**Nox5, a novel target in diabetes research?**

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**Introduction**

The Nox family of NADPH oxidases is a group of enzymes that generate reactive oxygen species (ROS). Nox5 is of interest in diabetic kidney disease research for numerous reasons:

- It generates superoxide, which can cause oxidative stress ensuing in renal damage1,2.
- It is the only isoform that operates independently of the cytosolic subunit p22phox.3
- Compared to the other Nox isoforms, it has a more localized tissue distribution, and is expressed in podocytes, which are intimately involved in the progression of renal disease2.
- It appears to be upregulated in patients with DKD as compared to non-diabetic controls using immunofluorescence detection.

We hypothesize that Nox5 is upregulated in response to diabetic stimuli. The goal of this study is to confirm this in vitro by stimulating human podocytes with Ang II or TGFβ and quantifying changes in gene expression and superoxide production using real-time PCR and lucigenin assays respectively. Expression of the other Nox isoforms present in human glomerular podocytes will also be measured.

**Methods**

- Podocytes in culture were stimulated with AngII or TGFβ
- Cells were lysed and scraped after predetermined periods of time
- For lucigenin assay, samples were loaded in a 96 well plate and luminescence was detected
- For gene expression, RNA was isolated using Qiagen’s RNeasy Micro kit
- First strand synthesis was performed using RT-PCR
- Relative gene expression was measured using qPCR

**Results and discussion**

AngII stimulation of hPODS for 6h provoked a 3 fold induction of Nox5, and a 30% increase in ROS production compared to an unstimulated control. For gene expression, results were normalized against GAPDH, a housekeeping gene. In the ROS assay, results were normalized to the protein concentration. AngII did not induce significant induction of Nox1 or Nox4.

In considering the lucigenin assay, it is important to note that it provides a measure of total superoxide production in the podocyte and is therefore not specific to Nox5 generated ROS. Nox1 and Nox2, also present in podocytes, produce superoxide that may be detected in the assay. Note that Nox4 produces primarily hydrogen peroxide which is not detected by this assay.

**Conclusion**

Stimulation of hPODS in vitro with AngII and TGFβ caused increased gene expression of Nox5 and increased production of ROS which agrees with our anticipated results. Increased gene expression of Nox4 was also observed. Although more research is required, preliminary data suggests that Nox5 might be an appropriate target in attempts to slow the progression of diabetic kidney disease.

**Future steps**

- Stimulation of hPODS under other diabetic conditions, including high glucose and stretch
- siRNA knockdowns of NOX5 and p22phox in hPODS to confirm the functional contributions of the various NOX isoforms to ROS production
- In vivo characterization of NOX5 in a line of transgenic mice with the human gene inserted under control of the nephrin promoter

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**Acknowledgements**

Thank you to the Touyz lab for their lucigenin assay protocol. Thank you to Dr. Kennedy, the principle investigator, along with Dr. Holterman, my supervisor, and the other members of the Kennedy lab. Thank you to Jessyn Niergarth for the illustrations.

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**Contact information**

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