

A systems biology approach for identifying deregulated molecular pathways in proximal tubular epithelial cells characterizing progressive proteinuric nephropathies

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Background

We will present results of a systems biology analysis utilizing gene expression data from kidney biopsies of patients with chronic kidney disease. We previously identified gene expression profiles in renal tubule cells differentiating patients with chronic kidney disease and healthy volunteers [1]. In the current analysis we identified differentially expressed genes and deregulated pathways in a patient cohort showing a progressive course of disease (PD) as compared to samples from patients with stable disease (SD).

Methods

A core vector of differentially expressed genes (DEGs) separating PD and SD samples following purely statistical criteria was generated [2]. Pathways enriched in DEGs were identified using the PANTHER Classification System. In particular expression values of pathway members of the *hypoxia via HIF regulation* and the *VEGF signaling* pathway were evaluated. A literature search on HIF target genes and regulatory proteins of HIF and VEGF was conducted for further extending the gene set under study.

For deriving functional interrelations between genes of these pathways we computed a metafunction among gene pairs including: gene expression data, protein-protein interactions as provided by OPHID (Online Predicted Human Interaction Database), gene ontology terms, and transcription factor binding site profiles following *in-silico* predictions as provided by the oPOSSUM tool. Genes indicating functional relevance in PD as derived on the basis of the computational Systems Biology procedure were then prospectively evaluated via real-time PCR.

Results

149 transcripts were identified as differentially expressed when comparing SD and PD samples. 122 of these clones, representing 113 unique genes based on UniGene IDs, showed a more than 2 fold induction, and 27 cDNA clones showed a more than 2 fold repression in PD samples when compared to SD samples. Among the most enriched biological pathways linked to PD were the *hypoxia response via HIF activation* and the *VEGF signaling* pathways. These pathways were of particular interest since *hypoxia* and *VEGF* have been described to be involved in various animal models of progressive renal disease, and these functionalities are supposed to play a role in human glomerular sclerosis and progressive tubulointerstitial fibrosis. Several key mediators of hypoxia response like HIF-1a, HIF-1b and CREBBP showed a significant up-regulation in PD and showed strong functional dependencies to other players of respective pathways following our metafunction. Interestingly proangiogenic VEGF, a key target gene of HIF, was down-regulated whereas negative regulators of the cell-cycle like CDKN1A (p21) were significantly induced by HIF also validated in real-time PCR experiments. Low expression levels of positive VEGF regulators like EGF, IGF1, or HIF-2a provide an explanation for the down-regulation of VEGF.

Conclusion

We identified various members of the *hypoxia via HIF activation* and the *VEGF signaling* pathway being up-regulated in PD samples. Our data furthermore indicate that hypoxia in progressive renal disease patients does not lead to angiogenesis as VEGF was down-regulated but leads to cell-cycle arrest.

References

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2. Perco P, Rapberger R, Siehs C, Lukas A, Oberbauer R, Mayer G, Mayer B. *Transforming omics data into context: bioinformatics on genomics and proteomics raw data. Electrophoresis.* 2006 27(13):2659-75.