighboring healthy kidney tissue. This extensive dataset will be analyzed in the coming project period to define the extracellular matrix cocktail that we will use to scaffold primary tumor cells and fibroblasts. Primary tumor cells have been isolated and cryopreserved pending finalization of the scaffolding system. One challenge that has been identified is in the isolation of tumor associated fibroblasts; in our hands these cells are not liberated using published dissociation techniques and we are in the process of empirically revising existing methods.

15. SUBJECT TERMS
Clear cell renal cell carcinoma, ccRCC, tumor stroma, tumor model, precision medicine

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified		19b. TELEPHONE NUMBER *(include area code)*

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1. INTRODUCTION

Clear cell renal cell carcinoma (ccRCC) is the 10th most common cancer. Surgical removal of the tumor is the primary treatment for the majority of patients, and is curative in over 50% of cases. However, therapeutic options are limited for the large number of patients whose cancer recurs. Chemo- and radiotherapies are ineffective, and the current focus is on inhibitors of specific pathways. Broadly, these are targeted to angiogenesis (eg Bevacizumab, Sunitinib) or to metabolism (eg Temsirolimus). It is anticipated that these drugs target pathways in tumor cells, cancer associated fibroblasts, endothelial cells and inflammatory cells. In most cancers, each of these populations plays an important role in determining pathogenesis; in ccRCC the cancer cell – vasculature communication has been the focus of particular attention because of the highly angiogenic nature of the tumor. However, little is known regarding the role of tumor associated fibroblasts. To study this poorly explored but potentially clinically important component of ccRCC we propose to generate an assay system with which we can understand the influence of tumor fibroblasts on tumor aggressiveness using primary patient-derived fibroblasts and tumor cells. We can efficiently generate ccRCC tumor models using porous 3D silk scaffolds with and without fibroblast incorporation, and these synthetic tumors are morphologically similar to patient tumors. Silk scaffolds allow for a controllable 3D structural support for cell growth and cell-cell interactions that is easily modified to mimic the native biophysical and biochemical environment. We have developed conditions for the growth of kidney tissue in the cortex of the kidney, where we see vigorous vascularization by the host. We propose to develop an orthotopic tumor modeling system with the highest possible fidelity by recreating multiple parameters of the tumor-of-origin such as cellular composition, metabolic profile, stiffness of the substrate and extracellular matrix (ECM) composition. Our project will generate high-fidelity patient-specific 3D tumor models in which patient-derived stromal cells and cancer cells are scaffolded on ECM matched to the tumor of origin. This technological platform will provide a high-fidelity assay system in which we can stringently evaluate the influence of cancer associated fibroblasts on tumor aggressiveness, and it will also provide a platform for a precision-medicine approach to determining drug susceptibility for individual patients.

2. KEYWORDS

Clear cell renal cell carcinoma, ccRCC, tumor stroma, tumor model, precision medicine

3. ACCOMPLISHMENTS (Italicized headings correspond directly to those in the SOW)

What were the major goals of the project? What was accomplished under these goals?

Specific Aim 1: Develop synthetic ccRCC tumors using prototype cell lines

Subtask 1 Submit documents for HRPO approvals covering all proposed animal experiments in the proposal

Documents were submitted and HRPO approval was obtained within the first 3 months of the award.

Subtask 2 Submit documents for ACURO approvals covering all proposed animal experiments in the proposal

Documents were submitted and ACURO approval was obtained within the first 3 months of the award.

Subtask 3 (Aim 1A): Retrieve 10 ccRCC tumors for use in the project from University of Pittsburgh Health Sciences Tissue Bank
10 samples were retrieved from the University of Pittsburgh Tissue bank, and in our first determination we found that approximately half of the collections were too small or had a fatty composition that made them very challenging to work with. For this reason, we expanded the number of tumors obtained from the tissue bank to 33 in order to obtain the highest quality material for the costly experiments proposed in this study. Table 1 provides a summary of the tumor samples that we obtained:

Subtask 4 (Aim 1A): Proteomic analysis of ECM from ccRCCs by SWATH mass spectrometry
We have performed 2 SWATH experiments in this study in the first year. First, we performed a pilot analysis on 3 tumor samples with 3 matched healthy tissue samples in technical triplicate. In this series of experiments, we troubleshot the technology and determined the best way to visually represent the data. Important aspects that we learned from this run was the need to incorporate internal normalization standards and the usability of the bubble plot to represent the data. This run gave us some candidates with big differential expression between tumor and normal, but we hesitate to draw conclusions about proteins with more subtle differential expression from this analysis. A summary of our pilot experiment is shown in Figure 1.

Based on our analysis of the pilot, we then performed a SWATH mass spectrometry analysis of 7 tumor versus matched healthy control tissue in technical triplicate. These samples were carefully selected so that they were all of the same stage (III) and grade (II) and histological quality. Data has undergone quality control and preliminary analysis, which is shown in Figure 2. We will finish the analysis within the coming month using the pipeline that we developed in our pilot experiment.

Subtask 8 (Aim 1B): Measurement of elastic modulus of ccRCCs using molecular calipers **and**
Subtask 9 (Aim 1B): Formulation of scaffolds mimicking tumor modulus by iterative variations in silk formulation followed by measurements of elastic modulus using molecular calipers
A range of silk percentages were investigated to determine their ability to mimic the extracellular environment of the ccRCC tumor. The Young's modulus of the silk scaffolds increased as the silk percentage increased (Figure 3). Additionally, the Young's modulus of each silk group was significantly different from all other silk groups ($p < 0.05$). The Young's moduli of the tested tumors had overall greater variation than the silk scaffolds (Table 2). Due to the range of Young's moduli measured (Table 3) from the tumors, both the 9% and 12% silk scaffolds are comparable to the Young's moduli of the ccRCC tumors.

Specific Aim 2: Determine how ccRCC aggressiveness is influenced by stromal recruitment
Subtask 1 (Aim 2A): Isolation and culture of cells from ccRCCs using established cell culture methods.
Tumor cells were cultures from 11 different tumor samples. For each cell isolation from a tumor, we also isolated cells from healthy neighboring tissue. Examples of matched samples are shown in Figure 4. Isolation was performed largely as published with some laboratory-specific modifications. Our success rate of establishing cell cultures from tumors is approximately 60%,

which is in line with published work. In almost all cases, the healthy neighboring cells grow out efficiently, but outgrowth of the tumor cells is the limiting step. To ensure that we can validate cell identity following outgrowth, we have developed a panel of molecular markers for use in flow cytometry, shown in Figure 5. Outgrowth of interstitial fibroblasts has been surprisingly challenging, and we have found that published methods do not work in our hands. Therefore, we are testing a variety of new methods based on work that we have done previously. In the interim, we will start our experiments using prototype immortalized fibroblasts as specified in the proposal, and we will also use primary human kidney fibroblasts purchased from a vendor.

What opportunities for training and professional development has the project provided?
Nothing to Report

How were the results disseminated to communities of interest?
Nothing to Report

What do you plan to do during the next reporting period to accomplish the goals?

Subtask 5 (Aim 1A): Construction of ECM-containing scaffolds. Incorporation of patient-defined matrix blends into silk will be tested by mass spectrometry.

Subtask 6 (Aim 1A): Testing tumor cell growth on ECM-containing scaffolds in vitro by measuring proliferation and migration of 786-O and NRK 49F cells.

Subtask 7 (Aim 1A): Testing tumor cell growth on ECM-containing scaffolds in vivo by measuring proliferation and invasiveness of 786-O and NRK 49F cells engrafted in scaffolds under the kidney capsule of immunodeficient mice.

Subtask 10 (Aim 1B): Testing effects of 786-O fibroblasts on modulus in vitro by evaluating if seeding of 786-O with or without NRK 49F cells into scaffolds alters their elastic modulus as measured using molecular calipers.

Subtask 11 (Aim 1B); Testing modified scaffolds in vivo by comparing proliferation and invasiveness of 786-O cells seeded into modified scaffolds with 786-O seeded into unmodified scaffolds.

Subtask 6 (Aim 2B): Cell biological verification of VHL mutant induced pluripotent stem cells by measuring their capacity to form kidney tissue following directed differentiation through molecular marker analysis.

Table 1: Renal Cell Carcinoma sample collection and cell isolation. Tumors and adjacent normal kidneys were collected from the University of Pittsburgh Medical Center and Maine Medical Center for use in this study. Cell isolation for *in vitro* culture modeling experiments was performed on samples meeting the following criteria; obtained within 18hrs of surgery and weight >0.5g. Success of isolation determined in ability to culture cells to minimum passage number (1 complete passage from 10cm culture dish).

Sample Collection Statitics		
Samples	33	
ccRCC	25 (75.8%)	
Sex	F	7 (28%)
	M	13 (52%)
	NA	5 (20%)
Race	White/Non-hispanic	22 (88%)
	NA	3 (12%)
Age	Average	65.92
	Median	68.5
Grade	G1	4 (16%)
	G2	8 (32%)
	G3	8 (32%)
	G4	4 (16%)
	NA	1 (4%)
Stage	I	10 (40%)
	II	3 (12%)
	III	9 (36%)
	IV	1 (4%)
	NA	2 (8%)
Isolation Statistics		
Criteria met for isolation	Normal	15 (60%)
	Tumor	18 (72%)
Succesfull culture	Normal	87.50%
	Tumor	94%

Table 2. Young's modulus of silk scaffolds and ccRCC tumors

Test Group	Young's Modulus (Pa)	Coefficient of variance (%)
3% Silk	152 ± 42*	28
6% Silk	1041 ± 234*	22
9% Silk	2887 ± 538	18
12% Silk	4030 ± 625	15
ccRCC Tumor	3235 ± 1140	35

Data presented as mean ± standard deviation and is representative of two (9% Silk), three (12% silk), or four (all other groups) independent experiments with 3 samples. Young's moduli of 3% and 6% silk are significantly different than Young's modulus of ccRCC tumor ($p < 0.0001$) as determined by ANOVA with Tukey post-hoc.

Table 3. Young's moduli of ccRCC tumors

Tumor Sample	Young's Modulus (Pa	Coefficient of variance (%	n
S79	3247 ± 563	17	3
S80	1862 ± 1470	79	2
S132	3878 ± 697	18	4
S250	3284 ± 1385	42	2
S282	2557	N/A	1
S340	2739 ± 1474	54	4
Average	3066 ± 1128	37	16

Data presented as mean ± standard deviation.

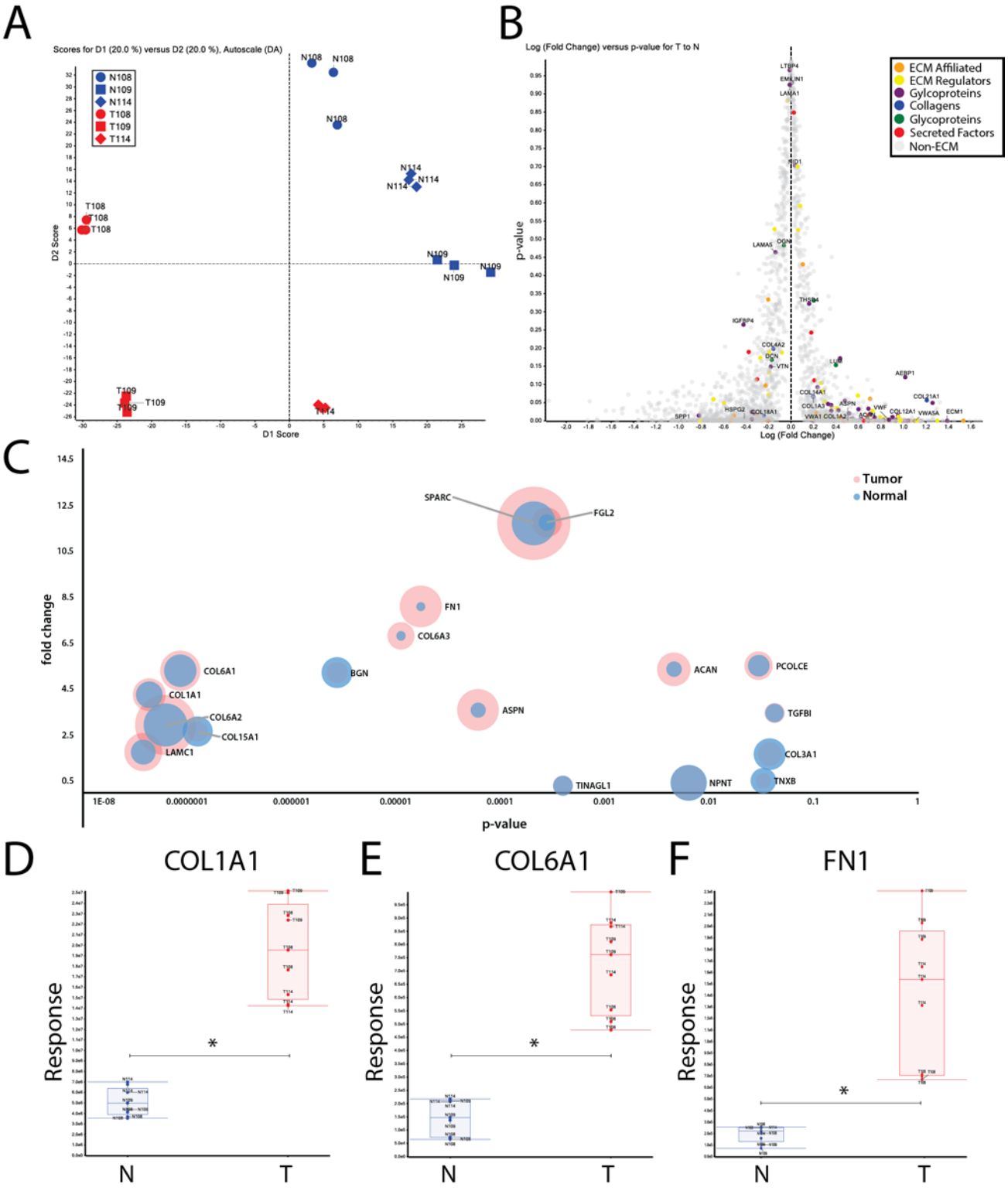

Figure 1: ccRCC Extracellular Matrix Analysis through Mass Spectrometry. A) Unweighted, unsupervised Principle Component Analysis of three sets of ccRCC tumors

and adjacent normal tissue. Normalization calculated using NMR methodology. Samples grouped by name alone, showing tight clustering of tumor and adjacent normal samples. B) Tumor vs. adjacent normal SWATH analysis conducted via SCIEX Analyst, presented as volcano plot. Of 2188 identified proteins, 149 matricellular proteins were identified and labeled through cross-referencing with the Matrisome database. C) Differentially expressed matricellular proteins were quantified through mass spectrometry data-dependent acquisition and analyzed in MaxQuant. Matricellular proteins comprised chromatograms of tumor and adjacent normal 15.96% and 7.98%, respectively. Extracellular matrix targets for use in modeling experiments were identified through combination DIA and DDA analyses, visualized using a bubble plot. Differentially expressed targets were identified using cutoff criteria of p=<0.05 and percent chromatogram cutoff of 0.1%. Bubble size in plot is proportional to percentage each target contributes to the chromatogram. D-F) Heterogeneity of selected targets was further analyzed and presented as box-and-whisker plots. "*" indicates groups are significant different (p<=0.05).

A

Mass Spectrometry Sample Statistics		
Samples	14 (7 Normal, 7 Tumor)	
ccRCC	14 (100%)	
Sex	F	1 (14.3%)
	M	5 (71.4%)
	NA	1 (14.3%)
Race	White/Non-hispanic	7 (100%)
Age	Average	63.29
	Median	68
Grade	G1	0
	G2	2 (28.6%)
	G3	2 (28.6%)
	G4	3 (42.8%)
Stage	I	0
	II	0
	III	7 (100%)
	IV	0

B

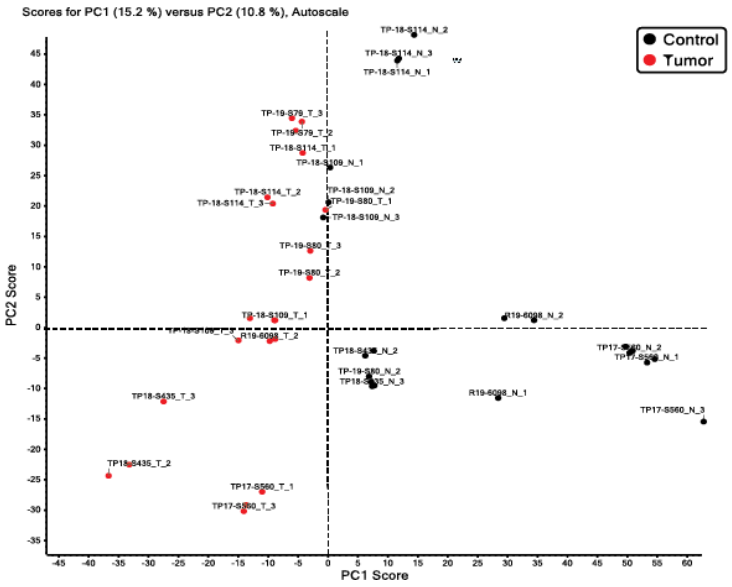

Figure 2: Expanded Mass Spectrometry Analysis based on tumor stage. A) Stage III tumors were selected for expansion of mass spectrometry analysis. B) Unweighted, unsupervised principle component analysis of grouped tumor and adjacent normal (control) samples.

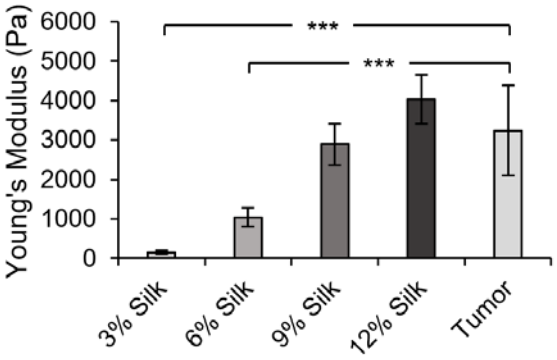

Figure 3. Young's modulus of silk scaffolds and ccRCC specimens. Data presented as mean ± standard deviation and is representative of two (9% silk), three (12% silk), or four (all other groups) independent experiments with 3 samples. Young's moduli of 3% and 6% silk are significantly different than Young's modulus of ccRCC tumor ($p < 0.0001$) as determined by ANOVA with Tukey post-hoc.

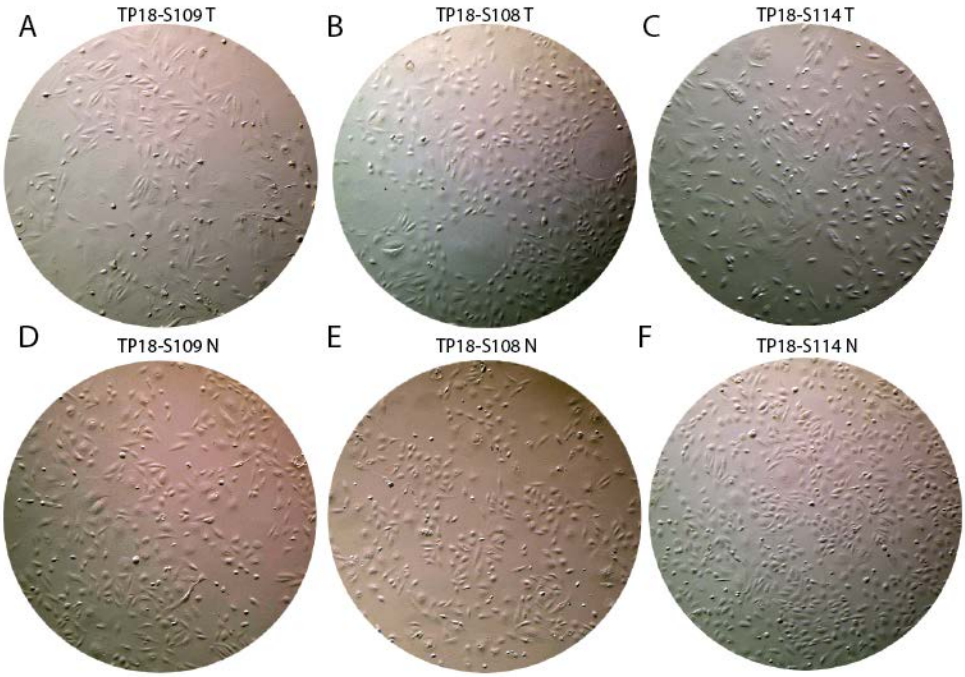

Figure 4. Examples of tumor (A,B,C) and matched normal (D,E,F) cells.

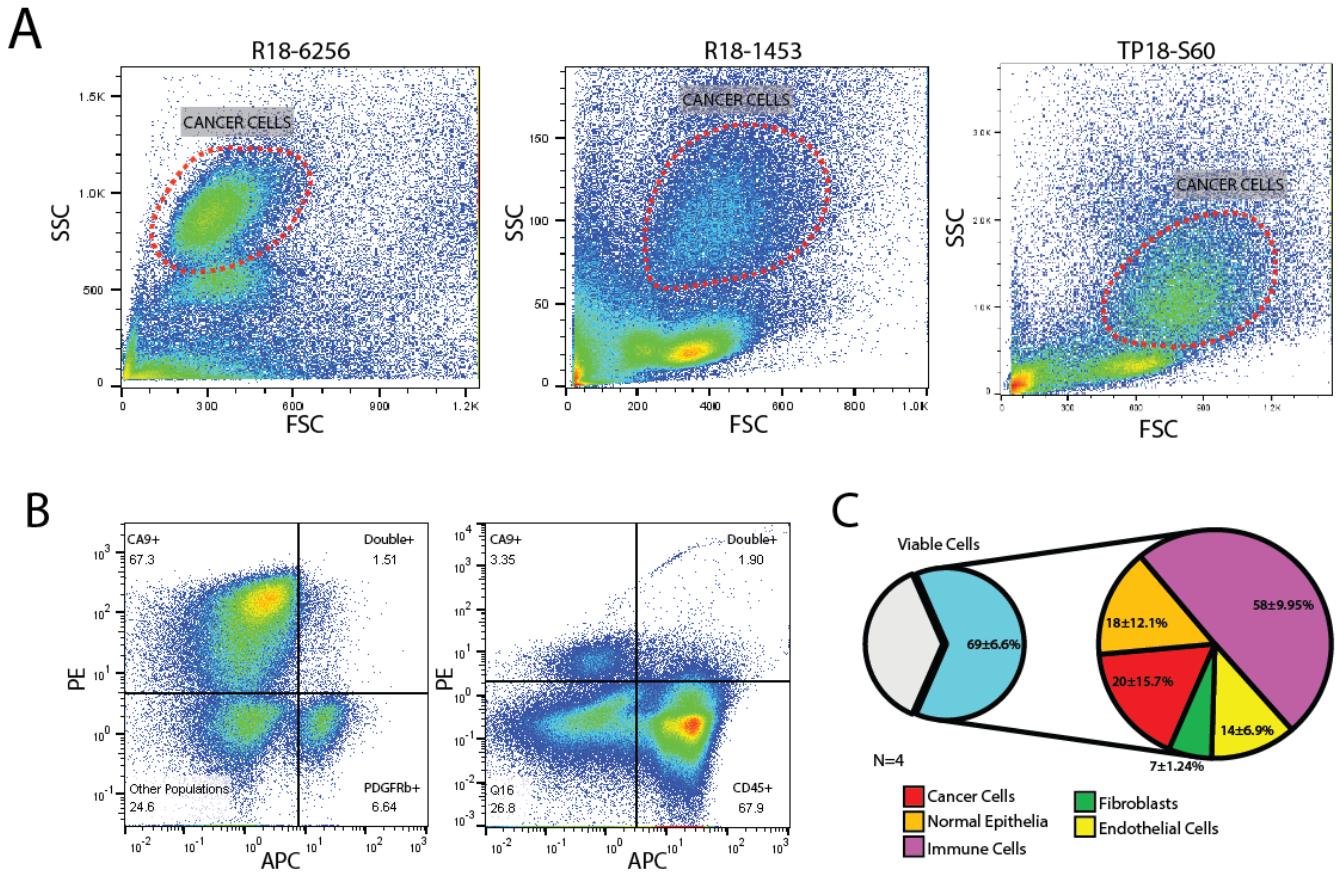

Figure 5: Flow Cytometry Analysis to define ccRCC tumor microenvironment. A) Flow cytometry analysis of single-cell suspension of tumors from three ccRCC tumor samples. Red dotted gate identifies tumor cells based on marker expression and morphological parameters, highlighting heterogeneity between tumors. B) Representative marker profile of tumor TP18-S60 shows gating efficiently distinguishes tumor cells (CA9+) from stromal components such as fibroblasts (PDGFRb+) and immune cells (CD45+). C) Summarized tumor microenvironment from the analysis of ccRCC samples (N=4). Only viable cells (Hoechst+/PI-) were used in establishing this expression profile.

4. Impact

What was the impact on the development of the principal discipline(s) of the project?

Nothing to Report

What was the impact on other disciplines?

Nothing to Report

What was the impact on technology transfer?

Nothing to Report

What was the impact on society beyond science and technology?

Nothing to Report

5. CHANGES/PROBLEMS

Nothing to Report

6. PRODUCTS

Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Leif Oxburgh
Project Role:	PI
Research Identifier:	0000-0002-2825-7663
Nearest person month worked:	1
Contribution to Project:	Coordination of the research project, preparation of documents for HRPO and ACURO approval, experiment planning and analysis, reporting.
Funding Support:	N/A
Name:	Kyle Bond
Project Role:	Doctoral Student
Research Identifier:	N/A
Nearest person month worked:	11
Contribution to Project:	Processing patient tumor material, isolation and propagation of primary cells, preparation of samples for mass spectrometry, experiment planning and analysis, preparation of figures.
Funding Support:	N/A

Name:	Jeannine Coburn
Project Role:	Co-Investigator
Research Identifier:	0000-0001-6354-5436
Nearest person month worked:	1
Contribution to Project:	The WPI research team fabricated silk scaffolds for in vitro renal cell culturing at Maine Medical Center Research Institute and performed mechanical testing on silk scaffolds and renal cell carcinoma biospecimens.
Funding Support:	N/A
Name:	Sunder Sims-Lucas
Project Role:	Sub-award Invesigator
Research Identifier:	0000-0003-1908-4809
Nearest person month worked:	1
Contribution to Project:	We provide kidney tissue samples and histological support for the project
Funding Support:	N/A
Name:	Elina Mukherjee
Project Role:	Research Technician
Research Identifier:	N/A
Nearest person month worked:	1
Contribution to Project:	We provide kidney tissue samples and histological support for the project
Funding Support:	N/A

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Oxburgh, Leif

R24DK106743 (Oxburgh) 7/1/15-6/30/20 3 calendar
NIH/NIDDK $269,333/yr
"Application of progenitor niche signals to ex vivo nephrogenesis"
The goal is to establish an arrayed, three dimensional, heterogenous culture system for ex vivo nephron differentiation.
Contact: Deborah Hoshizaki, Program Official
Deborah.hoshizaki@niddk.nih.gov
301-594-7712

PREVIOUSLY ACTIVE, NOW CLOSED
3R24DK106743-03S1 (Oxburgh) 9/21/17-8/31/18 1.8 calendar
NIH/NIDDK admin supplement $ 162,117/yr
“Engineering erythropoietin-producing cells”
The goal of this administrative supplement is to develop an EPO-producing cell from adult stem cells. The long-term goal is a cell-based therapy for anemia of chronic kidney disease.
Contact: Deborah Hoshizaki, Program Official
Deborah.hoshizaki@niddk.nih.gov
301-594-7712

PREVIOUSLY ACTIVE, NOW CLOSED
R01DK106743 (Oxburgh) 4/1/13-9/30/18 (NCE) 1.5 calendar
NIH/NIDDK $217,500/yr
“Defining the progenitor cell niche of the developing kidney
The goal is to identify signaling pathways stimulating proliferation of undifferentiated nephron progenitor cells.
Contact: Deborah Hoshizaki, Program Official
Deborah.hoshizaki@niddk.nih.gov
301-594-7712

PREVIOUSLY ACTIVE, NOW CLOSED
P30GM106391 (Friesel, PD) 9/1/13-5/31/19 (NCE) 1.2 calendar
NIH/NIGMS Phase III COBRE in Stem and $69,854/yr
Progenitor cell biology and regenerative medicine”
“Molecular Phenotyping Core”
The goal of this core is provide services for analysis of in vitro and in vivo gene and protein expression.
Role: Core Lead
Contact: Hongwei Gao, Program Official
Hongwei.gao@nih.gov

PREVIOUSLY ACTIVE, NOW CLOSED
P30GM106391 (Friesel, PD) 9/1/13-5/31/19 (NCE) 1.2 calendar
NIH/NIGMS Phase III COBRE in Stem and (salary only)
Progenitor cell biology and regenerative medicine”
“Administrative Core”
The goal of this core is to provide the administrative oversight for this Center that advances biomedically relevant discoveries in stem and progenitor cell biology.
Role: Core Investigator
Contact: Hongwei Gao, Program Official
hongwei.gao@nih.gov

National Science Foundation (C Henry, PI) 9/1/17-8/31/20 0 calendar
#1726541 Major Research Instrumentation Grant 497,479 (for instrumentation only)
Title: MRI: Acquisition of a digital light sheet microscope Leica TCS sP8 DLS: Bringing light sheet microscopy to Maine for research and STEM education
Principal Investigator: Clarissa Henry, University of Maine, Orono
Goal: The goal is to establish the first collaborative imaging facility with a light-sheet microscope in Maine. Analysis of biological processes within intact organs and/or living organisms is fundamental to understanding development and aging. The process of acquiring three-dimensional and/or time-lapse data in vivo has traditionally been hampered by technical difficulties that can be overcome, in large part, by using light-sheet microscopy. This award will facilitate cutting edge research and promote collaboration between researchers at the University of Maine, Maine Medical Research Center Institute, The Jackson Laboratories, and Mount Desert Island Biological Station.
Role: Co-Principal Investigator
Contact: Robert Fleischman, Division of Biological Infrastructure
rfleisch@nsf.org
703-292-7191

Sims-Lucas, Sunder
PREVIOUSLY ACTIVE, NOW CLOSED
K01DK096996 (Sims-Lucas) 07/01/2013-06/30/2018 6.60 calendar months
NIH $103,109 Annual Direct Costs
Renal Stroma Derived Endothelial Precursors are Critical for Renal Development
Kidney birth defects are the most common cause of kidney disease in children often leading to early death. We have mouse mutants that show how blood vessel cells are important in how the fetal kidney forms. A greater understanding of how blood vessel cells contribute to kidney birth defects will lead to new therapies for children with these diseases.
Role: PI
Contact: Tracy L Rankin, Program Official
rankint@mail.nih.gov
301-594-4748

PREVIOUSLY ACTIVE, NOW CLOSED
R03DK110503 (Sims-Lucas) 04/01/2017-03/31/2019 0.90 calendar months
NIH $50,000 Annual Direct Costs
Kidney derived endothelial progenitors play a critical role during kidney injury
We hypothesize that stromally derived endothelium are critical mediators for kidney injury.
Role: PI
Contact: Tracy L Rankin, Program Official
rankint@mail.nih.gov
301-594-4748

U24DK110791 (Dhir) 09/15/2016-05/31/2021 1.80 calendar months
NIH $103,494 Annual Direct Costs
University of Pittsburgh as the GUDMAP Tissue Hub and Collection Site
The goal is to act as both the GUDMAP Tissue Hub and Tissue Gathering site to build upon the pre-existing specialized collection 11g abilities of HSTB and provide high quality genitourinary samples to members of the scientific community including those within GUDMAP.
Role: Co-I
Contact: Deborah Hoshizaki, Program Official
Deborah.hoshizaki@niddk.nih.gov
301-594-7712

UG3DK114861 (Kellum) 07/01/2017-06/30/2022 0.60 calendar months
NIH $6,432 Annual Direct Costs
PReCISE AKI (Phenotyping REnal Cases In Sepsis and surgery for Early Acute Kidney Injury)
The Goal of this research is to propose to develop and deploy similar methods in this application. Careful clinical phenotyping will be essential to the goals of this UG3/UH3 consortium.
Role: Co-I
Contact:Cindy Roy, Program Official
cindy.roy@nih.gov

THIS AWARD
W81XWH-18-1-0620 (Oxburgh) 09/01/2018-08/31/2021 1.08 calendar months
DOD $18,332 Annual Direct Costs
Modeling the effects of stroma on clear cell renal cell carcinoma
The Goal of this research is to to study the poorly explored but potentially clinically important influence of tumor fibroblasts on ccRCC aggressiveness we propose to develop an assay system in which tumors will be generated in a controlled manner using primary patient-derived fibroblasts, primary tumor cells, and extracellular matrix scaffolding that closely mimics that of the tumor of origin.
Role: Co-I

Jeannine Coburn

Project Title: Development Methods for Delivery and Retention of Therapies with Oncological Applications (NEW AWARD)
Source of Support: Boston Scientific
Total Award Amount: $54,000
Total Award Period: 01/08/18 – 12/30/22
Location of Project: WPI
Months of Time Committed to Project: 0

Project Title: Site-directed genotypic targeting of pancreatic neuroendocrine tumors (NEW AWARD)
Source of Support: University of Chicago (University of Iowa)
Total Award Amount: $12,560
Total Award Period: 09/01/18 – 08/31/19
Location of Project: WPI
Months of Time Committed to Project: 0

Project Title: Modeling the effects of stroma on clear cell renal cell carcinoma (THIS AWARD)
Source of Support: Maine Medical Center (Department of Defense)
Total Award Amount: $29,030
Total Award Period: 09/01/18 – 08/31/21
Location of Project: WPI
Months of Time Committed to Project: 0.9 SUM

What other organizations were involved as partners?

Organization Name: University of Pittsburgh
Location of Organization: 123 University Place
B21 University Club
Pittsburgh, PA 15213-2303
Partner's contribution to the project (identify one or more)

- Other – subawardee

Organization Name: Worcester Polytechnic Institute
Location of Organization: 100 Institute Road
Worcester, MA 01609-2280
Partner's contribution to the project (identify one or more)

- Other - subawardee

KC170016: Modeling the effects of stroma on clear cell renal cell carcinoma

PI: Leif Oxburgh, Maine Medical Center

Budget: $553,824 **Topic Area:** Kidney cancer **Mechanism:** W81XWH-17-KCRP-IDA

Research Area: 0406 **Award Status:** 9/01/18 – 8/31/21

Study Goals:

To study the poorly explored but potentially clinically important influence of tumor fibroblasts on ccRCC aggressiveness we propose to develop an assay system in which tumors will be generated in a controlled manner using primary patient-derived fibroblasts, primary tumor cells, and extracellular matrix scaffolding that closely mimics that of the tumor of origin.

Specific Aims:

Aim 1: Develop synthetic ccRCC tumors using prototype cell lines **1A.** Incorporate tumor-specific ECM into the synthetic tumor scaffold **1B.** Adjust biomaterial stiffness to generate a scaffold with high physical fidelity

Aim 2: Determine how ccRCC aggressiveness is influenced by stromal recruitment **2A** Define the influence of fibroblast investment on tumor aggressiveness *in vivo* **2B.** Understand if fibroblasts participate in transformation of *VHL*-mutant epithelial cells

Key Accomplishments:

Publications:
Patents:
Funding Obtained: