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Studying channel and receptor physiology in podocytes

Kidney International (2008) **73,** 364; doi:10.1038/sj.ki.5002669

To the Editor: We would like to congratulate Shankland *et al.*[1] for their very apt and extensive review regarding the culturing of podocytes in which they have diligently put forth the evolution of this technique. Research in the area of renal glomerular physiology has already seen many technical developments over the past 100 years, and the latter are still evolving. This is mainly due to the complicated and multicellular structure of renal glomeruli and the manner in which their different cells are naturally arranged and interact. In fact, the complexity of glomerular architecture has hindered the progress of detailed and sophisticated research in this area. Cell culturing methods have been a vital ingredient for these advances in renal glomerular research. Podocytes, in culture, present a unique system to study the biology of these cells but, as was acknowledged by Shankland *et al.*,[1] this is not the final frontier. With every advance in research, there is always the need for yet a newer and better model that more closely approximates the actual native subject of interest. Past studies have shown that cultured preparations do not always accurately capture all the features of the *in vivo* conditions. For example, cultured cells have been widely reported to express smooth muscle α-actin, whereas this protein is not found *in vivo* as shown by Egler *et al.*[2] For this reason, it is highly speculative to extrapolate the results obtained in cultured podocytes (which are highly constrained within their cell cycle) to their native *in vivo* counterparts (which are highly differentiating cells).[3] It is far more preferable to work with intact native structures rather than with cultured cells, in order to minimize this confounding factor. With this in mind, we have been optimizing a system to look at the different glomerular cells, including podocytes and mesangial cells, in an intact and native environment in real-time using confocal microscopy.[4] In particular, we use 100 μm thin renal cortical slices from mice, rats and humans. After loading these with the Ca^{2+} fluorescent dye fluo-4, changes in fluorescence representing changes in intracellular Ca^{2+} concentrations, brought about by administration of different agonists such as angiotensin II and endothelin-1, can be captured and recorded. Further studies are underway using specific cell markers to distinguish between different glomerular cell types.

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Diagnosing Alport syndrome using electron microscopy of the skin

Kidney International (2008) **73,** 364–365; doi:10.1038/sj.ki.5002682

To the Editor: Patey-Mariaud de Serre *et al.*[1] reported that comparing α5(IV) with α2(IV) expression in skin biopsies is useful in diagnosing X-linked Alport syndrome (AS). However, α5(IV) staining variation can confuse the diagnosis and requires a different analytical approach. Morphological changes in the skin basement membrane determined using electron microscopy (EM) might provide additional information with which to diagnose AS. We found such changes using EM in a male patient with end-stage renal failure and AS. His mother had been diagnosed with chronic kidney disease and had died at the age of 58 years of malignant lymphoma. Hematoproteinuria had persisted in our patient since childhood, but a renal biopsy had not been performed. He had sensorineural hearing loss, but no ocular changes. We suspected X-linked AS and performed a skin biopsy. Standard immunofluorescence staining showed normal

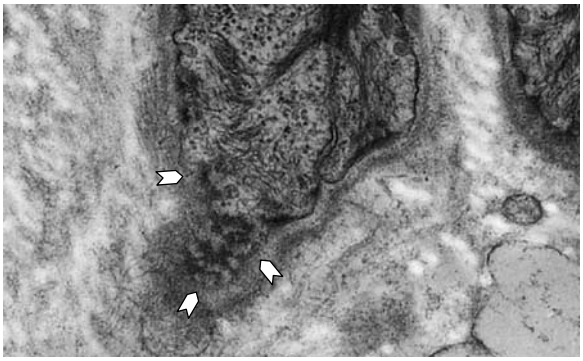

Figure 1 | Electron microscopy of the skin (original magnification × 10 000) showed discontinuity of the lamina densa (arrowhead).

α2(IV) expression and segmental distribution of α5(IV) in the skin basement membrane, and the EM findings showed discontinuity of the lamina densa (Figure 1). These findings indicated a diagnosis of AS. Although no morphological changes are supposedly associated with the skin basement membrane in AS, this conclusion has been based on very few skin biopsies.[2,3] The COL4A5 mutation is heterogeneous in AS,[4] which might cause highly variable morphological changes in the skin basement membrane and in α5(IV) staining.

Although this is the only single confirmation of AS based on EM findings of one skin biopsy, we believe that EM examination in addition to immunofluorescence staining should provide a more definitive diagnosis.

DISCLOSURE

The authors state no conflict of interest.

ACKNOWLEDGMENTS

This study has not received any financial support.

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