Role of MiRNA-204 in Renal Protection Against Immune Mediated Kidney Injuries

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Background & Purpose:

Podocytes are complex cells in Bowman's capsule possessing foot processes that make up the slit diaphragm to prevent the passage of large macromolecules, allowing them to remain in circulation. A variety of glomerulonephritides (GN) are involved in immune complex (IC) buildup within podocytes, though little is known about how podocytes interact with immune complexes. Previous studies revealed that in immune mediated renal diseases podocyte antigens are often the target of autoantibodies involved. However, the precise molecular effects of immune complexes on podocytes remains unknown. The purpose of this study is to determine if modulation of lysosomal function after an immune challenge is mediated by miR-204, a microRNA that is expressed in podocytes and has been shown to be protective in a variety of kidney diseases. We examined the effects of IC treatment on miR-204 expression in cultured podocytes and investigated miR-204 modulation of key lysosomal genes and lysosomal function. We hypothesize that upregulation of miR-204 after an immune challenge will lead to downregulation of lysosomal function, resulting in decreased lysosomal stress and podocyte protection.

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Materials & Methods:

-MiR-204 expression was examined in cultured mouse podocytes after treatment with immune complexes using miRNA-seq.
-Immunofluorescence and confocal microscopy methods were used to examine lysosomal morphology in podocytes after IC treatment and after modulating miR-204 levels using a miR-204 mimic or inhibitor.
-The expression of a lysosomal marker, LAMP1, and a key lysosomal enzyme, cathepsin D, was examined after miR-204 upregulation or inhibition using western blot analysis.
-Lysosomal activity was examined after an immune challenge by using fluorescent recovery after photobleaching (FRAP) to examine the activity of another lysosomal enzyme, cathepsin B.

Nephrotoxic Serum Nephritis Model:

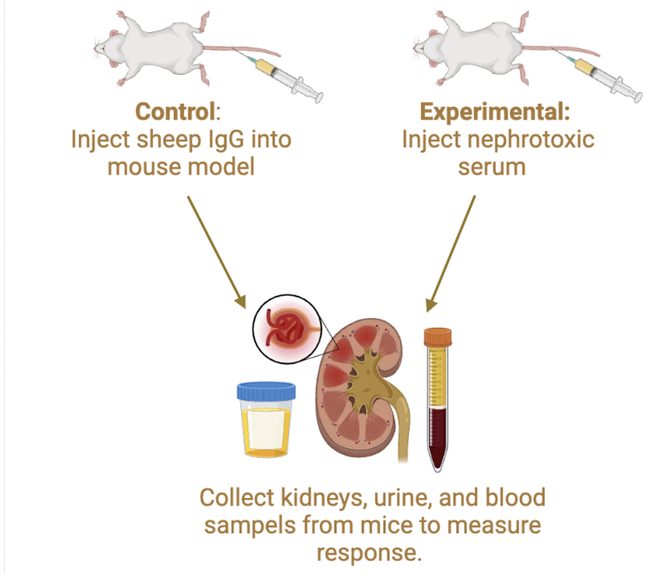

Figure 1: Nephrotoxic serum nephritis model. The control group will be treated with sheep IgG injections, while the experimental group will be given NTS injections.This technique will be used for our future research on this topic. (*Image generated on BioRender.com)*

Result Graphics:

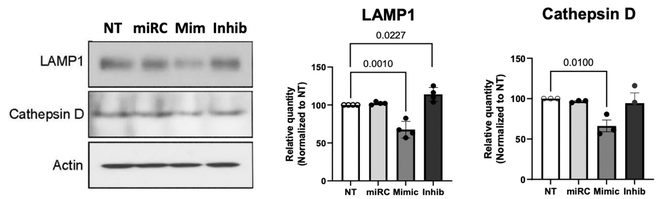

Figure 2: LAMP1 and Cathepsin D relative protein quantities when treated with miRC, Mim, and Inhib, when compared to NT.

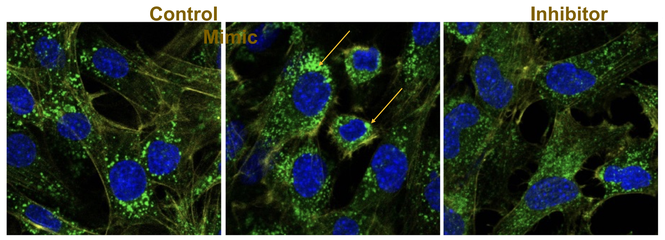

Figure 3: Mouse podocytes were cultured and treated with NTS, with either the mimic or the inhibitor miR-204. This image was developed with the use of confocal microscopy and it shows that the use of a miR-204 inhibitor induces the perinuclear clustering of lysosomes, while the miR-204 mimic induces lysosomal dispersal. LAMP1, a lysosomal marker labeled green in these experiments, can be seen localizing in a perinuclear formation when treated with the miR-204 inhibitor, indicated by the gold arrows.

Blue staining (Hoechst) → Podocyte Nuclei
Green staining (488) → LAMP1

Results:

-MiRNA-seq showed that IC treatment induced a significant increase in miR-204 expression in cultured podocytes.
-Confocal microscopy revealed that in podocytes treated with ICs, lysosomes were larger and clustered around the nucleus, indicating that immune complexes induce lysosomal activation.
-FRAP revealed that sustained IC exposure decreased cathepsin B activity in podocytes, suggesting that prolonged IC exposure depletes lysosomal enzyme activity.
-Upregulation of miR-204 in podocytes using a miR-204 mimic led to a significant decrease in the expression of LAMP1 and cathepsin D, suggesting that these genes are targets of miR-204.
-Downregulation of miR-204 via treatment with a miR-204 inhibitor induced clustering of lysosomes around the nucleus, suggesting lysosomal activity.
-Upregulation of miR-204 induced lysosomal dispersion throughout the cytoplasm suggesting lysosomal down-regulation.

Conclusion & Next Steps:

Our data suggest that an immune challenge induces lysosomal activation and upregulation of miR-204 downregulates lysosomes through inhibition of genes involved in lysosomal function. We hypothesize that chronic lysosomal activation in podocytes is deleterious and that miR-204 exerts a protective effect by diminishing chronic lysosomal activation. Our next steps involve utilizing miR-204 KO mice; These miR-204 KO mice will be acquired from Dr. Sheldon Miller of the NIH. Immune complex disease in these mice will be induced by utilizing the Nephrotoxic Serum Nephritis model, injecting nephrotoxic serum into a vein in the tail. Following injection, renal function in response to the IC mimicking condition will be measured by utilizing blood and urine tests, as well as collecting the kidney from the mouse models.

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