

Properties of the glomerular endothelial cell surface layer in vitro and in vivo

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Abstract

A healthy kidney produces final urine that is practically devoid of proteins and other physiologically important solutes. Tremendous amounts of fluid are filtered every day through the glomerular filtration barrier which is the actual sieving site in the kidney. Failure of the filtering function leads to proteinuria, which is a feature common to nearly all kidney disease. In spite of this pivotal role, the central mechanisms behind proteinuria are still unexplained. The filtration barrier is a complex biological membrane composed of four different structures: the podocytes (epithelial cells), the glomerular basement membrane, the glomerular endothelium and the glomerular endothelial cell surface layer (ESL). During the last decade the focus for understanding the regulation of this selective sieve has rested heavily on the study of the podocytes, whereas the glomerular endothelium and the glomerular ESL has been more or less neglected as contributors to the permselectivity of the barrier. However, it is of fundamental importance to investigate all components of the filtration barrier in order to understand the pathophysiology of proteinuric kidney disease. The molecular structure of the glomerular ESL is largely unexplored, and available data about its constituents so far is only based on *in vitro* studies.

The aim of this thesis was to identify molecules located in the glomerular ESL with a functional significance for normal glomerular filtration *in vivo*, and to examine whether a disease-emulating milieu damages major structural glomerular ESL components and thus increases the glomerular permeability to proteins. We have developed a method for qualitative and quantitative assessment of the glomerular ESL in rats, which includes a brief injection of hypertonic sodium chloride into the renal artery. This displaces and elutes non-covalently bound components of the glomerular ESL which are then subsequently collected for further characterization with liquid chromatography-mass spectrometry. Morphological as well as functional effects have been characterized by electron microscopy and by universal methods analyzing charge- and size selectivity of biological membranes. A conditionally immortalized human glomerular endothelial cell line was used to study the effects of hyperglycemia on glomerular ESL proteoglycans. Functional alterations were analyzed in terms of protein restriction by measuring the passage of albumin across a human glomerular endothelial cell monolayer.

In conclusion, we have identified molecules from the glomerular ESL in rats that are essential for maintaining a normal glomerular barrier function. Further, we found that hyperglycemia was associated with an alteration of glomerular ESL proteoglycans which lead to an increased permeability for albumin. Overall, the observations in this thesis emphasize the importance of the glomerular ESL for the restriction of proteins in the glomerular filtration barrier.

Keywords: glomerular filtration, glycocalyx, proteinuria, glomerular endothelial cell surface layer, proteoglycans, glycosaminoglycans, glomerular endothelial cells, permselectivity and glomerular endothelial cell coat.

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- I. High glucose causes dysfunction of the human glomerular endothelial glycocalyx**
Singh A, Fridén V, Dasgupta I, Foster R.R, Welsh G.I, Tooke J.E, Haraldsson B, Mathieson P.W and Satchell S.C
Am J Physiol Renal Physiol 300:F40-F48 2011,
- II. The glomerular endothelial cell coat is essential for glomerular filtration**
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- III. Further identification of components of the renal capillary endothelial cell coat**
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