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The Role of YY1 in the Modulation of the Podocyte Molecular Phenotype in High Glucose Milieu

Biochemistry (BCHM)

RESEARCH PLAN

Rationale:

- Diabetic nephropathy is a complication of diabetes mellitus that imparts stress onto cells in the kidney deemed critical to proper kidney function. Over time, this complication destroys kidney cells and eventually results in kidney failure, necessitating hemodialysis or kidney transplantation. Understanding the underlying molecular mechanisms of the development of diabetic nephropathy is important to promoting understanding of diabetic nephropathy and potentially finding new biomolecular targets for therapeutic treatment (Aldukhayel 2017).
- Podocytes are cells in the Bowman's capsule of the nephron that act to mediate filtration through cellular projections called foot processes. By wrapping these foot processes around glomerular capillaries, they create a complex network that supports the capillaries. Podocytes secrete protein complexes that occupy the spaces between these foot processes, restricting the passage of larger particles out of the capillary and regulating filtration (Pavenstadt 2014).
- Parietal epithelial cells are located in the same compartments as podocytes. Compared to podocytes, which are terminally differentiated and do not divide, parietal epithelial cells are highly proliferative. In disease conditions, parietal epithelial cells have been hypothesized to migrate to the glomerular tuft, where podocytes are located, and differentiate into podocytes. This hypothesis is supported by evidence detailing a transitory state between the two cell types with overlaps in their molecular phenotypes (Andeen et al. 2015, Appel et al. 2009).

- In high glucose conditions reminiscent of diabetes, podocytes become stressed and have been shown to dedifferentiate, reverting from the expression of podocyte markers (differentiation) to parietal epithelial cell markers (undifferentiated/proliferative) (Kumar et al. 2019)
- APOL1 is a protein that has been implicated in the transition from parietal epithelial cell to podocyte. Low APOL1 has been associated with the parietal epithelial cell molecular phenotype, and high APOL1 with the podocyte molecular phenotype. Conditions that modulate the expression of APOL1 have been associated with changes to the molecular phenotype of the podocyte. High glucose conditions that have been shown to cause dedifferentiation of podocytes have been associated with a decrease in APOL1 expression (Kumar et al. 2018).
- YY1 is a marker of proliferation in various cell types that are highly proliferative, including cancer cells. As a transcription factor, it serves to mediate repression of genes that ultimately confer a phenotype highly characteristic of proliferative cells. In diabetic nephropathy, YY1 has been explored in renal tubular cells, where elevated YY1 expression has been associated with renal fibrosis that ultimately promotes the development of diabetic nephropathy. This highlights the potential for YY1 to be a therapeutic target for treatment of diabetic nephropathy (Stovall et al. 2013, Yang et al. 2019).

Research Questions:

- Do APOL1 and YY1 interact in some way?

- If so, what is the role of YY1 in modulating the podocyte molecular phenotype in high glucose milieu?
- Can the dynamics of the YY1-APOL1 interaction be explored through computational studies?

Methods:

- Computational (developed for project):
    - Retrieve sequences of relevant proteins from Uniprot database.
    - Model sequences using ITASSER webserver (reliable due to high accuracy of comparative modelling and *ab initio* methods).
    - Relax models through GalaxyRefine to remove steric clashes and decrease poor rotamer count and increase Rama-favored residues.
    - Dock relevant proteins to each other using ClusPro server, choose models based on highest cluster count.
    - Simulate proteins using GROMACS molecular dynamics package: minimization (steepest descent), NVT to 300K for 500 ps, NPT to 1bar for 500 ps, MD production run for 30-50 ns (time is not yet decided, will have to see when convergence is reached).
    - Visualize trajectories using VMD, plot data using GNUPlot.
    - Focuses of trajectory analysis will be RMSD (to determine production convergence), RMSF, and radius of gyration (both measures of stability).
- Experimental (adapted from Kumar et al. 2018):
    - Treating Human Podocytes:

1. Seed undifferentiated podocytes on collagen plates, differentiate for 10 days.
2. Incubate podocytes in 5 mM glucose media (low glucose) or 30 mM glucose media (high glucose).

- Protein Silencing:
    1. Combine siRNA or scrambled siRNA with Lipofectamine RNAiMAX reagent and dilute with Opti-MEM.
    2. Add diluted siRNA to Lipofectamine reagent and incubate.
    3. Transfect differentiated podocytes with siRNA-lipid complex at 60-80% confluence, incubate in Opti-MEM.
- Western Blotting Studies:
    1. Harvest and lyse cells in RIPA buffer (PBS with 0.1% SDS, 1% Nonidet P-40, 0.5% sodium deoxycholate, 100 mM KI).
    2. Determine protein concentrations using a Bio-Rad protein assay kit according to described methodology
    3. Load protein lysate extract onto gel lanes in poured 10% polyacrylamide gels (with 10% SDS), run gels in running buffer (30g Tris base, 144g glycine, 10g SDS in 1L water to create 10X running buffer, dilute to 1X) and transfer onto PVDF membranes using a Bio-Rad Trans-Blot Turbo Transfer System according to described methodology.
    4. Treat membranes with primary antibodies (APOL1 - rabbit polyclonal; YY1 - mouse monoclonal; CD2AP - rabbit polyclonal; Nephrin - rabbit

polyclonal; Podocalyxin - mouse monoclonal; all antibodies sourced from lab resources), followed by treatment with the corresponding secondary antibodies.

5. Develop blots using a Bio-Rad chemiluminescence detection system and take pictures of developed blots.
6. Confirm equal loading by probing for GAPDH (rabbit polyclonal) and B-actin (rabbit polyclonal) and the corresponding secondary antibodies.

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