A High-Content Screening Technology for Quantitatively Studying Podocyte Dynamics

Jochen Reiser, Ha Won Lee, Vineet Gupta, and Mehmet M. Altintas

Podocytes form the visceral layer of a kidney glomerulus and express a characteristic octopus-like cellular architecture specialized for the ultrafiltration of blood. The cytoskeletal dynamics and structural elasticity of podocytes rely on the self-organization of highly interconnected actin bundles, and the maintenance of these features is important for the intact glomerular filtration. Development of more differentiated podocytes in culture has dramatically increased our understanding of the molecular mechanisms regulating podocyte actin dynamics. Podocytes are damaged in a variety of kidney diseases, and therapies targeting podocytes are being investigated with increasing efforts. Association between podocyte damage and disease severity—or between podocyte recovery and the performance of therapeutic molecules—have been the venues of research for years. In this perspective, more standardized high–content screening has emerged as a powerful tool for visualization and analysis of podocyte morphology. This high-throughput fluorescence microscopy technique is based on an automated image analysis with simultaneous detection of various phenotypes (multiplexing) across multiple phenotypic parameters (multiparametric). Here, we review the principles of high-content screening technology and summarize efforts to carry out small compound screen using podocytes.

Key Words: High-Content Screening, Image Analysis, Podocyte, Drug Discovery

INTRODUCTION

Urine formation starts with the filtration of blood through the glomerular filtration barrier, which is a three-layer interface formed by fenestrated endothelial cells,[1] glomerular basement membrane (GBM),[2] and visceral epithelial cells, also known as podocytes.[3] Owing to its delicate structure and coordinated cell polarity, glomerular filtration barrier constitutes a size and charge selectivity that facilitates cation transport and filters all the small molecules (water, salt, glucose, amino acids, urea, etc.) out of the blood but retains cells, platelets, and large proteins, such as albumin.

As a key player of the glomerular filtration, podocytes are aligned on the external surface of GBM and cover glomerular capillaries neatly with numerous cytoplasmic projections, called foot processes (FPs).[4] These terminally differentiated epithelial cells form the final barrier to protein loss by interdigitating with the FPs of the adjacent podocytes and leaving between them the slit diaphragm (SD), narrow filtration slits that are bridged by modified adherens junction.[5] Podocyte's functions, which are vital to glomerular filtration, depend on a highly regulated actin cytoskeletal network that is formed either by a central actin bundle along the long axis of FPs or by a relatively short cortical network aligning at the cell periphery and anchoring the components of SD.[6]

Podocytes are the major targets of several agents or molecules such as toxins, reactive oxygen species, complements, and antibodies.[7] Injury to podocytes may physically alter this elaborate structure causing the flattening and retraction of the FPs as well as the disappearance of the filtration slits, a process called FP effacement. Therefore, efforts to reverse the podocyte damage and rescue glomerular filtration generally focus on actin regulatory pathways[8] and developing therapeutic agents that can ameliorate disruptions of actin organization.[9]

A successful disease-specific tailoring of therapeutics may be achieved by using an image-based screening, which enables to analyze a wide variety of phenotypes in cells. Such high-content screening (HCS) platforms employ fully automated microscopes and image analysis software, making it possible to quantify changes in cellular and subcellular properties including cell area, morphology, actin fiber, and focal adhesion intensity. We recently described a novel phenotype-based HCS using immortalized mouse podocyte cells and applied it to identify podocyte-protective small molecules.[10] This review aims to discuss the screening experiments and image analysis approaches, as this high-throughput technique is being used in the preclinical development of the drug discovery process.

Podocyte as a Direct Target of Drugs

Kidneys have arguably the most complex membrane system and solute trafficking in the body, which attracted researchers with interests in kidney biology for many decades.[11] This is mostly due to the multicomponent nature of the glomerular filtration system, with endothelial cells, GBM, and visceral epithelial cells (podocytes) participating in the filtration process.[4] The function of this elegant filtration system is maintained by the interplay among these core constituents as well as the immaculate arrangement of the structural proteins within the membrane. The integrity[12] and elasticity[13] are other fundamental concepts since the capillary pressures far exceed those in other organs. The mechanical support required for glomerular capillaries is mainly provided by

From the Department of Medicine, Rush University Medical Center, Chicago, IL.

Financial Disclosure: J.R. and V.G. are inventors on pending patent applications related to this study and have the potential for future financial benefit from them.

Address correspondence to Mehmet M. Altintas, PhD, Department of Medicine, Rush University Medical Center, 1735 West Harrison St., Cohn Research Building, Suite: 718, Chicago, IL 60612. E-mail: Mehmet_Altintas@rush.edu

1548-5595/$36.00

http://dx.doi.org/10.1053/j.ackd.2017.04.001

podocytes[14] since GBM and its associated cells are not rigid, but rather flexible.[15] Furthermore, endothelial cells lack sufficient cytoskeletal structure (and contractile system) as demonstrated by the electron microscopy.[16] Hence, among the principal components of the glomerular filtration barrier, podocyte deserves a special attention. And it really has: during the past decade, podocyte research has remarkably expanded, with more than 3000 published papers directed toward delineating the mechanisms regulating podocyte structure and function.

Owing to its strategic location, podocyte is the major target of various agents soluble in the blood, including toxic and immunologic compounds, reactive oxygen species, complements, and antibodies to podocyte membrane antigens.[7] Podocyte is also injured by other means, such as genetic deletions or mutations impacting the proteins of podocyte itself, SD complex and GBM structure, or charge distortion directly affecting its apical membrane domain.[7] Podocyte injury leads to reorganization of actin cytoskeleton from a dynamic state (characterized by parallel and contractile actin filaments) to a rigid state (represented by thicker stress fibers) and FP effacement (fusion or retraction of podocyte terminal processes).[17] Beyond these structural changes and phenotypic conversions, persistent injuries to podocyte might cause lethal alterations such as detachment from the underlying GBM[18] (as a relevant note, podocytes disappearing from the glomerular tuft can be still alive and recovered from the urine[19]) and death.[20] The loss of podocytes is an irreversible event causing the loss of glomerular filtration function since podocytes are postmitotic cells with a minimal capacity to replicate.[21] Once podocytes are lost, the remaining podocytes fail to completely cover the outer aspect of the GBM and become more vulnerable to any additional workload.[22] Potential mechanisms for podocyte replacement include the contribution of glomerular parietal epithelial cells and cells of renin lineage as podocyte progenitors; however, uncertainties still remain regarding the routes of the migratory event that brought those progenitors to the glomerular tuft and the formation of complex cytoskeletal structure (i.e., FPs and SDs) in these candidate cells.[23]

Notably, there is abundant evidence indicating that any abnormality or change in podocyte cytoskeleton may contribute to proteinuria and nephrotic syndrome,[24] and more strikingly, disorders affecting glomerular filtration are responsible for 90% end-stage kidney diseases at a cost of $30 billion per year in the United States alone.[25] Several pharmacological agents targeting podocytes are being evaluated such as corticosteroids, angiotensin I-converting enzyme inhibitors, angiotensin receptor blockers, peroxisome proliferator-activated receptors agonists, retinoids, and vitamin derivatives.[26-28] Despite tremendous research effort, there are still very limited therapeutic options to stop progressive decline in glomerular filtration rate.[9] Podocytes are therefore a promising target for investigating the pathogenetic mechanisms of kidney protection and screening new treatment options.

CLINICAL SUMMARY

- The podocyte is a key player of glomerular filtration and its function depends on its unique cellular architecture.
- Podocyte injury that involves the disruption or disorganization of its highly regulated actin cytoskeletal network is an early sign of various proteinuric kidney diseases.
- High-content screening assays quantify cellular phenotypes in an unbiased manner, and have the capability of testing thousands of experimental conditions and/or drugs simultaneously.
- Podocyte-based high-content screening offers an information-rich, multiparametric venue for the development of podocyte-directed therapeutics.

Cultured Podocytes Are Suitable Sources for Image Analysis

Fundamental observations of biological processes can be consistently simplified in two-dimensional cell culture conditions, where cells grow (and differentiate) in the presence of a defined medium and behave similar to the in vivo situation. Regardless of its limitation regarding the representation of the physiological microenvironment, in vitro assessment of cell viability, metabolism, and functionality is an important step to many aspects of biomedical research. Therefore, over the years, traditional in vitro cell culture helps researchers to predict the response of more complex organisms (e.g., tissues, organs) to potential pharmaceuticals.

Kidney diseases are mainly characterized by structural and functional changes in glomerulus that causes a gradual loss of kidney function (measured by glomerular filtration rate) and terminal kidney failure, if not treated promptly. Physical properties of cells constituting glomerular filtration barrier have been shown to have a significant role in various kidney diseases, as in many instances, podocyte cytoskeleton is disorganized and podocyte adhesion decreased when the cells are no longer healthy. In that sense, the availability of murine[29] and human podocyte[30] cell lines is crucial since the injury-induced cytoskeletal changes and injury-driven cell motility can be evaluated by well-defined protocols in cultured podocytes.

Using a temperature-sensitive transgene, conditionally immortalized podocytes are able to proliferate under permissive conditions (at 33°C and with the presence of interferon-γ podocytes are of mouse origin) and display characteristic cobblestone shape, whereas under nonpermissive temperature (37°C), the cells stop replicating and starts differentiating by developing a large arborized morphology containing well-developed processes.[31] Actually, this one-cell-thick podocyte monolayer cannot fully replicate the highly sophisticated in vivo kidney filtration barrier (i.e., neither podocyte–GBM interaction is represented with a great molecular detail by the interaction of podocyte and extracellular matrix [ECM] ligands in the tissue culture flask nor the tight connections of podocytes with each other through specialized cell-adhesion