Capturing the in vivo molecular signature of the podocyte

Peter Y Chuang1, Jia Fu2, and John C He1,3

1Division of Nephrology, Department of Medicine, Mount Sinai School of Medicine, New York, New York
2Research Institute of Nephrology, Jinling Hospital, Nanjing University School of Medicine, Nanjing, China
3Department of Medicine, James J Peters Veterans Affairs Medical Center, Bronx, New York

Over the past 15 years, the number of podocyte-related publications per year has increased exponentially (Fig 1). This trend coincided with the identification of nephrin1, a discovery that brought to light the pivotal role of the podocyte in glomerular filtration; and the availability of a conditionally immortalized podocyte cell line2, making podocytes accessible for studies in cell culture systems. The availability of genetic tools that allow podocyte-specific manipulation of gene expression in animals (i.e. podocyte-specific expression of Cre recombinase and reverse tetracycline transactivator) further hastened the pace of podocyte research. In spite of these innovations, there is still a critical gap in our knowledge as well as the ability to examine the molecular attributes of the podocyte in health and disease.

The podocyte is a terminally differentiated, highly specialized epithelial cell. It is clear that podocytes maintained ex vivo in cell culture systems do not completely recapitulate the morphologic and molecular phenotype of podocytes in vivo. Furthermore, in most disease models it is impossible to replicate the in vivo disease milieu under ex vivo culture conditions. Since ex vivo cultured podocytes are not adequate substitutes for in vivo podocytes, many have attempted to isolate primary podocytes from animals. However, none of the existing protocols for podocyte isolation provide sufficient quality and yield to permit types of omics-level analysis other than transcriptome profiling. This has been a major quandary in the field of podocyte research.

Intimate association of the three glomerular cell types—podocytes, mesangial cells, and endothelial cells—makes the isolation of podocytes from biological samples difficult. Since glomerular volume in man and mice accounts for less than 5% of the total renal mass and podocytes are only present in the glomerulus, glomerular extracts are enriched in podocytes relative to the rest of the kidney. Glomeruli can be isolated with >95% purity from man and rats using sieving techniques3 and from mice by perfusion of magnetic particles followed by magnetic isolation4,5. While some have used glomerular extracts to obtain a first approximation of the podocyte-specific attributes, the joint presence of mesangial and endothelial cells with podocytes in the glomerulus precludes definitive characterization of podocyte-specific features. To overcome this, attempts have been made to isolate primary podocytes from glomerular extract after enzymatic digestion to dissociate glomeruli into a single cell suspension. Following this, podocytes are distinguished from non-podocytes...
either by using antibodies against podocyte-specific antigens, such as nephrin\textsuperscript{6} and podocalyxin\textsuperscript{7}, or through the use of fluorescent markers that are expressed exclusively in podocytes of transgenic mice\textsuperscript{8}. Podocytes are separated from non-podocyte cells using fluorescence\textsuperscript{7,8} or magnetic\textsuperscript{6,8}-activated cell sorting techniques. A major limitation of these approaches, however, is the low yield of extraction.

In this issue, Boerries \textit{et al.}\textsuperscript{9} described a modified podocyte extraction procedure. This protocol improved the glomerular yield by approximately 2-to-3 fold compared to the original protocol of glomerular isolation by perfusion of magnetic particles\textsuperscript{5}. To distinguish between podocyte and non-podocyte glomerular cells, Boerries \textit{et al.} generated a bitransgenic line of mice with podocyte-specific expression of a green fluorescence protein by breeding a Cre-reporter strain of mice (\textit{mT/mG}) to mice with podocyte-specific expression of Cre-recombinase (\textit{hNPHS2Cre}). Glomeruli were isolated from bitransgenic mice (\textit{mT/mG;hNPHS2Cre}) using perfusion of magnetic particles and then were dissociated into a single cell suspension prior to fluorescence-activated sorting of podocytes. The yield of extraction was greater than $5 \times 10^5$ podocytes per mouse, which was sufficient for transcriptome profiling as well as quantitative proteomic analysis when freshly isolated podocytes from two mice were pooled. Bioinformatic analyses of the transcriptomic and proteomic datasets revealed that gene regulation programs pertaining to endoplasmic reticulum, ubiquitination, cytoskeleton, nuclear elements and mitochondria, peroxisome and protein transport, and cell junction were enriched in podocytes.

This study by Boerries \textit{et al.} contributes to the study of podocyte biology in several important ways. Their modified isolation protocol improved on the yield of podocyte extraction, which allowed quantitative proteomic analysis to be performed on isolated podocytes. Comparison of the transcriptome and proteome revealed a lack of perfect correlation between the protein and transcript levels for some genes, which suggests that these genes are regulated at the post-transcriptional level. Since the regulation of gene expression is not fully represented by the transcriptome alone, proteomic data provides another layer of information. Although the transcriptomes of isolated podocytes have been published by others, none of those studies have juxtaposed their results with prior publications. Boerries \textit{et al.} compared the transcriptome of isolated podocytes to other publically available transcriptomes of podocytes from different sources: adult mice, developing mouse embryo, and cultured podocytes. Boerries \textit{et al.} also performed additional bioinformatics analyses of the transcriptomic and proteomic datasets. The combined datasets predict several biological functions, not previously described, that could be important for the maintenance of the podocyte.

While the approach taken by Boerries \textit{et al.} to isolate podocytes is an improvement from prior protocols, it remains imperfect. All existing podocyte isolation protocols require enzymatic digestion at $37^\circ$C to separate glomeruli from surrounding non-glomerular structures and to dissociate podocytes from non-podocyte glomerular cells. Mechanical disruption and enzymatic digestion at $37^\circ$C are likely to alter the molecular signature of the podocytes during the isolation process. Unfortunately, no alternative approach exists to avoid enzymatic digestion at $37^\circ$C. Another point to consider is the use of transgenic expression of a fluorescent tag for podocyte sorting as opposed to antibody-based sorting.
Genetic tagging of podocytes has the disadvantage of time and cost associated with backcrossing and breeding when studying models of kidney disease where the disease is due to a genetic alteration (i.e. db/db model of diabetes, Tg26 model of HIV associated nephropathy, or CD2AP null mice). However, this will need to be balanced with the higher yield and purity of the transgenic approaches compared to antibody-based methods. Finally, we also need to be cognizant of the fact that although bioinformatic-based analyses by Boerries et al. yielded predictions about biological processes that are enriched in the podocytes, these predictions will require experimental confirmation before they can be considered biologically valid and functionally important.

This study by Boerries et al. is the first to characterize the transcriptome and proteome of podocytes using cells that were procured in a way that was least likely to perturb their in vivo molecular signature. These datasets are valuable resources for the podocyte research community and can be used to establish the core transcriptome and proteome of the podocyte. Application of the podocyte extraction protocol to disease models followed by downstream characterization of the proteome, transcriptome, and epigenome will facilitate the progress of research and further our understanding of podocytes in kidney health and disease.

Acknowledgement

PYC is supported by NIH 5K08DK082760, FJ is supported by Chinese 973 fund 2012CB517601, JCH is supported by NIH1R01DK088541, P01-DK-56492, and VA Merit Award.

References

1. Kestila M, Lenkkeri U, Mannikko M, et al. Positionally cloned gene for a novel glomerular protein--nephrin--is mutated in congenital nephrotic syndrome. Molecular cell. 1998; 1:575–582. [PubMed: 9660941]
2. Mundel P, Reiser J, Zuniga Mejia Borja A, et al. Rearrangements of the cytoskeleton and cell contacts induce process formation during differentiation of conditionally immortalized mouse podocyte cell lines. Experimental cell research. 1997; 236:248–258. [PubMed: 9344605]
3. Yamamoto, T. Isolation and Enrichment of Glomeruli Using Sieving Techniques. In: Thongboonkerd, V., editor. Renal and Urinary Proteomics: Methods and Protocols. Germany: Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim; 2009.
4. Assmann KJ, van Son JP, Koene RA. Improved method for the isolation of mouse glomeruli. Journal of the American Society of Nephrology : JASN. 1991; 2:944–946. [PubMed: 1751795]
5. Takemoto M, Asker N, Gerhardt H, et al. A new method for large scale isolation of kidney glomeruli from mice. The American journal of pathology. 2002; 161:799–805. [PubMed: 12213707]
6. Murakami A, Oshiro H, Kanzaki S, et al. A novel method for isolating podocytes using magnetic activated cell sorting. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2010; 25:3884–3890.
7. Akilesh S, Huber TB, Wu H, et al. Podocytes use FcRn to clear IgG from the glomerular basement membrane. Proceedings of the National Academy of Sciences of the United States of America. 2008; 105:967–972. [PubMed: 18198272]
8. Brunskill EW, Georgas K, Rumballe B, et al. Defining the molecular character of the developing and adult kidney podocyte. PloS one. 2011; 6:e24640. [PubMed: 21931791]
9. boerries. Molecular fingerprint of the podocyte reveals novel gene and protein regulatory networks. Kidney international. 2012
Figure 1.
Number of PubMed citations for the term *podocyte* from 1987 to 2012. Conditionally immortalized murine podocytes and congenital nephropathy of the Finnish type were reported in 1997 and 1998, respectively.