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Analysis of hypoxia inducible transcription factor DNA-binding and hypoxic gene regulation in primary human renal tubular cells

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Background:

Oxygen tensions fluctuate physiologically in the kidney with very low oxygen levels especially in the medulla. Hypoxia inducible transcription factors (HIF) initiate adaptive mechanisms to hypoxia in order to secure cell survival. Animal studies have shown that genetic or preconditional pharmacological HIF stabilisation leads to a better outcome in models of acute kidney injury. The precise mechanisms responsible for the protective effect and the relevance of the HIF system in human kidneys remain unknown.

Methods:

Healthy human kidney tissue from patients undergoing tumor nephrectomy was used for a primary cell culture. HIF protein was stabilised by hypoxia (1%) or the hypoxia mimetic dimethyl oxalylglycine (DMOG). Chromatin immunoprecipitation (ChIP) was employed to identify HIF and RNApol2 binding sites. Gene expression analyses by Immunoblotting or qPCR was used for validation.

Results :

Immunoblots of kidney tissue incubated with DMOG showed increased HIF stabilisation over time (Fig1). Immunhistochemistry revealed the colocalisation of HIF-1α and the 11β-hydroxy steroid dehydrogenase (11β-HSD) in the distal connecting tubule (DCT) and the collecting duct (CD). Therefore, we used the distal primary cells to further explore the HIF-system (Fig. 2). Immunoblot analysis and qPCR confirmed induction of HIF and well established HIF-target genes (e.g. EGLN3) by hypoxia or DMOG (Fig.3). To identify direct transcriptional targets of HIF we employed ChIP experiments using specific antibodies against HIF-1α and HIF-1β subunits and RNA polymerase 2. qPCR analyses showed a robust enrichment of HIF at regulatory DNA elements and induction of the transcriptional machinery at known HIF-target gene loci (EGLN3, HIG2) across cultures of distal tubular cells from different individuals (Fig.5).

Conclusion:

We show that in human kidneys HIF stabilisation appears predominantly in distal tubular cells. Transcriptional activity is conserved at established target genes across several individuals. Therefore, this experimental setting appears to be suitable for further testing of potential protective mechanisms of the HIF system in human kidneys and to explore new therapeutic options in human kidney disease.

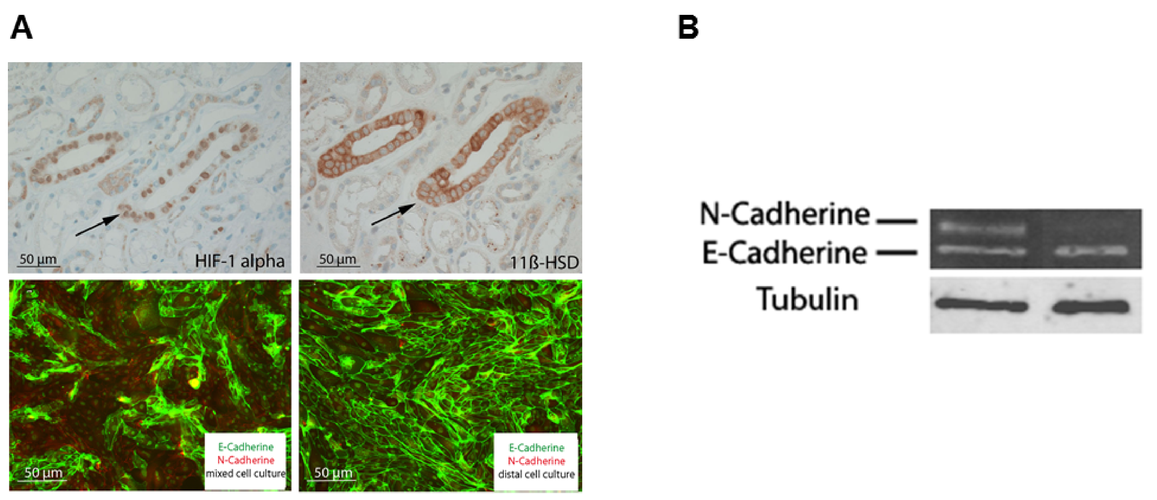

Fig.2 A Immunhistochemistry showing the colocalistaion of HIF-1α with the 11ß-HSD in the collection duct. Immuncytochemistry of primary tubular cells, with separation into proximal (N-) and distal (E-cadherin) tubuluar cells **B** Immunoblot of separated primary cell culture showing high purity of the distal cell culture in which only E-cadherin is detectable.

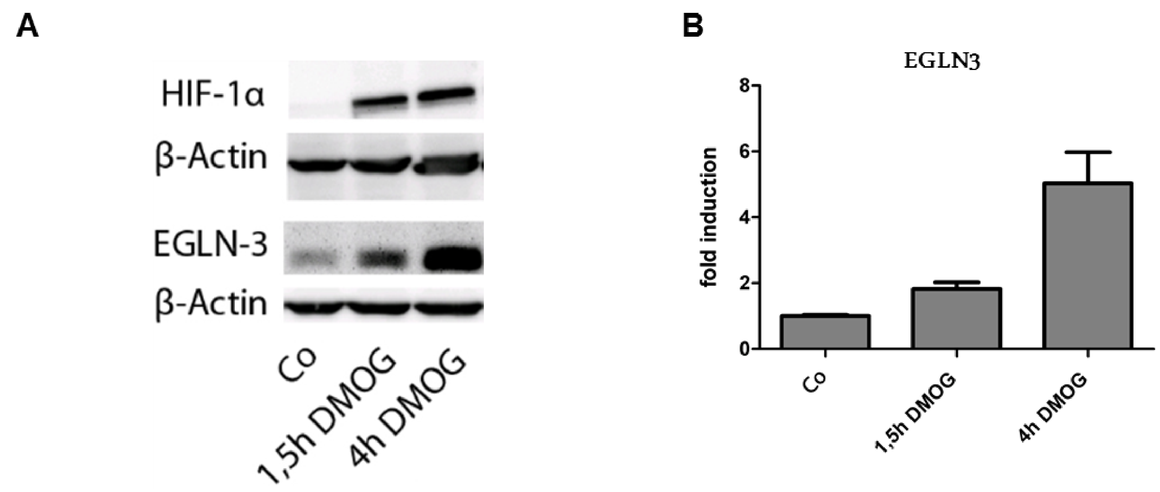

Fig.3 A Immunoblot of a distal primary cell culture showing HIF stabilisation and induction of EGLN3 over time. **B** qPCR of a distal primary cell culture: RNA induction of the HIF Target EGLN3 over time.

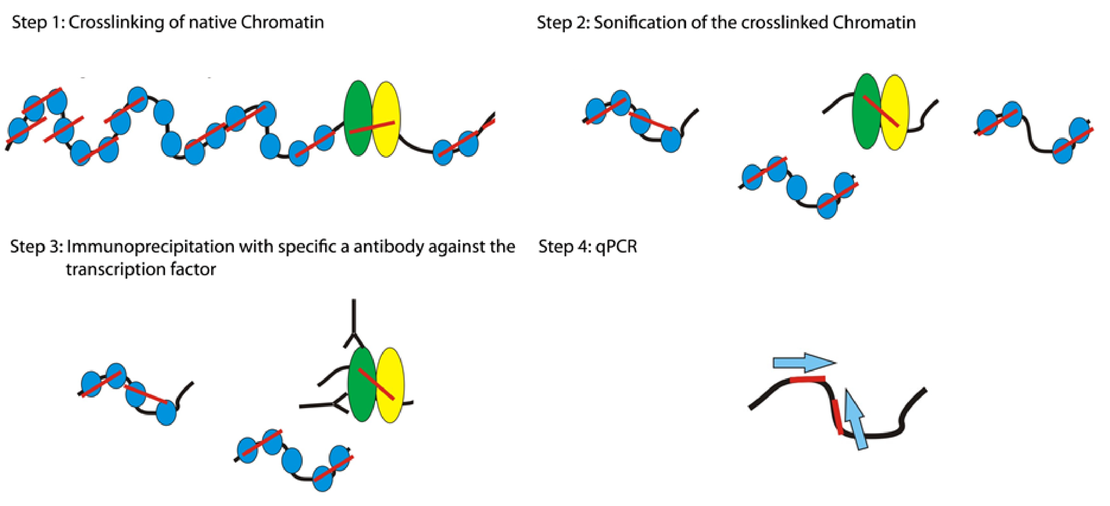

Fig 4. Schematic view of the chromatin immunoprecipitation.

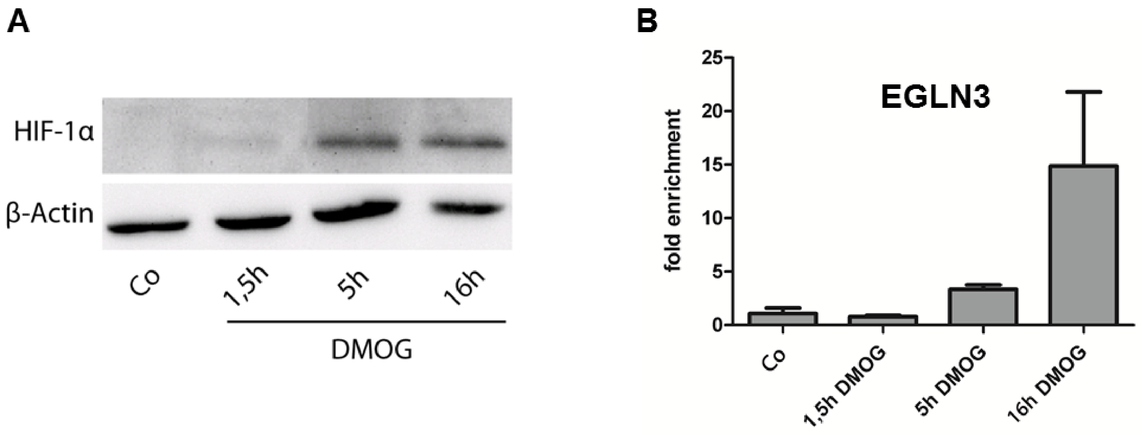

Fig.1 A Immunoblot of kidney tissue show an increasing HIF stabilization over time afte incubation with DMOG. **B** qPCR of kidney tissue with induction of the HIF target EGLN3.

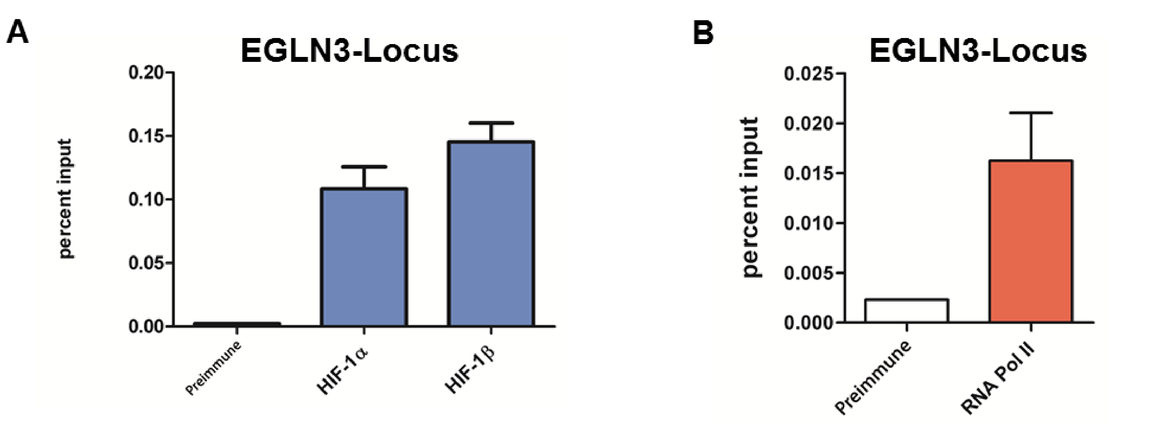

Fig.5 A In ChIP qPCR experiments of a distal primary cell culture epoxsed to DMOG a strong enrichment of HIF-1β and HIF-1α at the EGLN3 locus was messured. **B** ChIP qPCR with a RNApol II antibody also showing an increased enrichment at the EGLN3 locus which corresponds to increased transcription activity.

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