Renal cell carcinoma microenvironment discovery project (REMEDY)

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Title:
Renal cell carcinoma microenvironment discovery project

Nickname: REMEDY

Investigators:

Dr. Antonio Finelli (Principal Investigator)
Associate Professor of Surgery
Head, Division of Urology, University Health Network
University of Toronto
Antonio.Finelli@uhn.ca
416-946-2851

Dr. Masoom Haider
Professor of Medical Imaging
University of Toronto
Haider.Masoom@sinaihealthsystem.ca
416-480-6100, ext. 4341

Dr. Adrian Sacher
Assistant Professor of Medicine
University of Toronto
Adrian.Sacher@uhn.ca
416-946-4501, ext. 3550

Dr. Trevor Pugh
Assistant Professor of Medical Biophysics
University of Toronto
trevor.pugh@utoronto.ca
416-581-7689

Dr. Laurie Ailles
Associate Professor of Medical Biophysics
University of Toronto
laurie.ailles@uhnresearch.ca
416-581-7868

Dr. Gary Bader
Professor of Molecular Genetics
University of Toronto
gary.bader@utoronto.ca
416-978-6888

Dr. Kieran Campbell
Assistant Professor of Molecular Genetics
University of Toronto
Lunenfeld-Tanenbaum Research Institute
kierancampbell@lunenfeld.ca
416-586-4800, ext. 5824

Dr. Neil Fleshner
Staff Urologist
Princess Margaret Cancer Centre
Neil.Fleshner@uhn.ca
416-946-2899

Dr. Girish Kulkarni
Staff Urologist
Assistant Professor University of Toronto, Department of Surgery
Princess Margaret Cancer Centre
Mount Sinai Hospital
Toronto General Hospital
Toronto Western Hospital
Girish.Kulkarni@uhn.ca
416-946-2246

Dr. Nathan Perlis
Staff Urologist
Assistant Professor University of Toronto, Department of Surgery
Princess Margaret Cancer Centre
Toronto General Hospital
Nathan.Perlis@uhn.ca
437-533-0228

Dr. Rob Hamilton
Staff Urologist
Princess Margaret Cancer Centre
Toronto General Hospital
Rob.Hamilton@uhn.ca
416-946-2909

Dr. Jason Lee
Staff Urologist
Assistant Professor, University of Toronto, Department of Surgery
Toronto General Hospital
Jason.Lee2@uhn.ca
416-340-3855

Study Overview

Abstract: To date, therapies that provoke an immune attack against the tumor, termed immunotherapies, are the only forms of treatment that can initiate long-term durable responses in patients with metastatic renal cell carcinoma (RCC). However, as many patients do not respond to therapy, a significant need exists to identify new immunotherapy targets in order to extend the number of patients obtaining long-term benefit from these drugs.

Background: Historically, long-term cures for patients with metastatic RCC was only achieved with cytokine immunotherapies, such as high-dose interleukin-2. However, this was associated with a poor response rate (7-8% of patients) and high-toxicity including mortality risk (~5%), curbing enthusiasm for this approach.[1] More recently, interest in using immunotherapy for RCC has been re-established with data demonstrating the efficacy and tolerability of drugs blocking immune checkpoints to reactivate anti-tumor T-cells, such as the PD-1.[2] However, resistance to these agents are common, with studies in murine models demonstrating the need to co-target many immune cell populations in order to obtain broad responses to immunotherapy.[3] These combination approaches require a comprehensive molecular characterization of the tumor immune microenvironment, in order to determine the complex cellular interactions at play currently limiting existing immunotherapy approaches. The feasibility of comprehensively dissecting the tumor immune microenvironment to determine novel drug targets has recently been established in mouse models and human tumors using a combination of flow cytometry and RNA sequencing.[4]
Rationale: To date, while it is clear that a comprehensive molecular characterization of the RCC immune microenvironment is required to identify new immunotherapy targets to improve clinical responses, such studies are in their infancy. Herein, we propose to utilize established state-of-the-art multi-dimensional technologies to characterize the RCC tumor immune microenvironment at a detailed molecular level. This discovery dataset will fuel the development of novel biomarkers and therapies.

Hypothesis :
1) A comprehensive molecular characterization of human RCC tumor specimens is feasible through a combination of single-cell RNA sequencing, cell surface proteomics, whole genome sequencing as well as radiological and pathological assessment.

2) A comprehensive molecular characterization of human RCC tumor specimens will identify novel protein targets that can be targeted to improve immunotherapy.

Study Aims :

1) To assess the molecular profile, including differential gene expression and unique protein epitopes , of RCC tumor microenvironment cell populations through single -cell RNA sequencing .

2. To relate data obtained from the tumor microenvironment molecular profile of each RCC to its pathological and radiological imaging features.

3. To relate the data obtained from the tumor microenvironment molecular profile of each RCC to its genetic mutational profile.

4. To generate patient -derived cell lines and xenograft models of studied samples to facilitate the discovery of new biomarkers and therapies.

Significance: This study will identify new targets on cells within the RCC tumor microenvironment that could be perturbed to improve anti-tumor immune responses. This knowledge would allow for the development of new immunotherapeutic strategies for RCC, with the potential to improve long-term cure rates for this disease. The study plans to enroll 300 participants.

Experimental Overview

Aim 1: To assess the molecular profile, including differential gene expression and unique protein epitopes, of RCC tumor microenvironment cell populations through single -cell RNA sequencing. Rationale: In order to profile the transcriptome and cell surface proteome, fresh viable cells are required in order to minimize ischemic changes and ensure cell state, viability and phenotype are maintained. To achieve this, a renal mass incisional or needle core biopsy will be performed after nephrectomy (radical or partial) and tissue will be immediately and rapidly processed for downstream experimental applications including flow cytometry, single cell RNAseq and cell surface proteomics.

Experimental Design:

1. Tumor, blood and urine sample collection :

Tumor : After nephrectomy, the specimen will be transported to the Surgical Pathology suite by Sally Zhang. One or more incisional biopsy (~2 cm x 2 cm) will be taken by the UHN Biobank PA on shift. The biopsies will be performed in a manner that will not interfere with final pathological assessment of the tumor. To facilitate this, all cases will be discussed prior to surgery with the study pathologist Dr. Christopher Ma. This protocol has been reviewed and approved by our previous and current study pathologists Drs. Andrew Evans, Joan Sweet,Christopher Ma, and Susan Prendeville. The biopsy specimens will be placed immediately in culture media (M199), or snap-frozen in liquid nitrogen, and transported to the laboratory on ice. Sally Zhang will transport samples.

Laboratory work will be performed in Laurie Ailles lab, conducted by Jalna Meens and Sally Zhang (Biosafety #56597).

Blood and urine : In order to determine tumor specific gene expression changes within the tumor tissue we will also perform single-cell RNA sequencing on blood (i.e buffy coat) and urine tissue from each patient. These samples will be collected at the time of surgery from each patient while they are anesthetized. This will be a one-time sample collection. These samples will be processed for single-cell RNA sequencing in an analogous manner to the tumor tissue. Consent for these samples is not optional, and the patient cannot participate in this study if they do not consent for collection of blood and urine.

Note, a portion of each tumor, blood and urine sample will also be snap frozen for subsequent DNA sequencing (described below).

Additionally, if a patient enrolled in this study is undergoing renal tumor biopsy as part of their standard of care diagnostic work-up, we will obtain an additional biopsy core to be used for research purposes. The rationale is that this tissue can be subjected to the molecular characterization described here and aid in the development of precision medicine approaches. Obtainment of these specimens will not interfere with the normal pathology assessment of the kidney tumor and will only be obtained if adequate diagnostic tissue has been collected. Patients will be consented for this as outlined in our consent form. Once the biopsy core is obtained, the tissue will be processed in an analogous fashion to the samples obtained during surgery as described above.

Note, all patients will be consented in the surgeon's office prior to surgery.

2. Single cell suspension generation: A single cell suspension will be generated from the tumor biopsy specimen by first mincing the tissue with scalpels and digested with 500U/ml collagenase IV, 100U/ml collagenase I and 200 mg/ml DNAase I for 30 minutes in a 25 ml Erlenmeyer flask (continuous stirring) at 37C and 5% CO2. Cells will then be passed through a strainer (70 um), separating isolated single cells from remaining tissue fragments. The single cell suspension will be kept on ice to minimize ischemic changes. A portion of the biopsied sample will be persevered and snap frozen using liquid nitrogen for subsequent pathological assessment and whole genome sequencing. Urine and blood samples are already in single cell suspension format and do not require the above processing step.

3. Flow c ytometry: Cells isolated in aim 1 will be subjected to multi-color flow cytometry sorting, using an established panel of antibodies. Cell surface staining will be completed in the presence of anti-Fc receptor antibodies and 2% fetal calf serum to prevent non-specific binding. Fixable live/dead zombie dye will be used to assess cell viability. Following cell surface staining, cell sorting will be performed using a BD FACSAria sorter to isolate populations of interest.

4. Single Cell RNA sequencing: 200,000 cells for each tumor single cell suspension, blood or urine sample will be sorted (as above) and maintained at 4 degrees to minimize ischemic changes. Samples will be delivered to the Princess Margaret Genomics Core facility and technicians within the facility will prepare and perform single-cell RNA sequencing using the 10X genomics platform via established protocols. There will be no left over patient material after this step.

5. Illumina sequencing : scRNAseq libraries will be submitted to the Princess Margaret Cancer Centre for sequencing. Sequencing will be performed on an Illumina Novaseq according to established protocols. All samples will be de-identified prior to submission. Samples and data generated prior to this version of the protocol will continue to be shared with Celsius as per collaboration contract states. No further testing of the samples will be conducted by Celsius. All sample information that will be shared with Celsius including the single-cell genomics data is outlined in Appendix 1. Note, there will be no sharing of any personal health information. This has been reviewed and approved by the UHN legal office (Lesley Rapport).

6. Cell surface proteomics : Tumor infiltrating cell sub-populations will be isolated by flow cytometry as described above and assessed for cell surface protein composition through standard proteomics approaches. This will include both liquid chromatography mass spectrometry, high throughput flow cytometry, and CITE-seq as previously described.[5-7]

7. Data analysis : Functional genomic and proteomic sequencing results will be analyzed using computational approaches at the Bader Lab in the Donnelly Centre. The raw gene counts from the tissue samples will first be input into an informatics pipeline to identify various cell populations present with the tumours. Dr. Bader's computational team will perform deeper analysis on these cell populations to reveal tangible molecular characteristics such as cellular states, mutation profiles, and cell-cell interactions. These aspects are crucial to understanding the disease and guiding further research.

Aim 2 . To determine the radiological and pathological features associated with the derived tumor microenvironment molecular data from aims 1 -2.

Experimental overview:

1. Radiological assessment overview: A comprehensive radiological assessment of each tumor will be obtained

Following nephrectomy, the tumor sample will be taken for an MRI at Mount Saini Hospital in order to map geographically where the tumor biopsies were obtained (details described below in section 2). Radiomic features will be extracted from the MRI images and established machine learning algorithms will be employed to determine features associated with our derived tumor microenvironment molecular signatures from aim 1-2.

Note, post-op tumor MRIs, will be performed at the MSH MRI facility under the supervision of Dr. Masoom Haider.

Additionally, standard of care radiological images including post-operative CT/MRI scans may be utilized to facilitate radiological-pathological-genomic correlations. In particular, these images will aid in co-registration of spatial regions within the tumor, and provide information on MRI-CT correlations. This will improve the translatability of any derived radiological biomarker signatures that we develop. Patients will be asked to provide consent for us to utilize these images. In addition, we will ask patients for their consent to utilize these images in future research. This will ensure that the information collected in this study can be utilized to its full potential.

2. Post-operative tumor MRI ：Following biopsy and surgical extirpation of the renal tumor, it will be placed in a sealed container in normal saline and transported to the MRI facility at Mount Sinai Hospital. The specimen will be transported by a designated UHN Biobank staff as well as our study Radiologist (Dr. Masoom Haider). The specimen will be submerged in saline, and an MRI will be performed. The specimen will then be transported back to the Toronto General Hospital pathology department. The maximum time from surgical extirpation to delivery at the Toronto General Hospital pathology department will be 90 minutes. This delay in clinical pathological assessment for research MRI purpose has been approved by the study pathologist (Drs. Andrew Evans and Joan Sweet, Christopher Ma previously) Dr. Susan Prendeville, and the TGH pathology chief Dr. Theo Van Der Kwast.

Imaging will be obtained in an orientation that include the biopsy sheath in plane. Quantitative T1, T2, diffusion and susceptibility mapping sequences will be performed for further co-registration with ex-vivo pathology. Using an appropriate marking device, a line will be drawn indicating the plane of sectioning for pathology. Once transported back to pathology sectioning will be done by a dedicated PA along the indicated plane of section to give a 5-6mm slice. Blocks will be taken with a grid pattern through the slice and labelled so that after staining they can be scanned and reconstructed back to a large field section. This will then be co-registered to the in vivo and ex vivo MRI for radiologic-pathologic correlation. A trial run will be done with a cadaveric bovine/porcine kidney to demonstrate feasibility. An example of this paradigm is shown in a liver study done ten years ago at UHN (Fig 1). Co-registration with ex vivio liver MRI of this colorectal metastases is shown in Fig 2.

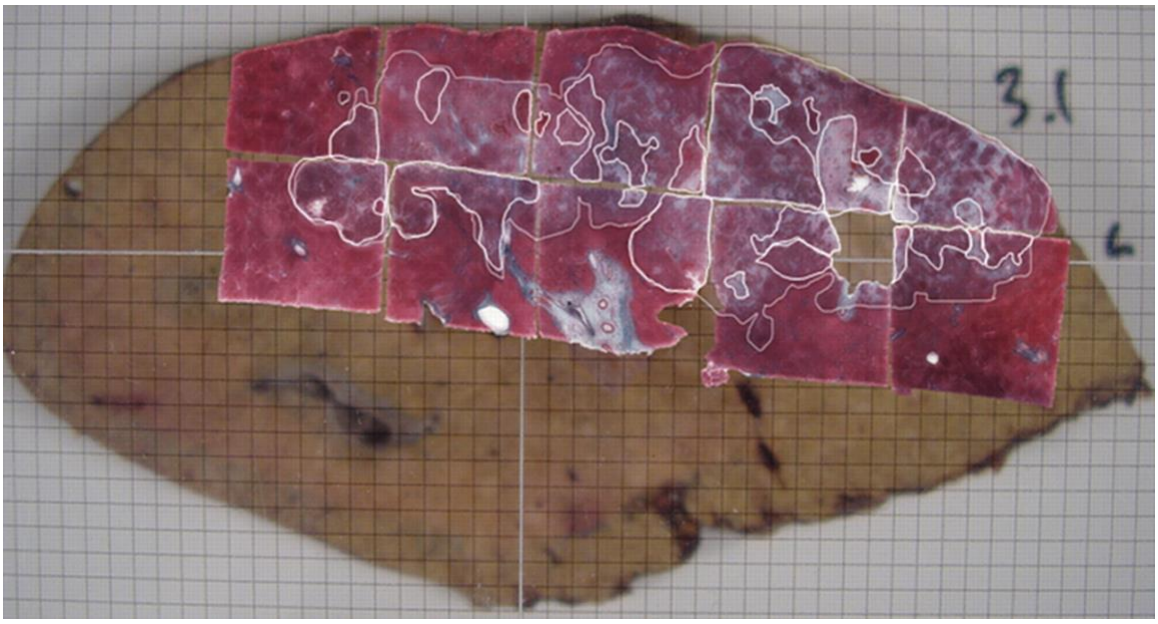

Fig 1. Example of path processing: Grid overlay with liver section further cut into squares to fit on standard slides and then reconstructed after scanning.

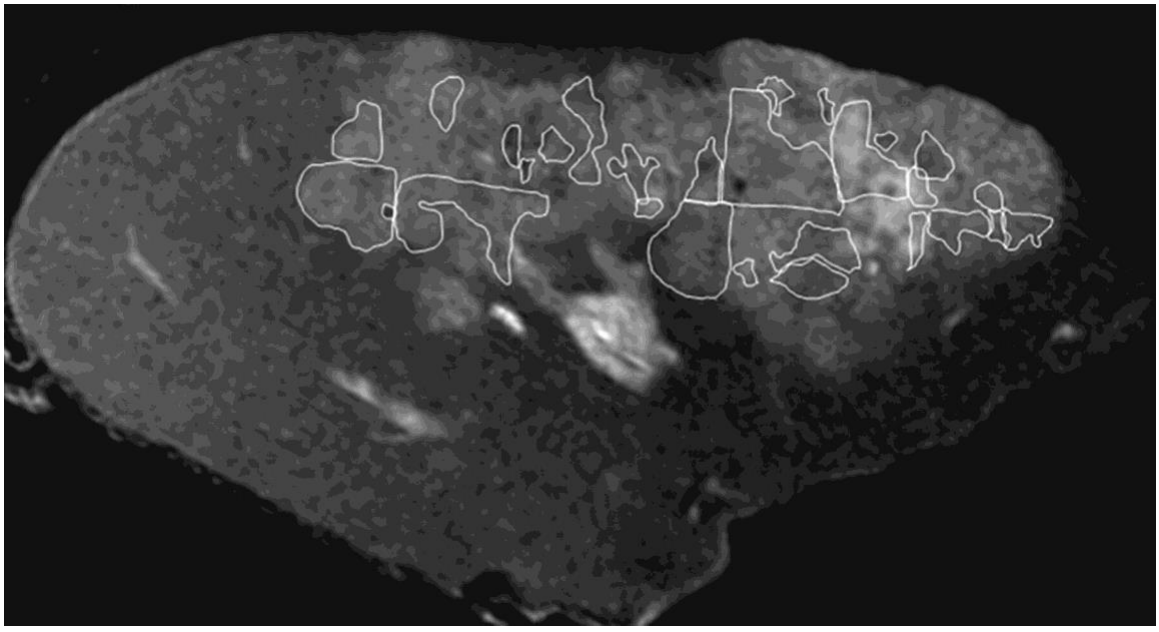

Fig 2. Example of path processing with imaging co-registration: T2 images of the sample plan as the pathologic section in Fig 1 showing the T2 signal correlates of areas of acinar necrosis in colorectal cancer

3. Pathological assessment of biopsy specimens : For each biopsy taken, a portion of the tumor material will be preserved, snap frozen in liquid nitrogen and submitted for standard pathological assessment. Digital images of the tumor

sections will be obtained and machine learning algorithms will be employed to determine digital pathology features associated with our derived tumor microenvironment molecular signatures from aim 1-2. This pathological assessment will be conducted for research purposes only.

4. Pathological assessment of renal tumor (surgic al) specimen: Following surgical extirpation and obtainment of an MRI image (Aim 3.3), the surgical specimen will be delivered to the Toronto General Hospital pathology department. Under the supervision of the study pathologist (Dr. Susan Prendeville), the specimen will be fixed and sectioned in a manner that matches the MRI imaging plane as close as possible. Histological sections will be scanned and reconstructed into whole tumor slices to facilitate radiological-pathological-genomic correlations. This pathological assessment will be conducted for research and clinical purposes, this protocol has been reviewed and approved by the study pathologist (Drs. Andrew Evans and Joan Sweet, Dr. Christopher Ma) Dr. Susan Prendeville.

Aim 3 . To determine the genomic features associated with the derived tumor microenvironment molecular data from aims 1 -2.

Experimental overview:

1. Whole genome sequencing on biopsy tissue , blood and urine . A portion of the tumor biopsy, urine and blood samples will be preserved and snap frozen in liquid nitrogen. In order to determine tumor specific mutations whole genome sequencing will also be performed on blood (i.e buffy coat) and urine tissue from each patient. These samples will be collected at the time of surgery from each patient while they are anesthetized. DNA will be extracted from all samples via established methods, and sequencing libraries will be prepared and sequenced at through the Princess Margaret Genomics Centre. All patient material storage will be organized through the UHN Biospecimen Services for this experimental aim. In addition, to ensure quick accessibility when needed, frozen tissue samples will be kept first in Ailles Lab, and later on moved to biobank for long term storage.

Aim 4. To generate patient -derived cell lines and xenograft models of studied samples to facilitate the discovery of new biomarkers and therapies.

Experimental overview:

1. Isolation of tumor fragments and cell lines from biopsy sam ple. At the time of final pathological assessment, a portion of the surgical specimen not required for pathological diagnosis will be dissected and placed in culture media (M199). This specimen will be transported to Dr. Laurie Ailles laboratory

(Princess Margaret Research Tower) and subjected to established protocols for generation of tumor fragments and cell lines [8, 9]. This will be performed by Jalna Meens and Sally Zhang under biosafety certificate # 56597.

Consent Procedure. Patients will be consented for this study prior to surgery or renal biopsy.

Inclusion Criteria

1. RCC or suspicious of RCC
6. 5mm slices, digital image available, biphasic CT. Di-com images available.

Exclusion Criteria

2. Pregnancy or Breast Feeding
3. Pheochromocytoma

Patient Risks

No risks exist for enrolment into this study.

References:

1. McDermott DF. Immunotherapy of metastatic renal cell carcinoma. Cancer 2009; 115: 2298-2305.
2. Motzer RJ, Escudier B, McDermott DF et al. Nivolumab versus Everolimus in Advanced Renal-Cell Carcinoma. N Engl J Med 2015; 373: 1803-1813.
3. Moynihan KD, Opel CF, Szeto GL et al. Eradication of large established tumors in mice by combination immunotherapy that engages innate and adaptive immune responses. Nat Med 2016; 22: 1402-1410.
4. Broz ML, Binnewies M, Boldajipour B et al. Dissecting the tumor myeloid compartment reveals rare activating antigen-presenting cells critical for T cell immunity. Cancer Cell 2014; 26: 638-652.
5. Elschenbroich S KY, Medin JA, Kislinger T. Isolation of cell surface proteins for mass spectrometry-based proteomics. Expert Rev Proteomics. 2010; 7: 141-154.
6. Gedye CA, Hussain A, Paterson J et al. Cell surface profiling using high-throughput flow cytometry: a platform for biomarker discovery and analysis of cellular heterogeneity. PLoS One 2014; 9: e105602.
7. Stoeckius M, Hafemeister C, Stephenson W et al. Simultaneous epitope and transcriptome measurement in single cells. Nat Methods 2017; 14: 865-868.
8. Gedye C, Sirskyj D, Lobo NC et al. Cancer stem cells are underestimated by standard experimental methods in clear cell renal cell carcinoma. Sci Rep 2016; 6: 25220.

9. Lobo NC, Gedye C, Apostoli AJ et al. Efficient generation of patient-matched malignant and normal primary cell cultures from clear cell renal cell carcinoma patients: clinically relevant models for research and personalized medicine. BMC Cancer 2016; 16: 485.

Appendix 1 Data elements collected in the study
All samples and information will be de-identified.

Clinical
Age
Sex
Co-morbidities
Relevant family history
BMI
Smoking status
Drinking status
Relevant disease history (previous and current)
- Previous renal tumour diagnoses and treatments
- Disease progression
- Metastatic status
- Others

Relevant treatment history
- Surgeries and physical procedures (e.g. renal biopsy)
- Radiation therapy: type, cycle/dose, location, duration, response
- Chemotherapy: type, cycle/dose, duration, response
- Systemic therapy: type, cycle/dose, duration, response
- Others

Radiological
CT of chest, abdomen, and pelvis
MRI of chest, abdomen, and pelvis
Ultrasound of chest, abdomen, and pelvis
Additional imaging (e.g. bone scans) in the case of metastasis

Pathological
Tumor location
Histology
Dimensions
Grade
Stage
Multifocality
Margin
Tumour extension and invasion
Tumor complexity
Additional features: sarcomatoid, rhabdoid, cystic, necrosis
Histological tissue staining features
Adjacent kidney tissue status