

Overall research theme:

**Pathogenetic mechanisms of diabetic angiopathy**

Latest update:

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Characteristics of the research group:

The research group combines competences in basic research and clinical medicine. It combines integrative research in whole transgenic mouse models with methods in cellular and molecular biology. Transgenic models include overexpression of hyaluronan in the media of large and small vessels and overexpression of TGF- $\beta$ 1 locally in the kidney. In vitro systems include culture of endothelial cells and smooth muscle cells. Methods for assessments include immunohistochemistry, electronmicroscopy, ELISA, Western, in situ hybridisation, RT-PCR, Northern, HPLC, and laser dissection. We are mostly focusing on extra-cellular matrix (ECM) modelling and the cellular consequences of changes in the ECM.

Running projects: Titles and abstracts:

#### **Hyaluronan in diabetic angiopathy:**

Vascular complications are the leading causes of morbidity and mortality in diabetes. We suggest the presence of a specific diabetic angiopathy, which contributes to the increased number of atherosclerotic plaques seen throughout the arterial tree in diabetic patients. Furthermore, this may explain the equal incidence of vascular disease in diabetic men and women. *Macro-angiopathy* is defined as non-atherosclerotic morphological and biochemical changes confined to the tunica media in macroscopically normal, large vessels. It is characterized by linear calcifications, accumulation of several connective tissue components, including hyaluronic acid (HA), and signs of dysfunction of the overlying endothelial cells. *Micro-angiopathy* is located to the arterioles and capillaries and develops specific facets in the kidney and retina. We hypothesize that changes in smooth muscle cells (SMC) in the tunica media – due to the diabetic milieu – lead to changes in the extracellular matrix (ECM). These changes may increase the susceptibility to atherogenic insults and thereby vascular disease. Hyaluronic acid is implicated in cell proliferation, cell migration, inflammatory responses and water-homeostasis. Thus, we investigate whether increased amounts of HA in the tunica media has any biochemical, morphological and functional consequences for the vascular wall in vivo. Furthermore, we are investigating the role of the HA receptor CD44 for smooth-muscle cell proliferation and migration in vitro.

#### **The role of PDGF in proliferative retinopathy:**

The *microangiopathy* is located in the capillaries and arterioles and the main change is accumulation of ECM. However, in the retina the microangiopathy also contains an abnormal activation and proliferation of specific retinal cell types, such as endothelial and glial cells, and the receptor for platelet-derived-growth-factor (PDGF) is overexpressed on the proliferating cells {Bek & Ledet 1996 ID: 600} {Bek & Ledet 1996 ID: 601} {Bek 1998 ID: 599}. We hypothesize that this growth factor may play a role in the pathogenesis of proliferative retinopathy. Thus, we are in the process of establishing transgenic mice with overexpression of PDGF in the inner layer of the retina targeted by the Brn-3b promoter.

#### **The role of TGF- $\beta$ in glomerulopathy:**

Transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1) contributes to the thickening of the glomerular basement membrane (GBM), abnormal deposition of extracellular matrix (ECM) therein and expansion of the mesangial matrix (MM) in several glomerular kidney diseases. By using transgenic mice with TGF- $\beta$ 1 expression targeted to the juxta glomerular apparatus we have found that exposure of the glomerulus to TGF- $\beta$ 1 in vivo induces aberrant deposition of fetal laminin  $\alpha$ 1-,  $\alpha$ 2- and  $\beta$ 1 chains and collagen type IV $\alpha$ 1/ $\alpha$ 2 in the GBM. We found that the cellular origin of at least laminin  $\alpha$ 1 and  $\alpha$ 2 chains may be the glomerular endothelial cells. We speculate that the endothelial cells

could contribute to TGF- $\beta$ 1-induced glomerulopathy. This is currently being examined further by a combination of microdissection, expressions array, immunoelectronmicroscopy and culture of isolated glomerular endothelial cells.

#### Recent publications related to the projects described above:

Wogensen, L., Nielsen, C.B., Hjort, P., Rasmussen, L.M., Nielsen, A.H., Gross, H., Sarvetnick, N., Ledet, T. Under control of the Ren-1<sup>C</sup> promoter, locally produced TGF- $\beta$ 1 induces accumulation of glomerular extracellular matrix in transgenic mice. *Diabetes* 48:182-192, 2000.

Krag S, Høj Nielsen A, Wogensen L. High plasma concentrations of prorenin in a transgenic animal of diabetic nephropathy with over-expression of transforming growth factor- $\beta$ 1 in the kidneys. *Clin Exp Pharm and Physiol* 2000; 27:724-726.

Krag S, Østerby R, Chai Q, Birch Nielsen C, Hermans C, Wogensen L. TGF- $\beta$ 1-induced glomerular disorder is associated with impaired concentrating ability mimicking primary glomerular disease with renal failure in man. *Lab Invest* 2000;80:1855-1868.

Hansen, C., Pedersen, L-M., Ledet, T., Heickendorff, L., Rasmussen, L.M. The production of hyaluronan and chondroitin sulphate proteoglycans from human arterial smooth muscle – the effect of glucose, insulin, IGF-I or growth hormone. *Eur. J. Endocrin.* 2001; 145:193-198.

Rasmussen LM, Schmitz O & Ledet T. Increased expression of vascular cell adhesion molecule (VCAM-1) in cultured endothelial cells exposed to serum from type 1 diabetic patients: No effects of high glucose concentrations. *Scand J Clin Lab Invest* 2002; 62: 485-494.

Hilpert J, Wogensen L, Thykjaer T, Wellner M, Schlichting U, Ørntoft TF, Bachmann S, Nykjaer A and Willnow T. Expression profiling confirms role of endocytic receptor megalin in renal vitamin D3 metabolism. *Kidney International* 2002; 62: 1672-1681.

Knudsen ST, Foss CH, Poulsen PL, Bek T, Ledet T, Mogensen CE & Rasmussen LM. E-selectin-inducing activity in plasma from type 2 diabetic patients with maculopathy. *Am J Physiol Endocrinol Metab* 284: E1-E6, 2003.

Chai Q, Krag S, Chai S, Ledet T and Wogensen L. Localization and phenotypical characterization of collagen producing cells in TGF- $\beta$ 1-induced renal interstitial fibrosis. *Histochem Cell Biol* 2003; 119:267-280.