Diabetic Complications Consortium

Application Title: Gene expression in the black bear kidney: A first step towards understanding recovery in mammalian kidneys

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1. Project Accomplishments:

Previous studies in bears suggest they have unique features in the kidney that allow them to endure lower functioning during hibernation and recovery soon after hibernation. These features are likely in part encoded in the genome sequence and gene expression patterns unique to the bear. To better understand bear adaptations and physiology, we established the first de novo genome assembly of the black bear (*Ursus americanus*) with over 100x coverage. Subsequent RNA-Seq analysis of kidneys comparing gene expression profiles in black bears entering (late fall) and emerging (early spring) from hibernation identified almost two thousand differentially expressed genes. Among the genes with the largest expression differences were *IL16* (250-fold decrease in the spring) and *TNC* (20-fold increase in the spring), which could be involved in the bear's recovery after hibernation. In addition, RNA-Seq revealed 56 transcripts with seasonal differences in RNA editing. Among these transcripts was *SLC19A3,* which expresses a different variant with an amino acid change between fall and spring that could affect function. The identification of these differences in gene expression and RNA editing in the black bear kidney may provide new insights in the prevention and treatment of kidney disease.

The results of this pilot study are now being used in follow up studies in which we mimic the expression of these genes in a mouse model and measure the effect on the kidney after ischemia-reperfusion. An RO1 proposal is in preparation to support this work further.

2. Specific Aims:

Specific Aim 1. Determine a histological timeline of nephron recovery in the black bear.

Results: We collected 66 kidney samples within a 6-week period, soon after the bears reemerged from hibernation as well as a number of samples in the fall. For each kidney we performed a gross histopathological exam, quantified various features (e.g. glomerular size, Bowman space, % glomerulosclerosis), and a TUNEL staining to quantify apoptotic cells.
Although we observed several interesting pathological features in the spring that were not present in the samples collected in the fall, we did not see a decrease of these features over the 6-week period. Several of the quantified features, such as glomerular size and the size of the Bowman space strongly correlated with the age of the bear (as determined by the number of rings of the molar). However, TUNEL staining significantly decreased over the 6-week period. When plotting %TUNEL staining against time of collection (with the first bear set at day 1) we see a strong negative correlation (RSquare=0.60, Prob>F 0.005) suggesting recovery over time within the measured period.

Specific Aim 2. Identify genes that are differentially expressed in black bear kidneys as a function of nephron recovery.

Results: Kidney samples were collected in the fall, before hibernation, and in the spring, shortly after the bears emerged from hibernation. Approximately 60 million RNA-Seq reads were obtained from each sample before quality control. After appropriate quality control and correction for batch effect, we compared the spring samples to the fall samples and identified 1,937 differentially expressed genes. Of these, 487 genes were upregulated and 1,450 genes downregulated in the spring samples. We found homology with human for 369 of these 1,937 genes (19%). The largest down regulation (250-fold) in the spring kidneys is the gene encoding interleukin (IL)-16. IL-16 is a T-cell chemo attractant and is strongly expressed in the proximal and distal tubules. Inactivation of IL-16 by antibody therapy or IL-16 deficiency prevents ischemia-reperfusion injury and suggests it is a critical factor in inflammation-mediated renal injury. Another gene involved in inflammation in the kidney is *KLF5*, which is upregulated almost 8-fold. Expression of this gene has been localized specifically to the collecting duct and increases with renal injury in mice. The protein that is encoded by *KLF5*, has been shown to be a regulator of the response of collecting duct cells to renal injury by recruitment of macrophages. The ability to prevent azotemia is a special feature of hibernating bears and is not well understood. Our observation of an almost 8-fold decrease of *KLF12* provides a clue to the mechanism of this adaptation. This gene regulates UT-A, a urea transporter that is expressed in the inner medullary collecting duct. Tenascin C is an important component of the glomerular extracellular matrix. It has been shown to be upregulated during nephrogenesis and in glomerular disease. Furthermore, it plays an important role in the resolution of renal inflammation after induced glomerulonephritis. Our data shows a 20-fold increase in expression in the spring samples. This increase in *TNC* expression might indicate glomerular repair. We confirmed this last observation by performing in situ hybridization on spring and fall samples that were collected for Aim 1. There is clear *TNC* expression in the spring samples while we do not detect a signal in the fall samples.

The RNA-Seq data showed sequence differences between spring bears and fall bears, which suggests RNA-editing. RNA-editing is a post-transcriptional process that involves changes in specific nucleotides of the RNA molecules. To confirm this we sequenced the genomes of the same bears at low coverage. After comparing with the genome sequences and filtering for protein-coding transcripts we saw that RNA-Seq reads for 26 transcripts contain a different variant (between 10% and 95% of the total reads) from the genome sequence in the spring and reads for 30 transcripts have a different variant from their genome sequence in the fall. Of these 56 transcripts, 28 (50%) show A-to-I editing in which adenosine undergoes hydrolytic deamination to inosine, which is interpreted as guanine during translation. Eleven edited sites are in the coding region and four are predicted to lead to an amino acid change. Examples of amino acid changing editing are transcripts encoding SLC19A3 (thiamine transporter 2) and FMN1 (Formin 1). For *SLC19A3* we only see unedited transcripts in the spring, but in the fall 42% of the transcripts show a specific base change leading to a protein with glutamic acid at position 43 instead of aspartic acid (Figure 2). Changes in SLC19A3 might alter thiamine (vitamin B_1) clearance and reuptake in the proximal tubule. Similarly, we do not observe any editing in *FMN1* transcripts in the spring, but in the fall 72% of the transcripts show a base change leading to a

protein with proline at position 29 instead of leucine. Disruption of formin 1 leads to defects in kidney development and this gene could be involved in regeneration.

Although our study provides exciting new data, there are some limitations that need resolving in future studies. The first limitation is the inability to identify human orthologs for many of the genes/transcripts, which is a problem encountered with many genome-sequencing projects of different species. Although it is likely that human orthologs exist, the differences between the bear and human sequences are such that current algorithms are unable to confidently match them. In our study we were able to match 369 out of 1,937 differentially expressed transcripts (19%) to a human ortholog with the currently available methods. We expect this will improve over the next few years with the development of novel computational methods. The second limitation is the time of collection. The kidneys were collected in the early spring and we assume within a few weeks after hibernation, but the exact time between hibernation and collection is not known and this could affect the results. In our study we attempted to compensate for this by applying filters and only including transcripts showing upregulation or downregulation in all spring samples compared to fall samples, thereby perhaps excluding some genes relevant to the processes that we are interested in.

Despite these limitations, the current study opens the door to explore some of the evolutionary adaptations and unique features of the black bear in the context of human health. Our whole-genome assembly will be an important tool for genome-based studies and shows interesting differences with other mammalian genomes. We used this genome assembly combined with RNA-Seq from kidneys collected in two seasons to identify candidate genes that might be involved in the unique capability by black bears to recover from the physiological changes that take place in the kidney during hibernation. Not only did we identify differentially expressed genes, with fold changes ranging between 20-fold higher in the spring to 250-fold higher in the fall, we also have evidence for season-dependent RNA-editing. Whether this RNA-editing is a consequence of differences in body temperature, which has been shown to occur in fruit flies to make proteins more cold tolerant, or due to differences in renal physiology remains to be determined. To our knowledge, changes in RNA-editing in renal patients and/or animal models for renal disease have not been reported. Changes in RNA-editing have been shown to be a cause for human disease, but may also be a consequence of disease, involved in the disease process, and a possibility for treatment.

3. Publications:

Srivastava A, Sarsani VK, Sheehan SM, Seger RL, Barter ME, Lindqvist C, Brody LC, Mullikin JC, Korstanje R. De Novo Genome Assembly and RNA-Seq Shows Differences in Gene Expression and RNA Editing in Black Bear Kidneys Before and After Hibernation. *Under review by the Journal of the American Society of Nephrology*.