Animal Models of Diabetic Complications Consortium
(U01 DK076133)

Annual Report
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"Adiponectin and Nox4 in Diabetic Kidney Disease"

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Part A:

Principal Investigator's Summary

1. Program Accomplishments:

Hypothesis: Our original proposal was based on our novel findings in the decorin knockout diabetic mouse study, i.e. that the diabetic mice with renal disease also had low circulating adiponectin levels and increased Nox4 in their kidneys and glomeruli. We had thus proposed the generation of two new mouse models of diabetic nephropathy, namely, a decorin/adiponectin double knock-out and a smooth muscle-specific Nox4 transgenic. **Our hypothesis was that adiponectin and Nox4 are key modifier genes for diabetic nephropathy and diabetic cardiovascular complications.** Specifically the two new mouse models of Diabetic Nephropathy and Vascular Complications were:

a. Double knockout of adiponectin and decorin (*Apn/Dcn* dKO)
b. Nox4 transgenic with SM22 (*SM22/Nox4*)

Recent Progress and Major Accomplishments

Project a. In preparation for the double knockout study, we initially began to characterize renal function in the adiponectin KO (*Apn* KO) mouse with and without diabetes. We have now found that the *Apn* KO mouse has an elevated level of albumin/creatinine as early as 4 weeks of age. In association with albuminuria there is foot process fusion and increased Nox4 in the podocytes of the *Apn* KO mouse. Furthermore, with development of diabetes with the low dose streptozotocin protocol, the *Apn* KO mice develop a marked increase in albuminuria and increased urine hydrogen peroxide levels. Ongoing studies will further characterize the podocyte response to adiponectin and their adiponectin receptors and signaling pathways. Due to the intense interest in adiponectin in relation to obesity and diabetes complications, we propose to further characterize the diabetic adiponectin KO mouse and consider options in developing a mouse model to further explore this pathway. The original proposal to develop the adiponectin/decorin double KO mouse is still an option. Although eminently feasible, these studies may not increase our understanding of adiponectin's role in kidney disease and was not enthusiastically endorsed by the AMDCC steering committee. On the other hand, the study would provide further insights into the potential interactions between decorin and adiponectin and loss of two potent protective factors may pre-dispose to further features of advanced nephropathy. Another approach would be to corss the Apn KO with the adiponectin R1 or R2 KO mouse to further characterize the role of adiponectin and its major receptors in diabetic nephropathy.

Project b. The Nox4 transgenic mouse was proposed to be vascular smooth muscle specific based on our findings that Nox4 was increased in the vascular smooth muscle cells of the diabetic kidney in rats. However in the decorin KO diabetic mice, there is marked upregulation of Nox4 in mesangial cells and podocytes. In addition, podocyte Nox4 was increased in the *Apn* KO mouse and may thus play a major role in the early podocyte dysfunction associated with obesity and diabetes. Support for this concept are the studies by Bottinger's group showing that albuminuria can be decreased with apocynin, an NADPH oxidase inhibitor. If podocyte Nox4 is a major source of free oxygen radicals in podocytes then it would be appropriate to study a podocyte specific Nox4 transgenic mouse. We have recently begun a collaboration with Dr. Tom Leto of the NIH who has developed a construct for a tet regulated

Nox4 transgene. This construct has been supplied to us and we propose to develop a transgenic mouse with this construct and cross it with the podocin promoter mouse developed by Jeff Kopp. This mouse would then have an inducible upregulation of podocyte Nox4 and thus provide conclusive evidence regarding the role of Nox4 in podocyte dysfunction.

The studies to prepare a smooth muscle specific Nox4 construct has not been successful thus far. A construct provided from Andrea Eckhart at TJU contained the SM22 promoter. However we were not able to insert the Nox4 gene into this construct despite numerous attempts and approaches.

Plans for the Upcoming Year

In year two of the AMDCC project we plan to characterize the Akita diabetic *Apn* KO mouse and determine its response to exogenous adiponectin treatment. As such a model would have direct clinical relevence, a therapeutic approach would have novel implications in directing future therapies. The adiponectin receptors will be further characterized in podocytes and the adipo receptor KO mice will be obtained. Thus far an adipoR1 and adipo R2 KO mouse has already been prepared. We plan to obtain these mice and cross them with the adipo KO mouse that we already have.

For our second project we propose to initially characterize the Nox4 tet responsive construct in cell culture and then propose the generation of a podocyte specific inducible Nox4 mouse to the AMDCC steering committee.

Preliminary Milestones for 2009 and Beyond

By 2009 we plan to have available the double KO for adiponectin and its major receptors. These mice will be studied with development of diabetes by crossing with Akita mice. Podocyte function will be studies by isolating glomerular podocytes and measuring permeability characteristics. In addition, the podocyte specific Nox4 inducible mouse will be generated and we will be able to study its phenotype with and without diabetes. Additional studies with fat feeding will determine the role of the adiponectin/adipo R1/R2 axis and Nos4 in development of obesity related complications.

2. Collaboration:

With other AMDCC PIs

During the past year we have been completing a sub-contract with Dr. Bottinger and the Mt. Sinai group. Specifically we have studied the double KO decorin/LDL receptor mouse with diabetes. Surprisingly this mouse did not develop a severe renal phenotype. We are presently studying high fat feeding in these mice.

We hope o initiate studies with Dr. Moshe Levi with respect to fat feeding studies in future studies, possibly with the adiponectin KO mouse.

Additional studies to characterize our mice with respect to cardiovascular paprameters is a major goal of our projects. We plan to discuss mutual projects with Ira Tabas and Dale Adel.

With Jax We have had preliminary discussions with Dr. Ed Leiter regarding phenotyping and generating our diabetic mice on the appropriate strains. Once we have verified the Nox4 tet regulated construct we will plan to work with Jax to develop the transgenic mouse.

With the MMPCs At this point we have not yet sent samples to the MMPCs. We understand that the Washington group will be doing phenotyping of kidney histology and we plan to use these facilities to validate our scoring methods. At present we are continuing a collaboration with TJU to facilitate phenotyping and providing expertise in generating our mouse studies..

With other non-AMDCC PIs Drs. Bary Goldstein and Dr. Kevin Williams are co-investigators in our proposal. They will remain as collaborators with the formation of a sub-contract between UCSD and TJU. In addition, Dr. Peter McCue and Steve Dunn will provide phenotyping expertise.

3. Address previous EAC comments:

NOT APPLICABLE THIS YEAR

4. Publications:

Please list

1. Tchekneva, E., Rinchik, E., Polosukhina, D., Kadkina, V., Dunn, S., **Sharma, K.,** Qi, Z., Fogo, A., Breyer, M.. Generation of dominant ENU-induced mutations that predispose mice to diabetic nephropathy. ***Journal of the American Society of Nephrology***, 18:103-12, 2007

2. Zhu, Y., Usui, H., **Sharma. K**. Regulation of Transforming Growth Factor-beta in Diabetic Nephropathy: Implications for Treatment in Diabetic Nephropathy. ***Seminars in Nephrology***, invited review Mar;27(2):153-60, 2007

3. Scalia R, Gong Y, Berzins B, Zhao LJ, **Sharma K**. Hyperglycemia Is a Major Determinant of Albumin Permeability In The Diabetic Microcirculation: The Role of {micro}-Calpain. ***Diabetes***, 2007 Apr 19; [Epub ahead of print]

4. Williams, K., Qiu, G., Zhu, Y., Dunn, S., McCue, P., Bottinger, E., Iozzo, R**., Sharma, K.** Decorin deficiency causes an advanced and lethal nephropathy in diabetic mice. Manuscript in final revision, ***American Journal of Pathology***, 2007

5. **Sharma, K**, Qiu, G, Zhu, Y., Rao, S., Kataoke, H., Dunn, S., McCue, P., Chan, L., Falkner, B., Goldstein, B .Critical Link between Obesity and Kidney Disease. Manuscript in revision for ***Journal of Clinical Investigation***, 2007

6. Susztak, K., Bottinger, E., **Sharma, K.** Gene expression profiling in the investigation of diabetic nephropathy. Chapter in textbook of ***The Diabetic Kidney***, edited by Pedro Cortes and Carl Erik Mogensen, 2006.

7. Katoako, H., **Sharma, K**. Renal Handling of Adipokines. Chapter in textbook of ***Obesity Related Kidney Disease***, edited by G. Wolf, Karger Publishing, Contrib Nephrol. 2006;151:91-105.

8 Zhu, Y., Usui, H., **Sharma. K**. Regulation of Transforming Growth Factor-beta in Diabetic Nephropathy: Implications for Treatment in Diabetic Nephropathy. ***Seminars in Nephrology***, invited review Mar;27(2):153-60, 2007