Diabetic Complications Consortium

Application Title: Development of a Biobank of Human Diabetic Nephropathy

Principal Investigator: Agnes Fogo

1. Project Accomplishments:

We have developed the infrastructure to collect tissue from nephrectomies and autopsies, not used for clinical diagnosis, and link this tissue to a synthetic medical record derivative. Vanderbilt had an existing synthetic medical record derivative, BioVU, linked to patient DNA, with deidentified patients and records available for research. Our pilot tested the feasibility of obtaining tissue from patients undergoing nephrectomy for various reasons, or at autopsy, and collect a wide range of tissue samples from kidney, both frozen, in formaldehyde, and for EM, from cortex and medulla, to create a biorepository of patient material that can then be mined for study of kidney in patients with or without diabetes, and at varying points of clinical phenotypes, linking to the synthetic medical derivative. In the companion study awarded to Dr. Ray Harris, with Agnes Fogo as co-Investigator), we have developed optimal tissue harvesting and allocation for novel, state of the art MALDI imaging mass spectrometry (MALDI IMS) studies. We have developed the infrastructure, begun collection of tissues, and used this feasibility data from this pilot and the companion Dr. Harris pilot as the foundation of a recently funded major R24 grant from the NIDDK to bring together an interdisciplinary team. This R24 grant allows for a unique, collaborative effort among investigators in different areas of expertise, in this case those with experience in clinical and experimental studies of DN, tissue analysis and procurement, mass spectrometry, biochemistry and medical bioinformatics. By exploring molecular mechanisms underlying the identified changes in proteins, lipids and metabolites occurring in the course of human diabetic nephropathy, our overall goal for these studies, based firmly on the foundation of these pilot grants, is to define the molecular basis for human diabetic nephropathy and to identify potential targets for therapy by utilizing these new and powerful modalities.

2. Specific Aims:

Aim 1. We will test the feasibility of harvesting tissues from nephrectomy specimens and at autopsy, to create a tissue biorepository linked to a de-identified electronic medical record.

Our first major accomplishment was to set in place the infrastructure, oversight, consents and logistics to allow tissue collections, with approval from the BioVU ethics committee and IRB. This complicated process took longer than anticipated, and thus, we requested, and were granted, a no cost extension. Our next major accomplishment was to successfully begin tissue collections.

Our pilot aimed to collect remaining tissue from nephrectomy specimens not involved by cancer, and from autopsy, comparing tissues from patients without diabetes (control biopsies from transplant donors), or patients with diabetes with or without overt diabetic nephropathy (nephrectomy remnant tissue or autopsy).

To date, we have collected tissues from 53 cases, including 15 autopsies and 38 nephrectomies. Patients range in age from newborn to 84 years old, with most being adult, with 37 men, 16 women, 4 African

Americans, 1 Asian, and 49 Caucasian patients. Tissue was collected for frozen, possible RNA, lipids and RNA analysis, in paraformaldehyde, and in glutaraldehyde for possible EM studies.
We have also harvested frozen tissue from control biopsies from transplant donors with minimal or no abnormalities. (n=25 frozen tissue samples, suitable for mRNA/miRNA/ etc, and accompanying paraffin blocks, and n=130 paraffin block specimens from last 5 years, suitable for proposed MALDI-IMS).

Aim 2. We will test the feasibility of applying histology-directed mass spectrometry profiling, comparing feasibility of assessing profiles of metabolites, proteins, drugs and the lipidome, with comparison to the morphologic phenotype.
Additional preliminary studies have compared tissues prepared in different modes, using normal mouse kidneys. We compared tissue placed in Michel solution for 20 min or 24 hours prior to flash freezing as well as unfixed controls, sectioned at 12 μm and collected on to gold-coated stainless steel MALDI target plates. Serial sections were collected on standard microscope slides and stained with H&E to guide the application of matrix to areas of glomeruli and tubules. In the companion pilot study, led by Dr. Harris, mass spectra were collected from all samples using a Bruker Autoflex Speed mass spectrometer operated in linear positive ion mode. Michel solution contains a maleimide, which is reactive towards sulfhydryl groups, and tissue placed in this media showed shifts in peaks compared to controls without Michel exposure. Thus, we conclude that the portions of archival kidney biopsies exposed to Michel's are not compatible with mass spectral analysis.
We next assessed MALDI-IMS analysis for various tissue specimens. We compared tissues fixed in paraformaldehyde and paraffin embedded to those that had first been frozen and OCT embedded before fixation and paraffin embedding (mimicking the condition of donor biopsy flash frozen tissue, n=2 for each condition). All samples underwent on-tissue tryptic digestion using an acoustic robotic microspotter to apply enzyme and subsequent matrix in an array over the surface of each tissue section. Peptide mass spectra were acquired over the mass-to-charge range 600-4500. On average, 550 peptide peaks were observed from the fixed tissue specimens with a very high degree of similarity between those that had been fixed directly and those that had been frozen prior to fixation. Conversely, the frozen non-fixed tissue specimens showed less than 50 peaks and poor overall signal quality. We conclude that both samples that were directly fixed and those that were frozen prior to fixation can be used in clinical studies. Samples that have been flash frozen only cannot be compared to those that have been formalin fixed. We therefore are harvesting nephrectomy and autopsy tissues for these MALDI-IMS analyses by fixing in paraformaldehyde. In addition, frozen tissue aliquots are allocated for possible enzymatic, mRNA, microRNA and protein and lipid analyses from tissue homogenates.
We have done a test run of tissue harvesting from autopsy kidney. Tissue was harvested using the Cooperative Human Tissue Network (CHTN), allocating multiple aliquots of tissue for paraffin block, for EM, frozen tissue, from cortex and medulla. Frozen tissue samples were allocated in amounts suitable for protein/enzyme activity/mRNA/miRNA etc. analyses. Although tissue is inherently not an infinitely renewable resource, as is DNA, the multiple allocations from varying anatomic areas of the kidney, linked to display of the tissue histologic phenotype and the potential for linkage to BioVU, will allow tremendous power in exploring novel mechanisms of kidney disease. Aperio-scanned slides will be linked on a website available to investigators to illustrate the morphology of the tissue (see example of one case from 57 yo white man with nephrectomy for renal cell carcinoma in fig 1).
Our pilot run allowed us to set up specific details of size and number of tissue aliquots for each media, define barcoding/scanning/deidentifying procedures, and release of tissue for research, transport, and logistics of initial notification (see below). For our initial pilot study of human diabetic disease (companion pilot study), we identified 32 diabetic nephropathy (DN) biopsies done for cause. Patients were selected with at least 3 years of follow-up. Biopsies were chosen with a range of severity of diabetic lesions, spanning class I to IV, according to a recent classification of severity of DN (Tervaert 2010). Patients were further categorized as to progressors vs nonprogressors.

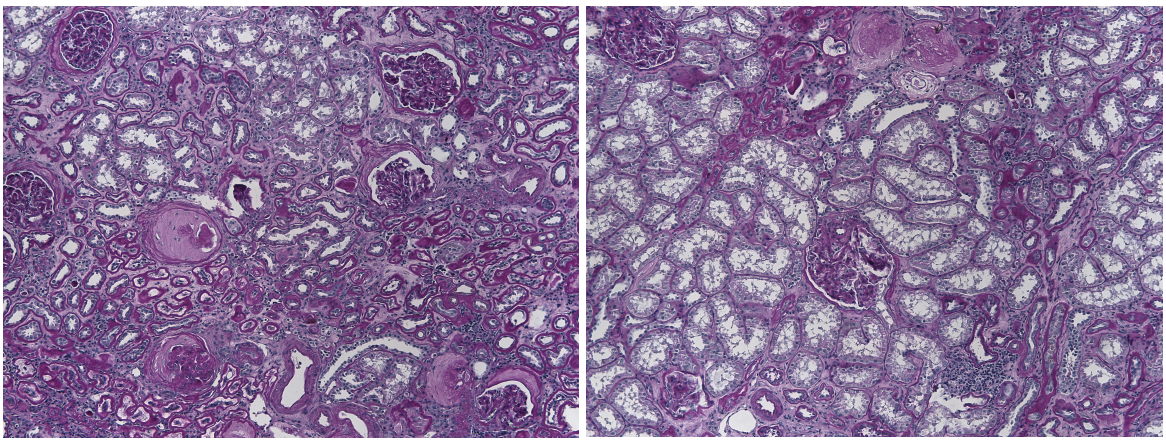

Fig. 1. Images from biorepository specimen from 57 yo WM, nephrectomy for renal cell carcinoma, from uninvolved area away from tumor. The kidney shows patchy interstitial fibrosis, some global glomerulosclerosis, and mild vascular sclerosis, findings consistent with mild arterionephrosclerosis.

We have done initial MALDI-IMS analysis of these tissues, in collaboration with the companion pilot grant, to further define whether differences in class of DN are mirrored by differences in peaks associated with morphologically detectable lesions. In future studies, supported by the R24 grant, we will mine these pilot findings to probe samples from patients with diabetes and kidney tissue from nephrectomies or at autopsies, and compare to those without diabetes.

Thus, we have developed a system that will allow allocation and de-identification of portions of tissue obtained from nephrectomy resections and at autopsy after diagnostic requirements are met. These samples are linked to de-identified electronic health records. This tissue procurement system builds on the existing deidentification program model and will provide a rich resource for studies of diabetic nephropathy. CHTN-VUMC IT and BioVU IT personnel have completed Stage I of the project, which required the development of web services to query the BioVU system for patient eligibility per the existing BioVU eligibility requirements (which excludes patients that have opted out). BioVU eligible patients are scheduled for tissue collection for targeted BioVU projects. Biospecimen collection details are pushed to the BioVU system and staff, the samples are distributed to the researchers and the bar code is destroyed in the CHTN DonorQuest system. This cycle allows BioVU to maintain the original collection data and build the virtual tissue resource linked to de-identified clinical data. Stage II of the proposed project will develop a standard set of clinical elements and patient driven data, extracted from BioVU records and organized to produce and support a query-triggered, centralized text-mining application.

Summary:

A current limitation of the BioVU approach has been that to date, only DNA samples have been collected. The current standard of care for diabetic nephropathy is to perform kidney biopsy only for diabetic patients with atypical disease features. Tissue samples from this patient population available for research are therefore limited. As a result of our pilot grants from the Diabetic Complications Consortium, we have been able to develop infrastructure to expand BioVU to include kidney tissue samples that will also be linked to the patients' DNA and medical information through the SD and we will utilize this infrastructure to procure samples for the kidney tissue biorepository that will be studied by MALDI-IMS. In addition, this biorepository will be available to be accessed by other investigators at Vanderbilt or at other institutions. Our recently funded R24 proposal will build on the foundation from these pilot studies.

3. Publications:

N/A