Nox5, a novel target in diabetes research?

Chelsea Nadeau-Towaij[1], Chet E. Holterman[1], Christopher R.J. Kennedy[1]
[1]Dept of CMM, University of Ottawa, Kidney Research Centre

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 damage[1,3].
- 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 disease[2].
- 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

Fig 1. AngII stimulation of hPODs – gene expression (n=3)

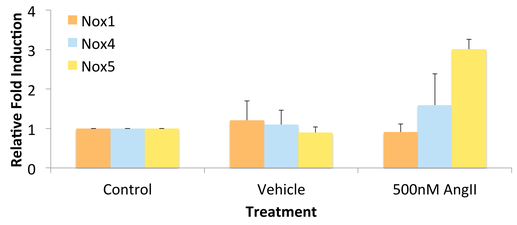
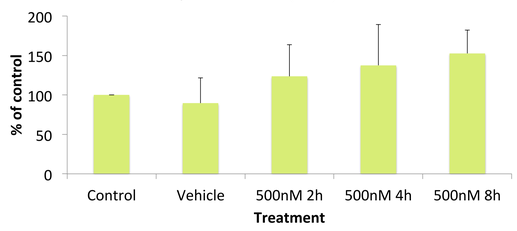


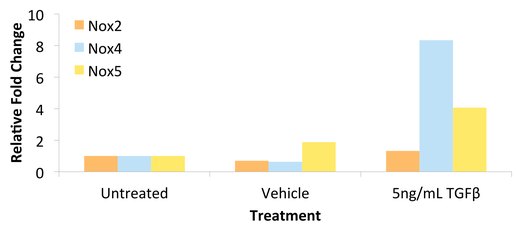
Fig 2. AngII stimulation of hPODS – ROS production (n=3)



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.

Fig 3. TGFβ stimulation of hPODs – gene expression



A 24h TGFβ stimulation provoked an 4 fold induction of Nox5 and an 8 fold increase in Nox4 expression. For gene expression, results were normalized against GAPDH, a housekeeping gene.

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

Literature cited

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Contact information

For more information:

The Kidney Research Centre's web-site:
http://www.ohri.ca/centrres/KRC/default.asp

Email:
cnade046@uottawa.ca