Review

Does the Notch signalling pathway play a role in hypoxia-induced renal fibrosis?

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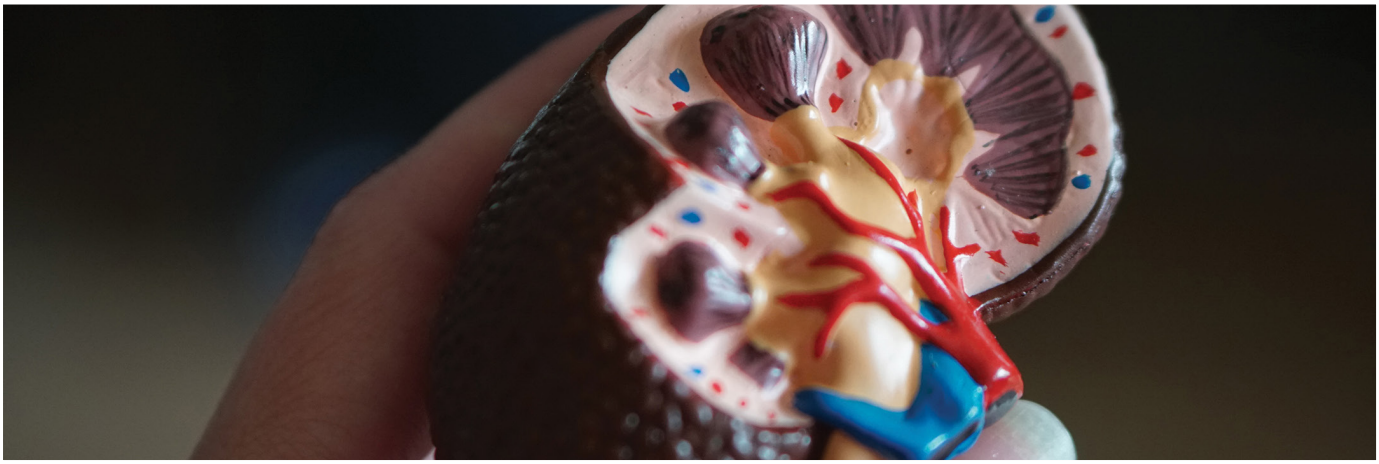

Abstract

Chronic kidney disease (CKD) has been estimated to affect millions of people in the United Kingdom. As such, much research has been conducted and the Notch signalling pathway (NSP) has been identified as a probable factor involved in triggering fibrogenesis in the kidneys, as a result of hypoxia. This review primarily aims to explore whether or not the NSP plays a role in hypoxia-induced renal fibrosis. Experiments have found that hypoxia triggers the NSP by up-regulating levels of the intracellular domain of the Notch receptor and expression of its ligand. In succession, in vitro experiments, which involve using components of an organism that have been removed from the usual biological surroundings, have concluded that the NSP produces changes typical of epithelial-to-mesenchymal transition (EMT). Conversely, in vivo experiments conducted on mice have noted that Notch-induced EMT is not a major contributor to renal fibrosis. While various experiments concluded that the NSP can be induced by hypoxia, the disparities in results of in vitro and in vivo experiments suggest that the NSP may not directly cause renal fibrosis. Therefore, more in vivo research should be conducted, with an emphasis on the molecular mechanisms of the NSP.

Abbreviations

CKD - Chronic kidney disease
CSL - CBF1 Suppressor of Hairless Lag-1
EMT - Epithelial-to-mesenchymal transition
FA model - Folic acid model
NECD - Notch extracellular domain
NICD - Notch intracellular domain
NSP - Notch signalling pathway
UUO - Unilateral ureteral obstruction

Introduction

Chronic kidney disease (CKD) refers to the gradual deterioration in the functioning of the kidney due to changes in its structure.[1] Since this disease is usually asymptomatic in the early stages, diagnosis typically does not occur until it has progressed to an advanced stage, making it difficult to treat effectively.[2]

The Notch signalling pathway (NSP) is highly conserved and is necessary for renal organogenesis, after which it is largely suppressed.[3] However, subsequent upregulation after kidney maturation has been associated with acute and chronic kidney damage.[3] This upregulation is facilitated by hypoxic conditions and results in renal fibrosis.[4] In essence, the NSP (**Figure 1**) involves the release of an intracellular component of a Notch receptor that serves to regulate transcription of Notch target genes.[5]

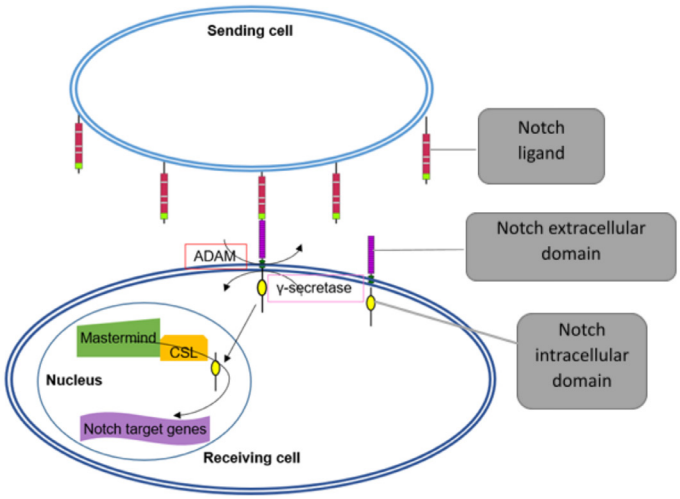

Figure 1. Diagram showing the Notch signalling pathway. Based on data from Mumm and Kopan (2000).[10]

The Notch receptor has two main components: the Notch extracellular domain (NECD) and Notch intracellular domain (NICD). The pathway

becomes activated when a Notch ligand from a sending cell binds to the NECD of a receiving cell. This promotes two proteolytic cleavages of the NECD and NICD, which are catalysed by an ADAM-family metalloprotease and γ-secretase, respectively.[1] The NICD becomes free within the cytosol of the receiving cell and enters the cell's nucleus, where it interacts with a DNA-binding protein known as CBF1 Suppressor of Hairless Lag-1 (CSL), its co-activator, Mastermind, and other transcription factors.[5] This process allows for Notch target genes to be transcribed.[1] Studying the intermediates of the NSP is hence important because it may signpost parameters that can be used to diagnose CKD and identify therapeutic targets.

While extensive research has been conducted on this topic, several uncertainties remain. The specific molecular mechanisms regarding hypoxia as a causative agent of renal fibrogenesis are not well elucidated. In particular, S2 cleavage remains an important aspect of further investigations since metalloproteases can be regulated by multiple external factors. Additionally, there is limited research about how the NSP operates under different temporal and spatial cellular resolutions. Temporal resolution refers to observations made over a period of time, while spatial resolution refers to observations made from a particular area or sample.

The primary aim of this review is to explore whether or not the NSP plays a role in hypoxia-induced renal fibrosis, by evaluating in vivo and in vitro experiments. The review will also consider some animal models that can be used to investigate this. Lastly, the interactions between the NSP and E-cadherin will also be briefly discussed.

Literature search

The sources utilised in this paper were acquired using the PubMed and Trip databases. Key words were extrapolated from the aim and included in the database searches. As such, the search term used was 'Notch AND hypoxia AND renal AND fibrosis'.

Filters were applied to refine the search to produce studies that have been published within the last twenty years. The titles and objectives of each study were then read to ascertain whether all the parameters of the search were discussed. Citation searches were also performed. Specifically, the article written by Sirin and Susztak, 2013, on the role of the NSP in the kidney was used to find primary research papers.[3] A flow diagram showing the identification process for eligible studies is shown in **Figure 2**.

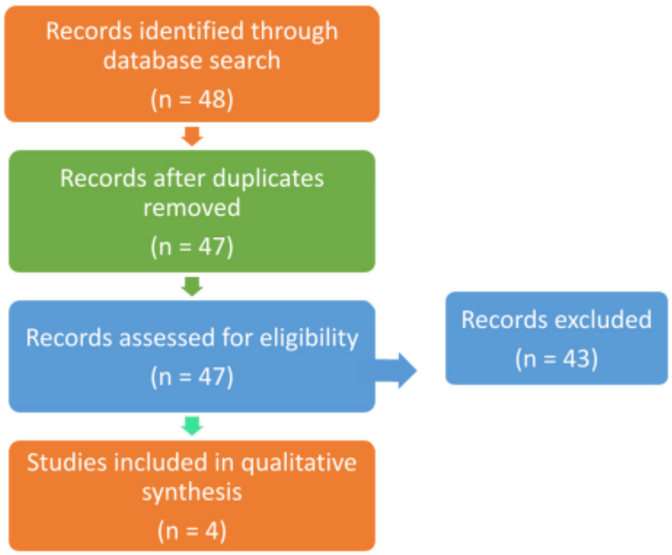

Figure 2. Diagram showing the identification process for eligible studies.

Discussion

Animal models of renal fibrosis Animal models are critical for biomedical research as they allow for the exploration of physiological functions and systemic interactions. Renal fibrosis can be studied using induced, spontaneous and in vitro models, amongst others. Specific to this review, two animal models will be discussed. The folic acid model (FA model) involves the administration of an injection of folic acid to mice. Folic acid precipitates in renal tubules, blocking the flow of luminal fluid through the nephrons and increasing the pressure within the tubules. This causes tubular epithelial damage and, consequently, acute renal failure, followed by renal fibrosis.

The unilateral ureteral obstruction (UUO) model involves the ligation of the ureter of mice. This prevents the flow of urine from the renal pelvis to the bladder and ultimately leads to acute kidney injury as a result of urea accumulation and pressure increase. Both models have limited temporal resolution but relatively high spatial resolution since immunohistochemistry is used to analyse the tissue.

How does hypoxia induce the NSP to cause renal fibrosis? The NSP regulates hypoxia-induced renal fibrosis via several synergistic mechanisms.[4] Firstly, hypoxia triggers the epithelial Notch pathway by up-regulating the levels of NICD and expression of the ligand.[6] A study conducted by Bielesz *et al*. noted that after 1 and 7 days of administration of folic acid to mice, transcript levels of Notch receptors (Notch 1-3) and the ligand (Jag 1) increased at least two-fold (**Figure 3**).[7] The results of this study were further supported by in vitro experiments done by Sahlgren *et al*., which produced statistically significant results ($p<0.05$).[6] These observations highlight that hypoxia can both induce the NSP and amplify pre-existing Notch signalling.

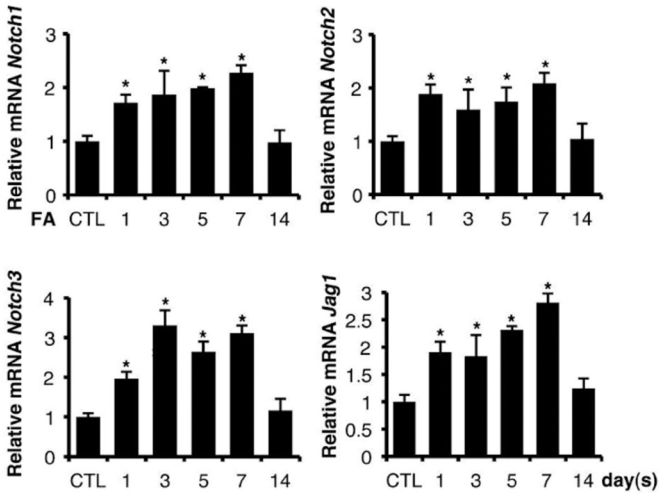

Figure 3. Graph showing the relative amounts of mRNA of different Notch receptors and a ligand. Graph showing the increase in transcript levels of the different Notch receptors (*Notch1*, *Notch2* and *Notch3*) and a Notch ligand (*Jag1*) after administration of folic acid (FA) in mice. CTL, control mice. Reprinted from Bielesz *et al*. (2010),[7] with permission from Elsevier.

Additionally, when the FA model was employed in conjunction with a γ-secretase inhibitor, the degree of fibrosis observed in histological samples was significantly less in comparison to the control.[7] This suggests that if the NSP is blocked, renal fibrogenesis decreases.

Sahlgren *et al*. performed experiments on cultured human cancerous ovarian cells and found that hypoxic conditions induced down-regulation of E-cadherin, up-regulation of fibronectin and N-cadherin, and morphological and molecular changes typical of epithelial-to-mesenchymal transition (EMT).[6] Fibronectin is an interstitial matrix component that precedes production of fibrillar collagens that eventually result in renal fibrosis.[8] E-cadherin is an adhesion molecule that contributes to epithelial cell behaviour and tissue formation;[8] its loss is often used as a marker of fibrosis. N-cadherin is expressed on mesenchymal cells.[9] Thus, the respective up-regulation and down-regulation of N-cadherin and E-cadherin promotes a mesenchymal phenotype for tissue, typical of renal fibrosis.

However, research done by Bielesz *et al*. showed that in vivo, Notch-induced EMT was not a major contributor to renal fibrosis.[7] This experiment was conducted using two animal models. In both cases, no decrease in mRNA or protein expression of Cadherin 1 was observed. No explanation was given for these results, though researchers referenced in vitro studies that obtained opposite findings. This highlights a gap in the literature that is limited by the external validity of in vitro studies.

Conclusions

In vitro experiments provide strong evidence to support that the NSP plays an important role in hypoxia-induced renal fibrosis. Both in vivo and in vitro studies reveal that hypoxia leads to an increase in the concentration of the NICD and the ligand.

With respect to EMT, the experiments conducted on cancerous ovarian cells and human renal tubular epithelial cells found that the NSP represses the expression of E-cadherin.

In conclusion, the NSP seems to play a role in hypoxia-induced renal fibrosis. However, fibrogenesis may not be a direct result of the pathway, but a result of interactions between the NICD and other mediators and transcription factors. As such, more in vivo research on the molecular knock-on effects of the NSP should be conducted to clarify this disparity, with a special focus on improving the temporal resolution of animal models used since CKD is a long-term illness. Finally, it is important to note that experiments involving murine species do not accurately represent the pathogenesis of CKD in a clinical setting due to anatomical and physiological differences between humans and mice.

References

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