Study Title: Collection of gastrointestinal malignant and non-malignant human samples

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1. **Title**
   Collection of gastrointestinal malignant and non-malignant human samples

2. **Principal Investigator**
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3. **Purpose**
   The purpose of the proposed research is to collect tissue, blood and fecal samples from patients undergoing standard of care for their gastrointestinal disease, including Inflammatory Bowel Disease (IBD), and Colorectal Cancer (CRC). Tissue and blood samples will be obtained during procedures that are part of normal treatment, including blood and fecal collection, surgical resection, and biopsy collection. Samples will be obtained from consenting patients at Seton Dell Medical Center at the University of Texas (SDMCUT), or other relevant facilities (see section 6.i below), and only tissue not required for histopathological analysis will be collected. Initially, the focus will be on IBD, and CRC, where there are extensive previous studies to draw from.

   The collected samples of the proposed study will be used to establish biomimetic human organ-on-a-chip platforms by leveraging microfluidic tissue culture technology. Another focus of the research will be study the human intestinal microbiome that is highly associated with the pathogenesis of human gastrointestinal diseases. We have developed the microchip technology to mimic the structure and physiological function of human intestine by integrating tools developed in a microfluidic device, tissue engineering, and clinical microbiology, using intestinal cell lines. To recreate more reliable intestinal disease models and to further investigate the host-gut microbiome interactions in our experimental platforms, we are transitioning to use human clinical samples. We will use tissue biopsies to culture human intestinal cells including epithelium, endothelium, connective tissues on-chip. We will obtain blood samples to isolate peripheral blood mononuclear cells (PBMC) that represent mixed population of white blood cells (WBC). Isolated WBCs will be co-cultured with intestinal cells. We will also further investigate any potential application of microbiome-related therapies such as fecal microbiota transplantation (FMT).

4. **Procedures**
   Tissue samples will be obtained from consenting patients at (SDMCUT), or other relevant facilities (see section 6.i below), in accordance with protocols approved by the University of Texas Institutional Review Board (UTIRB). Patients who meet eligibility criteria will have their tumors collected at the time of their surgical resection, or 6-10 additional biopsies collected during endoscopy (upper gastrointestinal endoscopy or colonoscopy). All samples will be obtained during a procedure that is part of normal treatment, with no variation from standard of care with respect to the performance of this research.

   During the initial clinic visit with surgeon, patient will be presented with educational materials related to this study and a copy of the consent for consideration, as well as contact information of research staff available to answer any questions or concerns for the patient. Consenting will occur at subsequent clinic visits if applicable, or may need to occur on the day

of surgery if no further clinic visits are needed prior to surgery. Every effort will be made to ensure the patient has had adequate time to consider consenting to participation, as well as any questions or concerns addressed. If consent is to be made on the day of surgery, extra precaution will be taken to ensure the patient is clear and comfortable with all aspects regarding participation in the study.

No more than 10 mL of whole blood will be drawn from each patient, kept in a heparin treated tube to prevent coagulation, and transported to Kim lab by a member of Dr. Kim's lab. PBMC will be isolated from the whole blood samples and subpopulation of the isolated immune cells will be further analyzed using a flow cytometry in the UT Core Facility. Blood collection will occur at a pre-op clinic visit or on the day of surgery.

Tissue will be used to verify disease status by the pathologist. Tissue samples not required for histopathological analysis will be used to generate organoid lines as follows. All biopsy samples should contain intestinal crypt possessing the intestinal stem cells. In IBD patients diagnosed with either ulcerative colitis or Crohn's disease (CD), biopsy during colonoscopy or surgical resection should be done in the fully inflamed region as well as the non-inflamed region but adjacent from the inflamed region. Amount of biopsy depends on the surgeon's consideration. The number of additional biopsies required for research are 3-5 each of affected region and normal, adjacent region of the intestinal lining, for a total of 6-10 additional biopsies. Each additional biopsy is <0.05 g. In CRC patients, we need biopsies obtained from CRC region as well as non-CRC but adjacent region. Collected human tissue samples (less than 5 g) will be placed in ice-cold phosphate buffered saline (PBS) containing antibiotics (100 U penicillin and 0.1 mg streptomycin/mL) and transferred on ice in a biosafety carrier to the designated location (BME 4.318 at UT Austin) within 30 min by a member of Dr. Kim's lab.

To grow intestinal organoids using biopsy samples, we will incubate the biopsies in 8mM EDTA for 1 hour with vigorous shaking. After transferring biopsies into 1-2 mL complete medium (Advanced DMEM/F12, penicillin/streptomycin, HEPES, glutaMAX, B27 supplement, N2 supplement, 50% L-WRN conditioned medium, murine epidermal growth factor (EGF), Jagged-1, SB 431542, Y-27632, A-8301), we will vigorously pipette to release intestinal crypts and filter the tissues using a cell strainer (cutoff size, 100 μm) at room temperature. We will collect crypts by centrifugation at 200×g for 5 min and remove the supernatant. We will resuspend the crypts (target crypt density, 200 crypts per 50 μL Matrigel) in the Matrigel solution (4 °C), drop 50 μL of crypt/Matrigel suspension into 24 well cell culture plate, solidify the crypt suspension to gel at 37 °C for 1 h, then add the pre-warmed complete medium. We will keep the well plate in the humidified 37 °C CO_2 incubator by changing medium every other day, and pass the sufficiently grown organoids (average size 500 μm in diameter) every 7 days into the new Matrigel.

Fecal samples will be collected during patient's visit at clinics, normally prior to surgery, because it is required to flash freeze the sample to minimize loss of viable anaerobic bacteria. Individual patients will collect fecal sample using the lidded sterile container and a sterile plastic stick or cotton swab. Because the samples are required to be proceeded further in an anaerobic glove box, we will flash freeze the collected samples and transport to the Kim lab (BME 4.318). The aliquot (5 g) of samples will be then diluted in 25 mL of sterile NaCl solution (0.85%, w/v; autoclaved or filter-sterilized by 0.22 μm) inside an anaerobic glove box at room

temperature, and then filtered sequentially using sieves (cutoff sizes at 100, 70, and 40 μm) to remove slurries. The filtered suspension will be centrifuged at 6,000× *g* for 15 min at room temperature. The concentrated fecal bacteria suspension (approximately 10^{10} cells/mL) will be mixed with sterile pharmaceutical grade glycerol (final concentration 10%, v/v in PBS) and stored in a deep freezer (-80°C) until use. Any biopsy sample that is not used on the day of collection will be discarded following the biosafety protocols (IBC-2016-00002). Fecal matter, blood samples and/or cells, and organoids derived from patient tissue samples will be stored in a liquid nitrogen for future use in this study, or for use in future studies.

5. Location

Patient consent and specimen collection will occur at (SDMCUT), or other relevant clinic or hospital facility (see section 6.i below).

All research related to this study will occur in BME 4.318 at UT Austin

For a multi-site study in which the University is the lead or coordinating institution, provide the following:

i. Tissue samples will be collected during surgical resection, biopsy collection, blood collection, or fecal collection at Dell Seton Medical Center at the University of Texas, other facilities in the Seton Healthcare Network, and St. David's Healthcare Partnership.

ii. Each participating institution has been confirmed to have an active FWA according to the current United States Department of Health and Human Services (USHHS) Office for Human Research Protections (OHRP) Database (FWA00004937, FWA00004937, FWA00006399, respectively).

iii. Dr. Declan Fleming will act as the PI contact at each institution.

iv. This study will operate in accordance with approval by the UTIRB, and site approval will be obtained from each institution. In addition, Dr. Fleming will communicate with the medical director at St. David's Medical Center as well as Seton Healthcare Network. Moreover, once approved by UTIRB, this protocol will be shared with Matt Cowperthwaite at St. David's IRB.

v. Dr. Jennifer McKinney will act as the contact and facilitator of communication between institutions, and will maintain the responsibility of addressing any unanticipated problems involving risks to subjects or others, and other study-related information.

6. Resources

Kenneth Rainin Foundation, Innovator Awards

Texas 4000

7. Study Timeline

Aug 2017 – Jul 2022

8. Measures

Subjects will be informed and consents will be obtained from the participants prior to the surgery according to the IRB protocol. Please see Appendix A for the patient demographic information to be gathered at the time the tissue sample is collected.

We need to verify if the biopsy/surgery tissue samples representatively reflect the disease status of patients. For instance, we have to collect biopsies isolated from CRC region & non-CRC but adjacent region for testing our hypothesized idea. The degree of CRC should be also identified to sort out different lines of CRC-derived organoids. For the IBD patients, same criterion is applied as described in CRC case. Disease status will be confirmed by the pathology staff.

To determine the gut microbiota from human fecal sample, total DNA from fecal sample will be extracted with PureLink Microbiome DNA Purification Kit (Thermo Fisher Scientific). The extracted metagenomics DNA will be quantitatively analyzed by 16S rRNA gene amplification in V4-V5 region. The raw DNA sequences will be analyzed by CLC genomics workbench v9.5.1. which includes the CLC Microbial Genomics Module v1.6.1. (Qiagen).

Upon the patients' specimen are grown into the intestinal organoids and cultured inside the microfluidic devices, their viability and differentiation-specific markers will be analyzed by live/dead assay and immunofluorescence staining using confocal microscopy (BME 4.318). Other assays to verify pathological characteristics will be performed following references published in scientific journals. Patients will be consented to be allowed to be contacted by clinical staff or research coordinator to obtain follow-up information regarding their disease status.

All the research results will be published in the appropriate scientific journals and will be presented at the scientific meetings.

9. Participants

a. Target Population

No special populations are targeted for accrual to this study. However, racial background may play a role in disease etiology, and a variety of races is optimal for this study to address that question. Thus, the patient's race information will be included with the sample.

b. Inclusion/Exclusion

The study will not exclude potential subjects from participation on the basis of ethnic origin or gender. Subjects recruited will include men, women, and all ethnic origins, provided they meet all eligibility criteria as follows:

a) Biopsy-proven colorectal cancer scheduled to have surgical resection of primary site, at participating and approved facilities. Since it is standard clinical care to resect certain suspicious gastrointestinal masses without a pre-existing biopsy, patients who are undergoing resections for highly suspicious masses believed to be cancer, may be consented.
b) Diagnosed with IBD or CD, scheduled to have biopsy and/or fecal collection.
c) Age ≥18 years old.
d) Ability to understand and willingness to sign a written informed consent document.

c. Benefits

There is no known benefit to subjects for their participation in this study.

d. Risks

Removal of tissue sample during surgery: There is low risk from participation in this study, as the subjects will not undergo any additional procedures for this research study. Tissue will only be taken when the patient is undergoing a surgical procedure as part of their standard care. The tissue samples will be obtained from tissue removal and will only be taken from tissue not needed for the patient's medical care. No additional tissue will be taken as part of this procedure, thus no additional risk is added to the patient, and no additional time for the patient under anesthesia.

Removal of tissue during endoscopy or colonoscopy: There is negligible increased risk to the patient, as the tissue collected will be a very small quantity, measuring approximately 0.03 g per additional biopsy, and obtained during a procedure that is part of normal treatment. Each additional biopsy specimen takes <15 sec to acquire, thus additional time for patient under anesthesia is expected to be <3 min. There is a very small additional risk of bleeding, infection, or perforation (0.016-0.2%) associated with acquiring the additional biopsy samples.

Blood sample: The risks of obtaining a blood sample are minimal, similar to any time blood is collected, and include pain, bleeding, bruising, infection, and skin irritation (from cleaning agents used to sterilize the skin, or bandages).

e. Recruitment

Up to 300 patients will be recruited from the various approved facilities. Because there exists a treatment relationship with the patient, no recruitment authorization will be required to be signed by the patient. There will be no study specific patient advertising or supplemental recruitment materials used for this study.

f. Obtaining Informed Consent

Patients with IBD or CRC scheduled for surgery or biopsy removal at an approved facility will be asked to participate in the study prior to the procedure. Subjects will be consented by qualified study personnel, i.e., a research coordinator and/or investigator. These

personnel will understand the research study and will have completed IRB training as directed by institutional requirements. The investigators, clinic nurses or research coordinator will provide the subject with information regarding the research study.

During the initial clinic visit with surgeon, patient will be presented with educational materials related to this study and a copy of the consent for consideration, as well as contact information of research staff available to answer any questions or concerns for the patient. Consenting will occur at subsequent clinic visits if applicable, or may need to occur on the day of surgery if no further clinic visits are needed prior to surgery. Every effort will be made to ensure the patient has had adequate time to consider consenting to participation, as well as any questions or concerns addressed. If consent is to be made on the day of surgery, extra precaution will be taken to ensure the patient is clear and comfortable with all aspects regarding participation in the study.

Consent will be obtained in a quiet setting prior to the initiation of any study procedures and the subject will be given ample time to consider the consent form and ask questions. Every effort will be made to ensure that the patient has an understanding of the consent formed based upon his or her verbal response.

Qualified personnel as listed above, prior to the initiation of study procedures, will obtain written consent from the eligible patient. For Spanish speaking subjects, a professionally-translated version of the consent will be provided. All subjects will receive a signed and dated copy of the consent form.

10. Privacy and Confidentiality

This project will require the use of a coded identifier which will have a direct link to protected health information (PHI). Identifying PHI, such as name, phone, and address will not be provided to research staff. A HIPAA authorization form will be provided to the patient at the time of consent. This authorization will designate the specific health information that will be required to be released. A signed and dated authorization will be collected by the qualified study staff at the time the subject is consented.

Paper records for this study will be maintained in locked cabinets in Dr. Kim's research laboratory. Electronic records for this study will be maintained on password-protected computers on which security is protected by firewalls. At the time of consent, each patient will receive a study-generated number that will be used to identify the patient. Each specimen will be given a specimen number that is related to the patient identifier. All PHI will be entered by the consenting staff into the secure, HIPAA compliant online database, REDCap. Restricted access to REDCap will be given to Dr. Kim and his staff (e.g., they will be given access to patient data except for name, phone number, and address). Any clinical information regarding study subjects will not be stored within the laboratory. Files containing patient data will be stored in a locked office and/or on a password protected computer with access granted only to those individuals listed on the HIPAA Authorization form.

Blood, tissue, and fecal samples obtained for this study will be retained in and used by Dr. Kim's lab for future research purposes, or they may be disposed of, or shared with other researchers. Specimens which are shared will be coded and will not have any identifying information. Patients will not be charged or receive compensation for use of reposited specimens, or from any commercial ventures based on this research or any research in the future. Results from future research will not be shared with the patient. Patients may contact the clinical or research staff at any time to request that samples be withdrawn from research use, and any samples still in possession at that time will be destroyed.

Confidentiality of the Data or Samples

a. Demographic data will be collected at the time of consent. Blood and tissue samples will be collected during procedures that are part of normal care for the patient as described above. Fecal samples will be collected during a normal clinic visit if possible (e.g., at the time of consent), or just prior to the procedure if applicable.
b. Samples and data will be securely stored in Kim lab facilities, which have access codes for the doors to the labs where the samples will be kept. Samples located in the lab will be labeled with the tissue type, specimen number, and coded patient identifier only.
c. Collected biopsy samples will be kept in the Kim lab no more than 1 day. After biopsies are grown into organoids, the organoids will be kept in a liquid nitrogen tank for the purpose of storage and furture studies by Dr. Kim, or other researchers. Collected blood and fecal bacteria will be stored in the Kim lab at -80°C for the purpose of storage and future studies by Dr. Kim, or other researchers.
d. Data and samples obtained in this study will be stored in Kim lab facilities, and may be shared with other researchers for future research use. All data and samples will be identified by the coded patient identifier, thus PHI will not be shared with any current or furture research staff. The utmost precautions will be taken to ensure that PHI is not compromised.
e. Any biopsy sample that is not used on the day of collection will be discarded following the biosafety protocols (IBC-2016-00002). Fecal matter, blood samples and/or cells, and organoids derived from patient tissue samples will be stored in a liquid nitrogen for future use in this study, or for use in future studies.

11. Compensation

No compensation will be provided to participants of this study.

Appendix A

Patient Demographics

Patient Demographics	
Name	
Medical Record Number	
LCI Research Number	
Date of Birth	
Age	
Gender	
Race/ethnicity	
Name of Physician or Surgeon	
Type of procedure	
Signed Consent	
Signed HIPAA	
Signed Repository Consent	
Specimen(s) given	
Tissue from	
Blood given	
Fecal sample given	
Type of Cancer/disease	
Diagnosis date	
Stage at diagnosis	
TMN at diagnosis	
Metastatic Disease date	
Stage at consent	
Previous surgery/procedure	
Date	
Comments	
Angio-lymphatic invasion	
Previous radiation	
Date	
Comments	
Previous Chemotherapy	
Date(s)/# lines	
Comments	
# organs with mets	
Current medications	
Comments	

Comorbidities	
Smoker	
Drinker	
Alcoholic intake amount	
Recreational Drug use	
Type	
Frequency	
Prior Cancer	
Comments	
Family history of cancer	
Comments	
Vegetarian diet	
Date last contact	
Red meat intake	
Frequency	
Antibiotics	
Type (if known)	
Date last use	
Probiotics	
Type (if known)	
Frequency	
Date last use	
Artificial sweetener	
Type	
Vitamins and minerals	
Type	