Developing an invitro model for immune mediated podocyte injury in Nephrotic Syndrome

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Background and Introduction

Minimal change disease (MCD) and focal segmental glomerulosclerosis (FSGS) are common causes of idiopathic nephrotic syndrome, accounting for 10-15% of cases in adults and a larger proportion of cases in children. [1,2]

The pathogenesis of MCD and FSGS is unknown but results in damage to podocyte cells, specialized epithelial cells which line the basement membrane, making up the filtration barrier of the kidney.[3]

We hypothesize that there is a a circulating factor present in MCD and FSGS patients which interacts with the immune system to mediate podocyte damage leading to nephrotic syndrome.

Objectives

- Aim 1: Evaluate patient and healthy control immunoglobulin specificity to podocyte cells
- Aim 2: Develop a method to analyze and quantify focal adhesion complexes in a human podocyte cell line
- Aim 3: Evaluate thymocyte mediated immunological effects on podocyte cell line using conditioned media

Methods

Conditioned media preparation

- Dissociated cells from thymus tissue were incubated with patient and healthy control plasma and immunoglobulins for 48 hours, and then media was harvested

Treatment of podocytes and quantification of focal adhesions

- Immortalized human podocyte cells were plated on type IV collagen coated coverslips and treated with conditioned media, TNF alpha (positive control), and FBS and culturing media (negative controls)
- Stained for paxillin (red) and vinculin (green)
- Cells imaged and focal adhesions quantified using Image J software and Focal Adhesion Analysis Server[4]

Results

Aim 1 Results

- No qualitative difference between the IgG binding specificity of patients (9327, 11153) versus healthy controls (11381, 13037, 13219) to podocytes cells

Aim 2 Results

- Podocyte focal adhesion complexes were successfully identified and quantified using Image J software and a Focal Adhesion Analysis Server[4]

Aim 3 Results

- No significant differences were found in the quantity of podocyte focal adhesion complexes between experimental groups

Results

Figure 1. Patient and healthy control antibody specificity to podocyte cells

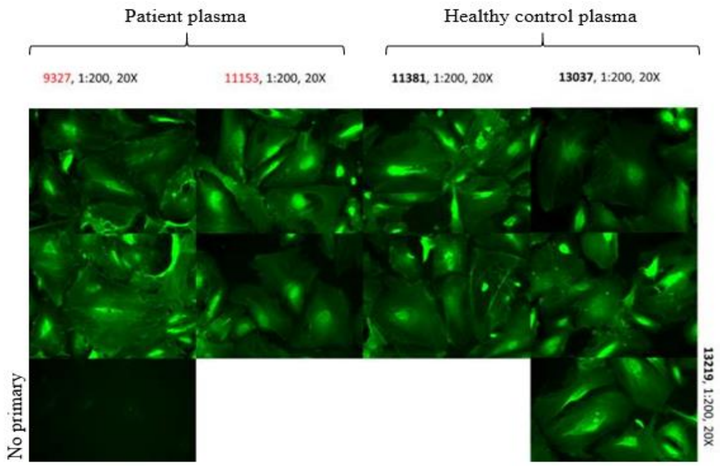


Figure 1. Patient and healthy control plasma was added to podocyte cells and then stained with anti-human IgG (green). No qualitative difference between the IgG binding of podocytes when exposed to patient plasma (9327, 11153) versus healthy control plasma (11381, 13037, 13219) was noted.

Figure 2. Method for the quantification of focal adhesions

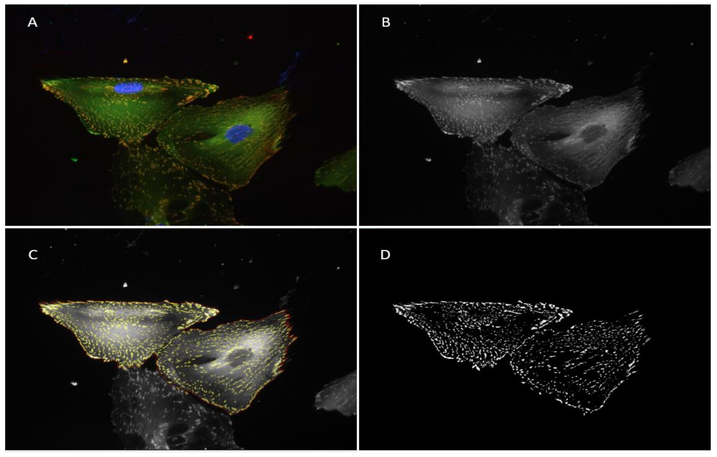
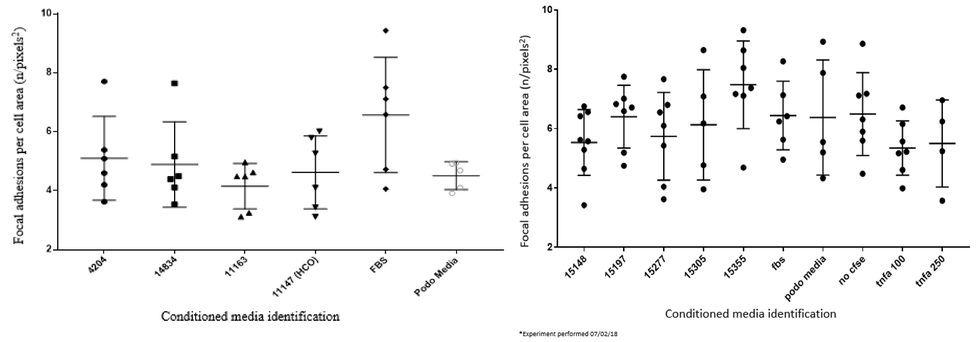


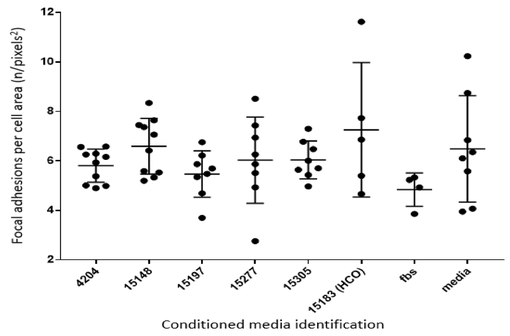
Figure 2. Focal adhesions were stained using vinculin (green) and paxillin (red). Focal adhesions were quantified using Image J software and Focal Adhesion Analysis Server

Figure 3. Evaluation of thymocyte mediated mechanism of podocyte injury via quantification of focal adhesion complexes



*Experiment performed 07/02/18

- Experiment performed 06/18/18
- Paxillin staining used for quantification



*Experiment performed 07/19/18
*Incubated with CM for 5 hours

Figure 3. Effect of patient conditioned media on quantity of focal adhesions per cell area. Six images were captured and quantified for each experimental condition. Paxillin staining was used for quantification of focal adhesions. A,B. podocytes incubated with conditioned media for 24 hours. C. podocytes incubated with conditioned media for 5 hours. No significant difference in quantity of focal adhesion complexes between experimental groups.

Summary

- There was no difference in direct IgG binding on podocytes between healthy controls and patients, indicating that there is no direct effect of a circulating factor upon podocytes.
- Cultured podocytes were not affected through a thymocyte mediated mechanism as there was not a significant difference in quantity of focal adhesions between experimental groups

Conclusions

- The methods established in this study provide a reproductible and unbiased method to analyze and quantify focal adhesion complexes in a human podocyte cell line using immunofluorescence microscopy combined with imaging analysis
- This study lays groundwork for future studies to investigate the role of circulating factors and immune mediators leading to glomerular disease
- The limitations of this study include the complications that arise from using an in vitro model system, an immortalized cell line, and frozen patient samples for experimentation.

References

1. Waldman M, Crew RJ, Valeri A. Adult Minimal-Change Disease: Clinical Characteristics, Treatment, and Outcomes. *Clinical Journal of American Society of Nephrology*. 2007;2(3):445-453. doi:https://doi.org/10.2215/CJN.03531006.
2. Eddy AA, Symons JM. Nephrotic syndrome in childhood. *The Lancet*. 2003;362:629-639.
3. Hara M, Yanagihara T, Kihara I. Urinary Podocytes in Primary Focal Segmental Glomerulosclerosis. Nephron. 2001;89(3):342-347. doi:10.1159/000046097.
4. Focal Adhesion Analysis Server. Focal Adhesion Analysis Server. https://faas.bme.unc.edu/. Accessed April 23, 2020.