Silencing TULP3 to Inhibit CDCA Pathway: A New Therapy for Polycystic Kidney Disease

Abstract

Polycystic Kidney Disease (PKD) is a leading cause of end-stage renal disease (ESRD) and remains an incurable genetic disorder. Autosomal dominant PKD (ADPKD), the most common form, arises from mutations in the PKD1 or PKD2 genes, which encode polycystin 1 (PC1) and polycystin 2 (PC2). These mutations lead to ciliary dysfunction and activation of the cilia-dependent cyst activation (CDCA) pathway, driving cystogenesis. Despite significant progress in understanding PKD, the downstream mechanisms of CDCA remain poorly defined, limiting therapeutic advances. This study focuses on silencing Tubby-like protein 3 (TULP3), a key regulator of CDCA signaling, in polycystin-deficient renal epithelial cells to inhibit cyst formation. Using short interfering RNA (siRNA) encapsulated in chitosan-based nanoparticles, this project proposes a targeted, non-invasive strategy to suppress TULP3 expression, disrupt CDCA signaling, and mitigate cyst growth. This approach aims to address critical gaps in PKD treatment by slowing disease progression.

I. Project Description

Statement of the Problem

Polycystic Kidney Disease (PKD) is the most common genetic kidney disorder, affecting over 12 million people worldwide. Nearly all cases progress to end-stage renal disease (ESRD), the final phase of chronic kidney failure, where patients require dialysis or kidney transplantation to survive [1]. Autosomal dominant PKD (ADPKD) is the most prevalent form, affecting 1 in 400 to 1,000 people [1]. ADPKD is characterized by progressive cyst formation, kidney enlargement, and complications in other organs, typically the liver. Current treatments are limited to dialysis, which provides only partial renal function, and kidney transplantation, which is constrained by organ shortages and long waiting periods. These limitations emphasize the urgent need for alternative therapeutic strategies.

ADPKD is caused by *PKD1* or *PKD2* mutations, impairing critical ciliary signaling pathways [2]. This results in ciliary dysfunction and activates the cilia-dependent cyst activation (CDCA) pathway, a driver of cystogenesis. Although the role of polycystins has been widely studied, the downstream mechanisms of CDCA remain poorly understood, limiting the development of targeted therapies.

Emerging research identifies Tubby-like protein 3 (TULP3) as a critical regulator of CDCA activation [3]. TULP3 appears to activate CDCA signaling independently of polycystins, making it a promising target for intervention [4]. This project hypothesizes that suppressing TULP3 expression reduces CDCA activity and inhibits cyst growth. To achieve this, short interfering RNA (siRNA) targeting TULP3 will be delivered using chitosan-based nanoparticles, which stabilize siRNA, prevent enzymatic degradation, and promote efficient renal uptake [5]. This approach aims to provide sustained TULP3 suppression, directly targeting the cellular mechanisms driving cystogenesis without requiring polycystin restoration.

If successful, this project could significantly advance PKD treatment by introducing a non-invasive, kidney-specific therapy that directly targets cystogenesis. By reducing reliance on

dialysis and transplantation, this approach could enhance patient quality of life and address an unmet need in PKD treatment. Additionally, this study could open new avenues for research into ciliary dysfunction and its role in other ciliopathies, broadening the therapeutic landscape for genetic kidney disorders.

Background

PKD and the CDCA Pathway

ADPKD is characterized by progressive renal cyst formation and kidney enlargement, often accompanied by cysts in other organs such as the liver [1]. ADPKD arises from mutations in the *PKD1* or *PKD2* genes, which encode polycystin 1 (PC1) and polycystin 2 (PC2), respectively. These proteins localize in the primary cilia of renal epithelial cells and are essential for cellular signaling, proliferation, and apoptosis. Loss of polycystin function disrupts these processes, leading to abnormal cell growth and cystogenesis [2].

A critical downstream effect of polycystin deficiency is the activation of the CDCA pathway, which drives cystogenesis in polycystin-deficient conditions. Under normal physiological conditions, PC1 and PC2 suppress CDCA activity, maintaining tubular integrity and preventing cyst formation. In their absence, CDCA signaling becomes dysregulated, triggering cytoskeletal remodeling, hyperproliferation, and aberrant extracellular matrix deposition, ultimately accelerating cyst growth [1]. Despite significant advances in understanding polycystin structure and function, the downstream signaling mechanisms of CDCA remain poorly characterized, presenting a major barrier to developing therapies that target the molecular drivers of cystogenesis [2].

TULP3: A Promising Target

TULP3 is a ciliary trafficking protein that functions as an adapter within the IFT-A complex. It facilitates the transportation of signaling molecules, including polycystins, to the ciliary membrane, which is critical for normal ciliary signaling and cellular homeostasis [3]. Notably, TULP3 is uniquely implicated in CDCA pathway activation independently of polycystins. Studies in *Tulp3* conditional knockout mouse models have demonstrated that loss of TULP3 significantly reduces CDCA activation and mitigates cystogenesis in polycystin-deficient kidneys [4]. These models provide critical insights into the consequences of TULP3 absence, which include diminished cyst formation and restoration of tubular architecture. Although conditional knockouts cannot be directly applied to adult organisms, they indicate the potential of TULP3 suppression as a therapeutic strategy, mimicking the effects observed in developmental models.

Furthermore, TULP3 mutations result in renal cyst formation without impairing ciliogenesis, highlighting its distinct role in ciliary signaling compared to other ciliopathy-associated proteins. These findings position TULP3 as a pivotal regulator of CDCA and a promising target for interventions aimed at reducing cystogenesis in PKD. However, the molecular cargoes and interactions modulated by TULP3 in the context of CDCA remain incompletely understood, requiring further investigation [3].

Gene Therapy and siRNA Delivery

Gene therapy modifies gene expression for disease treatment, using viral and non-viral delivery systems. Viral vectors, such as adeno-associated virus (AAV), adenovirus, and lentivirus, have been studied for renal cell targeting but face significant limitations. AAV offers limited

packaging capacity, adenoviral vectors trigger strong immune responses, and lentiviral vectors struggle to target non-dividing renal cells [6]. Among the available approaches, short interfering RNA (siRNA) technology is particularly attractive due to its precision in degrading specific mRNA transcripts, thereby preventing protein translation [7]. siRNA therapies are customizable, exhibit predictable pharmacokinetics, and have faster development pipelines compared to traditional viral therapies [8]. However, siRNA molecules are inherently unstable and susceptible to enzymatic degradation, limiting their therapeutic potential without effective delivery [7].

Encapsulation within chitosan-based nanoparticles addresses these limitations by enhancing siRNA stability, protecting it from nucleases, and promoting efficient renal uptake [5]. Chitosan, a positively charged polymer, forms stable complexes with siRNA, enabling passage through the glomerular filtration barrier while minimizing systemic degradation. Preclinical models have demonstrated up to 60% functional gene knockdown in renal proximal tubular epithelial cells using this delivery method, with no significant toxicity or immune activation [5]. These nanoparticles maintain normal kidney function markers, such as blood urea nitrogen (BUN) and creatinine, and do not provoke significant immune responses or cytokine release. Additionally, chemically modified siRNA combined with chitosan nanoparticles achieves sustained kidney accumulation, supporting their application for kidney-specific gene therapy [8]. Despite extensive research into polycystins, few therapies target pathways activated by their loss. TULP3 is an emerging regulator of CDCA signaling and presents a promising but underexplored target. TULP3 represents an emerging and promising target for addressing the molecular mechanisms underlying CDCA-mediated cystogenesis.

II. Research Plan

Overview of the Research Design

This study employs PKD models with *PKD1* or *PKD2* mutations, comparative models with alternative ciliary protein mutations, and wild-type (WT) controls. The comparative models will clarify TULP3's broader role in ciliary signaling beyond its interaction with polycystins. All groups will undergo evaluation for differences in cyst size, morphology, and kidney function. Chitosan-based nanoparticles will be used to deliver siRNA targeting TULP3, and outcomes will be analyzed through histological and molecular techniques.

The central hypotheses are:

1. Suppressing TULP3 in PKD rat models will reduce cystic growth and/or result in fewer cysts compared to untreated PKD models.
2. Suppressing TULP3 in non-PKD comparative models will either mimic WT physiology or reveal unforeseen biological effects, providing valuable insights either way.

Experimental groups include:

- WT controls, modelling normal polycystin expression and ciliary signaling.
- PKD models, with mutations in *PKD1* or *PKD2*, to replicate the cystic pathology of ADPKD.
- Comparative models, which retain functional PC1 and PC2 but carry mutations in other ciliary proteins, such as Nestin-Cre (targeting progenitor cells), Tam-Cre

(tamoxifen-inducible), BPK (biliary atresia with ciliary dysfunction), Lewis, or RCRC mutations. These models evaluate whether TULP3 silencing produces non-specific effects on ciliary signaling or cyst formation.

Comparative models with intact polycystin function but mutations in other ciliary proteins will help clarify TULP3's specific role in CDCA signaling. Studying these models will identify any off-target effects of TULP3 silencing, as ciliary proteins often interact within shared signaling networks. These findings will ensure that the therapeutic approach specifically addresses PKD pathology without broadly disrupting ciliary signaling. All animals will receive identical diets and environmental conditions. Outcomes will measure cyst size, count, and kidney morphology.

Materials and Methods

Animal Models

Sprague-Dawley (*Rattus norvegicus*) rats will serve as the model organism due to their well-characterized renal physiology and established use in PKD studies. Rats carrying *PKD1* or *PKD2* mutations will model PKD. Comparative models with mutations in alternative ciliary proteins (e.g., Nestin-Cre, Tam-Cre), PKD models, and WT controls will be genotyped to ensure accurate group categorization. Animal care and experimentation will comply with IACUC guidelines and institutional ethical standards.

WT, PKD, and comparative model rats will be divided into treated and untreated subgroups. Treated subgroups will receive intravenous injections of siRNA encapsulated in chitosan nanoparticles targeting TULP3 expression. Untreated subgroups will receive saline injections and serve as controls to establish baseline levels of cystogenesis and ciliary signaling. This experimental design evaluates the treatment's efficacy in reducing cyst formation and assesses its effects on ciliary signaling in non-PKD contexts.

Chitosan-Based Nanoparticle Synthesis and siRNA Preparation

Using methods adapted from Alameh et al. (2024):

1. Low (10 kDa) and high (120 kDa) molecular weight Chitosans will dissolve in nuclease-free water and HCl overnight at 5 mg/mL. 1H NMR and gel permeation chromatography will characterize their degree of deacetylation and molecular weights to confirm molecular weight distribution and degree of deacetylation.
2. Anti-TULP3 siRNA (HPLC-purified and endotoxin-free) will be prepared in sterile, nuclease-free water. Chitosan nanoparticles will form via electrostatic mixing at an amine:phosphate ratio of 5:1, ensuring stability and effective encapsulation.
3. Hyaluronic acid will coat the nanoparticles to improve stability and reduce toxicity. The particles will undergo sterile lyophilization with 0.83% trehalose and 5.8 mM histidine (pH 6.5) for long-term storage, then reconstituted before injections.

Nanoparticles will be administered intravenously at 0.25 mg/kg siRNA, with injection volumes standardized to 10 μL per gram of body weight. This approach ensures uniform dosing and minimizes variability in drug delivery. The intravenous route maximizes nanoparticle distribution and renal uptake, leveraging the kidney's natural filtration dynamics to enhance therapeutic localization.

Post-Treatment Analysis

After 6-8 weeks of treatment, rats will be sacrificed. Kidneys will be harvested, fixed in 10% neutral-buffered formalin, and analyzed. Imaging software will quantify cyst size, density, and kidney area affected. Tissues will undergo H&E staining to assess tissue integrity, fibrosis, and TULP3 knockdown efficacy. RNA and protein levels of TULP3 and CDCA pathway effectors will be quantified using Western blotting to validate target engagement.

Analysis and Expected Results

Statistical comparisons will evaluate cystic size and distribution among treated and control WT, PKD, and comparative models. One key metric is cyst size reduction, where treated PKD models should exhibit smaller and fewer cysts compared to untreated PKD models. Additionally, histological normalization will be considered, where treated kidneys should closely resemble WT histology, with restored tubular architecture and reduced fibrosis. Non-PKD comparative models may reveal compensatory mechanisms or off-target effects, providing additional insights into TULP3's role in ciliary signaling.

Statistical significance will be determined using ANOVA for multiple comparisons and t-tests for pairwise analysis. Technical challenges, such as incomplete siRNA delivery or off-target effects, will be mitigated through optimization of nanoparticle design and dosing protocols.

If successful, these results would validate TULP3 silencing as a viable therapeutic strategy for PKD, offering a novel pathway-specific intervention that bypasses the need for polycystin restoration. The observed normalization of renal architecture and reduction in cystogenesis would not only confirm TULP3's pivotal role in CDCA but also position siRNA-loaded chitosan nanoparticles as a platform for addressing other ciliary dysfunctions. Beyond PKD, this approach could inform therapeutic strategies for ciliopathies with overlapping molecular pathways, establishing a framework for targeted RNAi-based treatments in renal and systemic diseases.

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