BIOMOLECULES & THERAPEUTICS

Review
Biomol Ther 32(3), 291-300 (2024)

Novel Potential Therapeutic Targets in Autosomal Dominant Polycystic Kidney Disease from the Perspective of Cell Polarity and Fibrosis

Yejin Ahn[1] and Jong Hoon Park[1,2,*]

[1]*Department of Biological Sciences, Sookmyung Women's University, Seoul, 04310,*
[2]*Research Institute of Women's Health, Sookmyung Women's University, Seoul, 04310, Republic of Korea*

Abstract

Autosomal dominant polycystic kidney disease (ADPKD), a congenital genetic disorder, is a notable contributor to the prevalence of chronic kidney disease worldwide. Despite the absence of a complete cure, ongoing research aims for early diagnosis and treatment. Although agents such as tolvaptan and mTOR inhibitors have been utilized, their effectiveness in managing the disease during its initial phase has certain limitations. This review aimed to explore new targets for the early diagnosis and treatment of ADPKD, considering ongoing developments. We particularly focus on cell polarity, which is a key factor that influences the process and pace of cyst formation. In addition, we aimed to identify agents or treatments that can prevent or impede the progression of renal fibrosis, ultimately slowing its trajectory toward end-stage renal disease. Recent advances in slowing ADPKD progression have been examined, and potential therapeutic approaches targeting multiple pathways have been introduced. This comprehensive review discusses innovative strategies to address the challenges of ADPKD and provides valuable insights into potential avenues for its prevention and treatment.

Key Words: ADPKD, Kidney, Cell polarity, Fibrosis, Therapeutic target

INTRODUCTION

Polycystic kidney disease (PKD) is a common form of hereditary nephropathy (Arkhipov and Pavlov, 2019; Schonauer *et al*., 2020). It is characterized by the spontaneous growth of fluid-filled cysts throughout the renal tubules (Arkhipov and Pavlov, 2019).

Among these disorders, autosomal dominant polycystic kidney disease (ADPKD) significantly contributes to the incidence of chronic kidney disease on a global scale and is considered the fourth leading cause of chronic kidney disease worldwide (Sharma *et al*., 2019). It is the most frequent congenital genetic disorder leading to renal failure, with an estimated prevalence ranging from 1:400 to 1:1,000 individuals (Chow and Ong, 2009; Chebib and Torres, 2016). ADPKD is primarily a monogenic disease caused by mutations in *PKD1* (85% of cases) or *PKD2* (10-15% of cases), which encode polycystin-1 and polycystin-2, respectively (Tan *et al*., 2011; Chebib and Torres, 2016; Mangolini *et al*., 2016; Oh *et al*., 2021). These mutations lead to the autonomous development of cysts, predominantly beginning as tubular or ductal dilations in a small portion (approximately 1%) of nephrons (Arkhipov and Pavlov, 2019).

While a comprehensive cure for ADPKD is not currently available, ongoing drug trials and studies play a crucial role in advancing research for treatment and symptom management. Research on ADPKD treatment is ongoing, and it is important to remain alert to new developments and constantly changing research findings. In addition to the drugs used to relieve symptoms, new targeted research is needed to diagnose and treat this disease at an early stage.

Epithelial cell polarity plays a crucial role in creating and sustaining the structural and functional differences that are fundamental to normal renal structure and function. This involves ensuring that growth factor receptors, ion and fluid transporters, and channels are correctly targeted to either the apical or basolateral cell membranes (Wilson, 2011). The loss of epithelial cell polarity is associated with cell plasticity or the

Open Access https://doi.org/10.4062/biomolther.2023.207

Received Nov 27, 2023 **Revised** Dec 18, 2023 **Accepted** Dec 26, 2023
Published Online Apr 9, 2024

***Corresponding Author**
E-mail: parkjh@sookmyung.ac.kr
Tel: +82-2-710-9414, Fax: +82-2-710-9414

ability to differentiate into another cell type. In addition, abnormalities in the organization of polarization can result in a multitude of diseases affecting different organs. This is prevalent in PKD, where cysts originating from epithelial cells replace typical renal tubules (Wilson, 2011). In ADPKD, the cyst-lining epithelium exhibits abnormal cell polarity (Silberberg *et al*., 2005; Le Corre *et al*., 2015). Currently, cell polarity defects in polycystic kidneys are viewed as a cause of the disease; therefore, targeting such defects could have important implications.

In addition, in PKD, there is an increase in the production of the extracellular matrix (ECM), a reduction in its degradative capacity, and alterations in its composition (Norman, 2011). Collectively, these factors contribute to the development of fibrosis, resulting in a progressive decline in kidney function. Notably, changes in the epithelial cells within cysts appear to precede and drive modifications in the surrounding stromal tissue. These observations support the hypothesis that the development of fibrosis in ADPKD follows a biphasic pattern. Consequently, as cyst formation and fibrosis in ADPKD are interconnected events, slowing down fibrotic progression, potentially via anti-fibrotic strategies, may offer a beneficial approach to treat the disease.

Therefore, in this review, we summarize and present potential therapeutic targets that could lead to important advances in the management and treatment of ADPKD. Furthermore, the continual research efforts and the dissemination of research findings will contribute to the advancement of the field.

GENERAL TREATMENT OF ADPKD

Therapeutic approaches for ADPKD are challenging because of its complex nature. The ultimate size of the kidney is intricately related to the kinetics of cyst formation, the overall number of cysts, and the net cell growth rate within individual cysts. Both the absolute number of cysts and rate of cyst formation appear to be pivotal determinants that collectively influence disease progression and represent potential targets for therapeutic interventions (Grantham *et al*., 2008). Therefore, the treatment of PKD has focused on this area.

Additionally, many potential therapeutic targets have been implicated not only in cyst formation but also in vital physiological processes across the body, including cellular proliferation, growth, and tissue repair (Serra *et al*., 2010). Because of the high potential side effects of drugs targeting ADPKD, treatment options have traditionally been limited to addressing and alleviating symptoms and associated complications, and several drugs have been administered to patients on a limited basis. The most commonly used drugs include Tolvaptan and mammalian target of rapamycin (mTOR) inhibitors.

TOLVAPTAN AND VASOPRESSIN V2 RECEPTOR INHIBITION IN ADPKD TREATMENT

Tolvaptan, the first FDA-approved prescription medication for ADPKD, is a key intervention for inhibiting kidney cyst growth (Chebib *et al*., 2018). The FDA has approved tolvaptan to address the decline in kidney function among adults at risk of rapid progression (Hori, 2013; Lanke and Shoaf, 2019). The drug is only used for specific indications, and its approval, specifically for ADPKD, highlights its role in slowing the deterioration of kidney function in individuals predisposed to accelerated progression (Black and Sutton, 2013).

Tolvaptan plays a pivotal role as a vasopressin V2 receptor inhibitor and is a key factor in the regulation of water and salt reabsorption in the kidneys (Berl, 2015). Tolvaptan is a vasopressin receptor antagonist that specifically targets V2 receptors and disrupts the reabsorption of free water (Torres *et al*., 2012; Tamma *et al*., 2017). This mechanism ultimately leads to the excretion of diluted urine (Fujiki *et al*., 2019). Tolvaptan has proven efficacy in ADPKD and is now used intermittently to reduce urinary osmolarity by blocking V2R (Torres *et al*., 2012).

Additionally, this drug is closely related to the cAMP signaling pathway. In an ADPKD model, increased renal adenosine cyclic 3',5'-monophosphate (cAMP) is thought to promote cyst growth by secreting fluid into the cyst lumen (Torres and Harris, 2014). Similar to the findings in the mouse ADPKD model, the downregulation of cAMP signaling using this drug in patient populations reduces kidney weight, cyst enlargement, and the rate of progression of PKD (Tamma *et al*., 2017; Nakamura *et al*., 2018; Fujiki *et al*., 2019). Additionally, tolvaptan prevents vasopressin-induced relocation of AQP2 to the plasma membrane and inhibits osmotic water transport in the collecting duct principal cells expressing endogenous V2R receptors in patients (Tamma *et al*., 2017). This drug effectively reduces cyst growth rate and total kidney volume (TKV) in patients with ADPKD (Torres *et al*., 2012, 2017; Raina *et al*., 2021).

However, although tolvaptan is currently considered the gold standard for ADPKD treatment, complete disease suppression remains a challenge (Torres *et al*., 2012). Additionally, certain side effects associated with tolvaptan, including safety and hepatotoxicity concerns, may limit its therapeutic efficacy (Watkins *et al*., 2015; Bellos, 2021; Raina *et al*., 2021).

MTOR INHIBITORS IN ADPKD TREATMENT

Another target for ADPKD treatment is the mTOR pathway. This signaling pathway is involved in the regulation of cell growth and division. When the key causative gene encoding for polycystin-1 is mutated or malfunctions, as in ADPKD, the expression of RHEB is de-repressed, leading to mTOR activation and increased cell growth. mTOR signaling is activated and upregulated in the cystic epithelial cells of mouse models and the kidneys of patients with ADPKD (Shillingford *et al*., 2006; Mekahli *et al*., 2014). Therefore, dysregulation of the mTOR pathway has been proposed as a renal pathological characteristic of patients with ADPKD. Many drugs targeting this mechanism have been discovered and are currently in use (Pathomthongtaweechai *et al*., 2014; Su *et al*., 2022; Zhang *et al*., 2022).

Sirolimus, also called rapamycin, is currently used to inhibit mTOR signaling. This drug is an immunosuppressant whose use is limited to patients with ADPKD who have reached a severe stage and have received a transplant (Lorenz and Heitman, 1995; Sabers *et al*., 1995; Shillingford *et al*., 2006). Rapamycin treatment reduces cyst growth, inhibits epithelial cell proliferation and fibrosis, and increases apoptosis of cyst-lining cells (Shillingford *et al*., 2006, 2010; Liu *et al*., 2018; Holditch *et al*., 2019). This drug significantly reduces cyst size (Perico *et al*., 2010). However, no significant improvements

in kidney function are observed; for instance, no changes in blood urea nitrogen (BUN) levels are noted (Serra *et al*., 2010; Zafar *et al*., 2010; Braun *et al*., 2014). Therefore, this drug is permitted at low doses and is used to attenuate disease progression by focusing only on delaying renal failure (Wahl *et al*., 2006; Zafar *et al*., 2010; Shillingford *et al*., 2012).

Everolimus is an mTOR inhibitor. It inhibits cyst growth and restores cell polarity. This drug modulates intracellular signaling pathways and can effectively manage cyst growth in ADPKD. However, this drug has some limitations as a treatment option. Everolimus is effective in slowing the increase in TKV in patients with ADPKD but has the disadvantage of not slowing the progression of kidney damage (Walz *et al*., 2010; Zschiedrich *et al*., 2015).

CELL POLARITY DISRUPTION IN ADPKD

Epithelial cells are polarized along two orthogonal axes. The apical basal polarity is formed along the vertical axis based on the matrix, and planar cell polarity is formed perpendicular to the apical basal axis, defining it as a cellular tissue (Karner *et al*., 2006; Nigro *et al*., 2015). When cell polarity is accurately determined within a tissue, the positions of various channels or junctional proteins present in the cell are adjusted, allowing them to perform their correct functions and roles. Various reports suggest that cell polarity disruption may be the cause of ADPKD (Luyten *et al*., 2010; Riga *et al*., 2020; Papakrivopoulou *et al*., 2021), and it can be used as an additional targeted treatment strategy.

Renal cysts in ADPKD originate from the abnormal proliferation and incomplete differentiation of tubule cells and exhibit disruptions in cell polarity, including alterations in apical-basal polarity and extracellular matrix rearrangements (Calvet, 1993; Kunimoto *et al*., 2017; Xu *et al*., 2018a). Numerous cellular abnormalities, including cell polarity disruption, have been observed in the cyst-lining cells during cyst growth (Arkhipov and Pavlov, 2019; Tran Nguyen Truc *et al*., 2023). The role of these disruptions has been discussed previously (Sharma *et al*., 2019). Therefore, ADPKD may be associated with cell polarity problems. Studies related to cell polarity are essential for understanding the intrinsic properties of ADPKD, the process of renal cyst formation and growth, and potential therapeutic strategies (Tran Nguyen Truc *et al*., 2023). Research on cell polarity is currently underway to elucidate the intracellular processes and molecular mechanisms contributing to ADPKD and is expected to aid in the development of effective treatment strategies. In addition, the correct polarization of epithelial cells lining the renal tubules is important for inducing normal renal development and preventing cyst expansion. Therefore, interventions that restore the defective polarity to normal levels are potential therapeutic strategies for cystic kidney disease.

CELL POLARITY-RELATED POTENTIAL THERAPEUTIC TARGETS FOR ADPKD

Research on cell polarity is currently underway to elucidate the intracellular processes and molecular mechanisms contributing to ADPKD and is expected to aid in the development of effective treatment strategies. Therefore, several drugs and treatments have garnered significant, with a focus on the related mechanisms (Table 1).

Polarized junctional protein and related mechanism

ADAM10 activity: Cellular adhesion involves various protein complexes, such as tight junctions, adherens junctions, desmosomes, and gap junctions. Adherens junctions play a pivotal role in maintaining the structural integrity and polarity of renal epithelial cells. In kidney epithelial cells affected by ADPKD, the maintenance of cell polarity and cell-cell adhesion are disrupted. These are primarily associated with alterations in E-cadherin-mediated adherens junctions (Roitbak *et al*., 2004; Solanas *et al*., 2011; Xu *et al*., 2015).

The *PKD1* gene interacts with various signaling molecules, especially, polycystin-1 and Gα12, which play a role in regulating E-cadherin cleavage in renal epithelial cells. This affects cell polarity and cell-cell adhesion (Maretzky *et al*., 2005; Xu *et al*., 2015). Direct PC1-Gα12 interaction is involved in regulating the apoptosis of renal cystic epithelial cells (Yu *et al*., 2011). Mutation or deletion of the *PKD1* gene leads to the ac-

Table 1. Cell polarity-related therapeutic target compounds

Therapeutic target compound	Mechanism of action	Note	Reference
GI254023X	ADAM10 activity inhibition	Disruption of cell polarity and cell-to-cell contact signals caused by dysregulation of ADAM10 activity	Xu *et al*., 2015; Xiao *et al*., 2022
Tubacin	HDAC6 activity inhibition	Inhibits cyst growth Reduces cyst index, height/weight ratio, and BUN levels	Cebotaru *et al*., 2016; Yanda *et al*., 2017b
Trichostatin		Reduces ERK1/2 phosphorylation and alleviates cyst growth	Liu *et al*., 2012
Tubastatin-A (TSA)		Inhibits cyst formation and slows cyst growth	Cebotaru *et al*., 2016
ACY-1215 (ricolinostat) ACY-241(citarinostat)		Slows cyst growth in an ADPKD model Verification of drug combination feasibility	Yanda *et al*., 2017a; Lorenzo Pisarello *et al*., 2018; Pulya *et al*., 2021
Probenecid	ENaC activity inhibition	Pannexin-1 channel inhibitior Reduces sodium levels and fluid retention within the cyst Increases ENaC current and attenuates cyst formation	Arkhipov and Pavlov, 2019; Arkhipov *et al*., 2023

tivation of Gα12 (Yu *et al*., 2011), which results in decreased cell-matrix and cell-cell adhesion (Wu *et al*., 2016; Meyer-Schwesinger *et al*., 2022). Gα12 activation likely induces cystic growth of renal epithelial cells (Kong *et al*., 2009).

This process is associated with initiating the maturation of ADAM10. ADAMs (disintegrin and metalloproteinase) constitute a family of versatile proteins involved in both cell adhesion and proteolysis (Shiu *et al*., 2018; Wang and Cao, 2023). The ADAM substrate comprises molecules that play important roles in the plasma membrane by enhancing the release of E-cadherin (Kato *et al*., 2018; Yuan *et al*., 2020). The subsequent cleavage of the ectodomain fragment of E-cadherin results in the translocation of β-catenin from the cell membrane to the nucleus (Lichtenthaler *et al*., 2018). Ultimately, this process promotes the formation of bladder-like structures in renal epithelial cells by disrupting cell polarity and intercellular contacts (Yu *et al*., 2011; Meyer-Schwesinger *et al*., 2022).

Dysregulation of ADAM10 activity, leading to E-cadherin cleavage and subsequent β-catenin translocation, has the potential to disrupt cell polarity. Therefore, blocking this signaling pathway, especially by inhibiting ADAM10 activity, has emerged as a potential new therapeutic strategy for ADPKD. GI254023X can be used as an inhibitor of this activity (Xu *et al*., 2015; Xiao *et al*., 2022). Additionally, because of its potential to protect kidney tissues and promote regeneration, this inhibitor may be useful for treating kidney disease.

Histone deacetylase 6 (HDAC6) activity and tubacin: HDACs, or histone deacetylases, comprise a family of small molecules that play crucial roles in cellular processes by removing acetyl groups from histones and non-histone proteins, thereby modulating gene expression (Seto and Yoshida, 2014; Milazzo *et al*., 2020). They play a vital role in numerous crucial cellular processes, overseeing significant biological activities such as transcription, cell migration, proliferation, cell-cell interaction, and cell signaling (Valenzuela-Fernandez *et al*., 2008).

HDAC6 has garnered attention because of its increased expression and activity in ADPKD (Liu *et al*., 2012; Ke *et al*., 2018). This condition is characterized by disrupted planar cell polarity and defective apical-basal cell polarity, which contribute to cyst growth.

Some studies have indicated that HDAC6 inhibition, particularly with the specific inhibitor tubacin, can effectively reduce cyst growth in patients with ADPKD. These findings highlight that tubacin not only arrests cyst growth but also leads to cyst shrinkage in MDCK cells (Cebotaru *et al*., 2016; Yanda *et al*., 2017b). Furthermore, HDAC inhibitors exhibit a substantial reduction in key indicators, including cyst index, kidney/body weight ratio, and blood urea nitrogen (BUN) levels. This suggests their considerable potential as treatments. (Yanda *et al*., 2017b; Feng *et al*., 2018; Hao *et al*., 2020). Moreover, inhibition of HDAC6 using compounds such as trichostatin or tubacin results in the elevated acetylation of α-tubulin, reduced expression of EGFR, and restoration of proper localization of EGFR in renal epithelial cells and tissues with *PKD1* mutations (Liu *et al*., 2012). This outcome has been linked to a reduction in the phosphorylation of ERK1/2, which serves as a downstream target of the EGFR signaling pathway (Liu *et al*., 2012).

Additionally, Tubastatin-A (TSA), another HDAC6 inhibitor, demonstrates a similar effect in inhibiting cyst formation and slowing cyst growth (Cebotaru *et al*., 2016). Furthermore, treatment with other inhibitors, ACY-1215 (ricolinostat) and ACY-241 (citarinostat), has also been shown to slow cyst growth in an ADPKD mouse model. The effects have been studied in clinical trials (Yanda *et al*., 2017a; Lorenzo Pisarello *et al*., 2018; Pulya *et al*., 2021). In addition, various HDAC6 inhibitors, including ACY-1215 and ACY-241, have the potential to be used in combination with other drugs and are suggested as effective therapeutic strategies (Lorenzo Pisarello *et al*., 2018).

In conclusion, HDAC6 inhibitors, including tubacin, trichostatin, tubastatin-A, ACY-1215, and ACY-241, are effective in attenuating renal cyst growth in an ADPKD model. The associated mechanism involves inhibiting cell proliferation, reducing cAMP levels, and influencing the CFTR-mediated chloride currents. HDAC6 activity appears to be intricately linked to both planar and apical-basal cell polarity, suggesting potential therapeutic avenues for the management of PKD (Cebotaru *et al*., 2016). Although further studies are needed to fully elucidate the role of HDAC6 in these cellular processes and its implications in ADPKD, it has great potential as a therapeutic target.

Polarized channel and related mechanism

Epithelial sodium channel (ENaC) activity: ENaCs are expressed in various epithelia, including the aldosterone-sensitive upper renal unit, colon, and lungs, and play a key role in limiting the rate of electrogenic Na+ reabsorption by regulating the steep transepithelial Na+ concentration gradient (Bhalla and Hallows, 2008). The regulation of ENaC is necessary for salt and water balance in various Na+-transporting epithelia. The activity of ENaC influences apical-basal cell polarity and plays a role in cytoplasmic localization by trafficking to the apical and lateral membranes. In ADPKD, ENaC activity plays a key role in intracellular localization and prevention of metastasis in cases with polarity defects (Blazer-Yost *et al*., 2003). Accordingly, targeting ENaC activity has been proposed as a therapeutic strategy for ADPKD.

ENaC is responsible for the regulation of salt and fluid transport and affects cell polarity dynamics. The modulation of cell polarity may also influence cyst development in ADPKD. Epithelial Na+ channels play a pivotal role in regulating salt and fluid transport in the cells of various organs. In polarized cells, they respond to internal and external signals via fine regulation of membrane proteins. Studies on neurons and epithelial cells have shown similarities in the ability of these proteins to organize their membrane localization. Na+ transport in principal CD cells occurs primarily via ENaC, which requires tight control of Na+ transport to maintain systemic Na+ homeostasis. Additionally, CD principal cells tightly regulate apical and basolateral Na+ transport through the regulation of Na+/K-ATPase cell surface expression by Na+ apical entry (Vinciguerra *et al*., 2005).

Epithelial cells segregate transport and regulatory proteins, thereby maintaining the apical and basement membrane regions. Similar to various epithelial ion channels, trafficking of ENaCs ensures their movement to the correct apical membrane, contributing to the maintenance of cell polarity in epithelial tissues (Staruschenko *et al*., 2007).

Furthermore, ENaC acts as a rate-limiting factor in fluid absorption and must be cleaved by proteases to transport Na+ and prevent excessive mucosal fluid absorption. Protease inhibitors block this process, thereby inhibiting ENaC activity (Garcia-Caballero *et al*., 2009). These characteristics suggest

that targeting ENaC could be considered a treatment strategy for ADPKD.

Pannexin-1 channel activity and probenecid: Pannexin-1 plays a crucial role in the formation of large-pore membrane channels that facilitate the passage of ions and metabolites and promote ATP release (Chiu *et al*., 2018; Whyte-Fagundes and Zoidl, 2018; Wei *et al*., 2021). This channel not only interacts with various cytoskeletal proteins and influences cell polarity but also contributes to microtubule stability (Silverman *et al*., 2008; Xu *et al*., 2018b) Additionally, its interaction with actin and factors regulating cell surface localization and mobility are vital for maintaining normal cellular functions (Bhalla-Gehi *et al*., 2010; Wicki-Stordeur and Swayne, 2013). The preferential concentration of pannexin-1 channels in the apical membrane domain of polarized cells, particularly monolayer sheets or spheroids, facilitates diverse cellular actions. Mutations in this channel protein can lead to abnormal states, disrupt cell polarization, and interfere with intercellular contacts and polarization processes (Shum *et al*., 2019).

In the context of ADPKD, the cystic fluid contains elevated ATP levels due to abnormalities in renal epithelial cells. This aberration can hinder electrolyte reabsorption in the cystic lining cells, leading to the accumulation of cystic fluids. Abnormal ATP secretion into the cystic lumen is considered a pathogenic factor in ADPKD (Arkhipov and Pavlov, 2019; Sudarikova *et al*., 2021). The ability of probenecid to inhibit pannexin-1 activity has emerged as a promising approach for alleviating ADPKD pathogenesis (Garcia-Caballero *et al*., 2009; Arkhipov *et al*., 2023). The specific effect of probenecid as a pannexin-1 inhibitor in ADPKD has been studied, with a focus on ENaC activity and fluid retention within cysts.

Elevated levels of pannexin-1 in the human ADPKD cystic epithelium compared to those in normal collecting ducts suggest a potential correlation with ADPKD development. Therefore, the inhibition of pannexin-1 by probenecid may be a viable strategy to impede ADPKD progression, particularly as a specific target in the cyst epithelium.

Experiments involving probenecid administration have demonstrated increased ENaC currents, leading to the attenuation of cyst formation *in vitro* by reducing sodium levels and fluid retention within the cyst (Arkhipov and Pavlov, 2019; Arkhipov *et al*., 2023). Targeting pannexin-1 in the context of ADPKD has therapeutic potential, offering a promising avenue to slow disease progression by inhibiting pannexin-1 activity.

RENAL FIBROSIS IN ADPKD

Fibrosis in the kidneys of patients with ADPKD is characterized by the abnormal accumulation of fibrous tissue, which presents as a distinct and deleterious pathological feature (Rockey *et al*., 2015). The main cause of fibrosis is excessive accumulation of ECM proteins, such as fibronectin and collagen (Bulow and Boor, 2019; Fragiadaki *et al*., 2020).

Fibrosis is caused by the activation of myofibroblasts and epithelial cell proliferation, leading to the production of an abundant matrix and excessive deposition of fibrous tissue (Falke *et al*., 2015). This phenomenon significantly contributes to the decline in kidney function in patients with ADPKD; in severe cases, approximately 50% of patients are known to have accelerated progression to end-stage renal disease (Yuan *et al*., 2019; Gluba-Sagr *et al*., 2023).

Ongoing research is aimed at effectively inhibiting the progression of fibrosis and restoring kidney tissue distorted by cyst expansion. The development of interventions that can address the underlying fibrotic changes caused by cyst expansion is essential for the successful recovery of renal function.

RENAL FIBROSIS-RELATED POTENTIAL THERAPEUTIC TARGETS FOR ADPKD

Fibrosis in ADPKD disrupts normal renal morphology and reduces renal function, highlighting the importance of strategies that effectively inhibit fibrosis. This contributes to slowing the progression of fibrosis caused by cyst expansion and may lead to the development of new treatments (Table 2).

AMPK activity-related potential therapeutic targets

In ADPKD, hyperactivity of mTOR and cystic fibrosis transmembrane conductance regulator (CFTR) has been shown to play important roles as triggers in the progressive expansion of renal cysts (McCarty *et al*., 2009). The activity of these proteins can be inhibited by AMP-activated kinase (AMPK), and research on treatment strategies using this mechanism is in progress.

Metformin: Several studies have highlighted the potential therapeutic benefits of metformin in various kidney diseases, with a pronounced focus on its implications in ADPKD. In the context of ADPKD, metformin has been identified as an inhibitor of CFTR-mediated fluid secretion and cyst formation associated with mTOR, both of which are modulated AMPK (Takiar *et al*., 2011). Recent experimental and clinical investigations have consistently supported the hypothesis that metformin, which acts as a pharmacological activator of AMPK, may exert positive effects in the treatment of ADPKD (Song *et al*., 2021).

The role of metformin in ADPKD is to mitigate inflammation, as evidenced by the diminished leukocyte infiltration and the downregulation of pivotal inflammatory and renal injury markers in the pericapsular region of PKD variants following metformin treatment (Song *et al*., 2021; Pastor-Soler *et al*., 2022). Additionally, metformin-induced AMPK activation is correlated with the phosphorylation and inactivation of CFTR chloride channels, leading to reduced epithelial cytoplasmic secretion in ADPKD (Seliger *et al*., 2018). AMPK activation has been consistently associated with reduced renal fibrosis in various experimental kidney disease models, positioning metformin as a potential candidate for mitigating renal fibrosis (Satriano *et al*., 2013; Borges *et al*., 2020).

Furthermore, metformin administration triggers the activation of renal AMPK, enhances mitochondrial biogenesis, and initiates diverse anti-inflammatory and anti-fibrotic pathways independent of blood pressure or glucose effects. This results in a notable reduction in albuminuria levels and the expression of renal fibrosis markers, suggesting a potential therapeutic effect in the context of induced fibrosis (Borges *et al*., 2020; Sharma and Smyth, 2021). Metformin-induced phosphorylation of acetyl-CoA carboxylase (ACC) via AMPK has been established as a mechanism that reduces renal fibrosis, emphasizing the pivotal role of metformin in conferring anti-fibrotic effects in renal disease models (Lee *et al*., 2018).

Moreover, metformin's anti-fibrotic effects are closely tied to the inhibition of extracellular signal-regulated kinase 1/2 (ERK1/2) phosphorylation (Zheng *et al*., 2017). Metformin has

Table 2. Fibrosis related therapeutic target compounds

Therapeutic target compound	Mechanism of action	Note	Reference
Metformin	AMPK activity activation	Diminishes leukocyte infiltration and mediates the downregulation of pivotal inflammatory and renal injury markers Inhibits renal cyst growth Inhibits ERK1/2 phosphorylation and impedes the accumulation of extracellular matrix (ECM)	Liang *et al*., 2019; Borges *et al*., 2020; Song *et al*., 2021
PXL770		Inhibits renal cyst growth and suppresses various inflammatory signaling Attenuates macrophage infiltration and fibrosis Enhances mitochondrial biogenesis	Gluais-Dagorn *et al*., 2022; Dagorn *et al*., 2023
PF-06409577		Direct activator of AMPK Inhibits proliferation of cyst-lining epithelial cells and CFTR-regulated cystic fluid secretion by downregulating the mTOR pathway Interferes with the development of renal cysts and the fibrotic process	Cameron *et al*., 2016; Esquejo *et al*., 2018; Su *et al*., 2022
Nintedanib	RTK activity inhibition	Inhibits cyst epithelial cell proliferation and growth Involved in inactivating renal interstitial fibroblasts and suppressing the expression of ECM proteins	Liu *et al*., 2017; Feng *et al*., 2021; Jamadar *et al*., 2021
DM509	Farnesoid X receptor agonist soluble epoxide hydrolase inhibitor	Reduces the area of collagen-positive renal fibrosis Attenuates the expression of inflammatory genes by suppressing TNF-alpha inflammatory signals Reduces plasma cholesterol levels	Hye Khan *et al*., 2019; Stavniichuk *et al*., 2020; Imig *et al*., 2021

also demonstrated efficacy in preventing fibrosis by inhibiting ERK1/2 activity and impeding the accumulation of ECM in a mouse model (Liang *et al*., 2019).

AMPK is a promising therapeutic target that plays crucial roles in cellular energy sensing and regulation. These findings collectively underscore the potential of metformin to ameliorate various facets of ADPKD pathogenesis.

PXL770: PXL770 is a promising candidate for the treatment of fibrosis because of its ability to activate AMPK. This compound has demonstrated efficacy in suppressing various inflammatory signaling pathways, with reported success in mitigating liver fibrosis. In a mouse model of fibrosis, treatment with PXL770 reduced fibrosis-related indicators (Gluais-Dagorn *et al*., 2022).

Following validation in a liver fibrosis model, PXL770's potential was further confirmed in an ADPKD model. Treatment with PXL770 inhibited cyst growth in both mouse- and patient-derived cells. Notably, it mirrored the effects of metformin, such as the attenuation of macrophage infiltration and fibrosis via AMPK activation and the enhancement of mitochondrial biogenesis (Dagorn *et al*., 2023). These findings underscore PXL770's potential as a novel target with anti-fibrotic effects.

PF-06409577 (1H-indole-3-carboxylic Acid): PF-06409577, also known as 6-chloro-5-[4-(1-hydroxycyclobutyl)phenyl]-1H-indole-3-carboxylic acid, directly activates AMPK. It exerts its effects by downregulating the mTOR pathway, inhibiting the proliferation of cyst-lining epithelial cells, and regulating CFTR-regulated cystic fluid secretion (Cameron *et al*., 2016). In addition to its recognized efficacy in treating diseases such as diabetic nephropathy and nonalcoholic fatty liver disease (NAFLD) by reducing the expression of mRNAs associated with fibrosis markers, PF-06409577 interferes with the formation and expansion of cysts in a variety of models. Ultimately, this interferes with the development of renal cysts and fibrotic processes (Cameron *et al*., 2016; Esquejo *et al*., 2018; Su *et al*., 2022). Therefore, this compound is also one of the promising target substances that can play a role in delaying the progression of PKD.

Triple receptor tyrosine kinases (RTK) activity and nintedanib

Renal fibrosis involves the activation of fibroblasts and the deposition of ECM. This intricate process involves the activation of growth factor receptors, particularly receptor tyrosine kinases (RTKs), and their downstream signaling pathways, which regulate various cellular physiological processes, such as cell metabolism, growth, and differentiation (Liu and Zhuang, 2016).

The potential utility of inhibiting RTK activity in mitigating fibrosis has been demonstrated in various tissues such as the lungs, heart, and liver. Renal fibrosis can be suppressed by inhibitors targeting RTK activity.

Nintedanib is an RTK inhibitor known for its anti-fibrotic effects. It blocks the phosphorylation of several kinase receptors associated with unilateral ureteral obstruction (UUO). These receptors include platelet-derived growth factor receptor (PDGFR), vascular endothelial growth factor receptor (VEGFR), fibroblast growth factor receptor (FGFR), and epidermal growth factor receptor (EGFR) (Feng *et al*., 2021).

Moreover, nintedanib exhibits a significant anti-fibrotic effect in ADPKD. It inhibits cyst epithelial cell proliferation and growth, inactivates renal interstitial fibroblasts, and suppress-

es the expression of ECM proteins. These findings highlight the therapeutic potential of nintedanib for the treatment of fibrotic kidney diseases (Liu *et al*., 2017; Jamadar *et al*., 2021).

FXRA/sEHi activity and DM509

The farnesoid X receptor (FXR) is prominently expressed in both the liver and kidneys, demonstrating notable anti-fibrotic activity in various fibrosis models (Wang *et al*., 2010; Gai *et al*., 2017). Additionally, soluble epoxide hydrolase inhibitors (sEHi) have emerged as promising preventive measures against kidney fibrosis, even when administered as a treatment (Chiang *et al*., 2015; Kim *et al*., 2015).

Based on these findings, DM509, a compound that acts as a farnesoid X receptor agonist and sEHi, exhibited significant effects in a mouse model of renal fibrosis (Stavniichuk *et al*., 2020). DM509 treatment reduced collagen-positive renal fibrosis. Moreover, DM509 demonstrated the ability to attenuate the expression of inflammatory genes by modulating lipid levels and suppressing TNF-alpha inflammatory signals (Imig *et al*., 2021).

Furthermore, DM509 has been proven effective in alleviating fibrosis-induced kidney damage, including mediating a reduction in plasma cholesterol levels (Hye Khan *et al*., 2019). These findings suggest that DM509 is a potentially innovative renal anti-fibrotic agent.

CONCLUSION

In this thorough review, besides exploring tolvaptan and mTOR inhibitors for treating ADPKD, the investigation delves into potential therapeutic targets for the disease. The focus extends to understanding and targeting both cell polarity and fibrosis-related mechanisms.

This review highlights the complex nature of ADPKD and the need for a comprehensive approach to address its multifaceted characteristics. By delving into the intricacies of cell polarity and fibrosis, this review seeks to provide a nuanced understanding of disease mechanisms. This understanding will serve as a foundation for the development of targeted therapeutic interventions that can potentially modify the course of ADPKD.

ACKNOWLEDGMENTS

This work was supported by National Research Foundation of Korea (NRF) grants funded by the Korean government (MSIT) (NRF-2022M3A9B6082667 and 2022R1A2C3002899).

REFERENCES

Arkhipov, S. N. and Pavlov, T. S. (2019) ATP release into ADPKD cysts via pannexin-1/P2X7 channels decreases ENaC activity. *Biochem. Biophys. Res. Commun.* **513**, 166-171.

Arkhipov, S. N., Potter, D. L., Sultanova, R. F., Ilatovskaya, D. V., Harris, P. C. and Pavlov, T. S. (2023) Probenecid slows disease progression in a murine model of autosomal dominant polycystic kidney disease. *Physiol. Rep.* **11**, e15652.

Bellos, I. (2021) Safety profile of tolvaptan in the treatment of autosomal dominant polycystic kidney disease. *Ther. Clin. Risk Manag.* **17**, 649-656.

Berl, T. (2015) Vasopressin antagonists. *N. Engl. J. Med.* **373**, 981.

Bhalla-Gehi, R., Penuela, S., Churko, J. M., Shao, Q. and Laird, D. W. (2010) Pannexin1 and pannexin3 delivery, cell surface dynamics, and cytoskeletal interactions. *J. Biol. Chem.* **285**, 9147-9160.

Bhalla, V. and Hallows, K. R. (2008) Mechanisms of ENaC regulation and clinical implications. *J. Am. Soc. Nephrol.* **19**, 1845-1854.

Black, P. and Sutton, R. (2013) Commentary on: tolvaptan in patients with autosomal-dominant polycystic kidney disease. *Urology* **81**, 705-706.

Blazer-Yost, B. L., Esterman, M. A. and Vlahos, C. J. (2003) Insulin-stimulated trafficking of ENaC in renal cells requires PI 3-kinase activity. *Am. J. Physiol. Cell Physiol.* **284**, C1645-C1653.

Borges, C. M., Fujihara, C. K., Malheiros, D., de Avila, V. F., Formigari, G. P. and Lopes de Faria, J. B. (2020) Metformin arrests the progression of established kidney disease in the subtotal nephrectomy model of chronic kidney disease. *Am. J. Physiol. Renal Physiol.* **318**, F1229-F1236.

Braun, W. E., Schold, J. D., Stephany, B. R., Spirko, R. A. and Herts, B. R. (2014) Low-dose rapamycin (sirolimus) effects in autosomal dominant polycystic kidney disease: an open-label randomized controlled pilot study. *Clin. J. Am. Soc. Nephrol.* **9**, 881-888.

Bulow, R. D. and Boor, P. (2019) Extracellular matrix in kidney fibrosis: more than just a scaffold. *J. Histochem. Cytochem.* **67**, 643-661.

Calvet, J. P. (1993) Polycystic kidney disease: primary extracellular matrix abnormality or defective cellular differentiation? *Kidney Int.* **43**, 101-108.

Cameron, K. O., Kung, D. W., Kalgutkar, A. S., Kurumbail, R. G., Miller, R., Salatto, C. T., Ward, J., Withka, J. M., Bhattacharya, S. K., Boehm, M., Borzilleri, K. A., Brown, J. A., Calabrese, M., Caspers, N. L., Cokorinos, E., Conn, E. L., Dowling, M. S., Edmonds, D. J., Eng, H., Fernando, D. P., Frisbie, R., Hepworth, D., Landro, J., Mao, Y., Rajamohan, F., Reyes, A. R., Rose, C. R., Ryder, T., Shavnya, A., Smith, A. C., Tu, M., Wolford, A. C. and Xiao, J. (2016) Discovery and preclinical characterization of 6-chloro-5-[4-(1-hydroxycyclobutyl)phenyl]-1H-indole-3-carboxylic acid (PF-06409577), a direct activator of adenosine monophosphate-activated protein kinase (AMPK), for the potential treatment of diabetic nephropathy. *J. Med. Chem.* **59**, 8068-8081.

Cebotaru, L., Liu, Q., Yanda, M. K., Boinot, C., Outeda, P., Huso, D. L., Watnick, T., Guggino, W. B. and Cebotaru, V. (2016) Inhibition of histone deacetylase 6 activity reduces cyst growth in polycystic kidney disease. *Kidney Int.* **90**, 90-99.

Chebib, F. T., Perrone, R. D., Chapman, A. B., Dahl, N. K., Harris, P. C., Mrug, M., Mustafa, R. A., Rastogi, A., Watnick, T., Yu, A. S. L. and Torres, V. E. (2018) A practical guide for treatment of rapidly progressive ADPKD with tolvaptan. *J. Am. Soc. Nephrol.* **29**, 2458-2470.

Chebib, F. T. and Torres, V. E. (2016) Autosomal dominant polycystic kidney disease: core curriculum 2016. *Am. J. Kidney Dis.* **67**, 792-810.

Chiang, C. W., Lee, H. T., Tarng, D. C., Kuo, K. L., Cheng, L. C. and Lee, T. S. (2015) Genetic deletion of soluble epoxide hydrolase attenuates inflammation and fibrosis in experimental obstructive nephropathy. *Mediators Inflamm.* **2015**, 693260.

Chiu, Y. H., Schappe, M. S., Desai, B. N. and Bayliss, D. A. (2018) Revisiting multimodal activation and channel properties of Pannexin 1. *J. Gen. Physiol.* **150**, 19-39.

Chow, C. L. and Ong, A. C. (2009) Autosomal dominant polycystic kidney disease. *Clin. Med. (Lond.)* **9**, 278-283.

Dagorn, P. G., Buchholz, B., Kraus, A., Batchuluun, B., Bange, H., Blockken, L., Steinberg, G. R., Moller, D. E. and Hallakou-Bozec, S. (2023) A novel direct adenosine monophosphate kinase activator ameliorates disease progression in preclinical models of Autosomal Dominant Polycystic Kidney Disease. *Kidney Int.* **103**, 917-929.

Esquejo, R. M., Salatto, C. T., Delmore, J., Albuquerque, B., Reyes, A., Shi, Y., Moccia, R., Cokorinos, E., Peloquin, M., Monetti, M., Barricklow, J., Bollinger, E., Smith, B. K., Day, E. A., Nguyen, C., Geoghegan, K. F., Kreeger, J. M., Opsahl, A., Ward, J., Kalgutkar, A. S., Tess, D., Butler, L., Shirai, N., Osborne, T. F., Steinberg, G. R., Birnbaum, M. J., Cameron, K. O. and Miller, R. A. (2018) Activation of liver AMPK with PF-06409577 corrects NAFLD and lowers

cholesterol in rodent and primate preclinical models. *EBioMedicine* **31**, 122-132.
Falke, L. L., Gholizadeh, S., Goldschmeding, R., Kok, R. J. and Nguyen, T. Q. (2015) Diverse origins of the myofibroblast-implications for kidney fibrosis. *Nat. Rev. Nephrol.* **11**, 233-244.
Feng, L., Li, W., Chao, Y., Huan, Q., Lu, F., Yi, W., Jun, W., Binbin, C., Na, L. and Shougang, Z. (2021) Synergistic inhibition of renal fibrosis by nintedanib and gefitinib in a murine model of obstructive nephropathy. *Kidney Dis. (Basel)* **7**, 34-49.
Feng, Y., Huang, R., Guo, F., Liang, Y., Xiang, J., Lei, S., Shi, M., Li, L., Liu, J., Feng, Y., Ma, L. and Fu, P. (2018) Selective histone deacetylase 6 inhibitor 23BB alleviated rhabdomyolysis-induced acute kidney injury by regulating endoplasmic reticulum stress and apoptosis. *Front. Pharmacol.* **9**, 274.
Fragiadaki, M., Macleod, F. M. and Ong, A. C. M. (2020) The controversial role of fibrosis in autosomal dominant polycystic kidney disease. *Int. J. Mol. Sci.* **21**, 8936.
Fujiki, T., Ando, F., Murakami, K., Isobe, K., Mori, T., Susa, K., Nomura, N., Sohara, E., Rai, T. and Uchida, S. (2019) Tolvaptan activates the Nrf2/HO-1 antioxidant pathway through PERK phosphorylation. *Sci. Rep.* **9**, 9245.
Gai, Z., Chu, L., Xu, Z., Song, X., Sun, D. and Kullak-Ublick, G. A. (2017) Farnesoid X receptor activation protects the kidney from ischemia-reperfusion damage. *Sci. Rep.* **7**, 9815.
Garcia-Caballero, A., Rasmussen, J. E., Gaillard, E., Watson, M. J., Olsen, J. C., Donaldson, S. H., Stutts, M. J. and Tarran, R. (2009) SPLUNC1 regulates airway surface liquid volume by protecting ENaC from proteolytic cleavage. *Proc. Natl. Acad. Sci. U. S. A.* **106**, 11412-11417.
Gluais-Dagorn, P., Foretz, M., Steinberg, G. R., Batchuluun, B., Zawistowska-Deniziak, A., Lambooij, J. M., Guigas, B., Carling, D., Monternier, P. A., Moller, D. E., Bolze, S. and Hallakou-Bozec, S. (2022) Direct AMPK activation corrects NASH in rodents through metabolic effects and direct action on inflammation and fibrogenesis. *Hepatol. Commun.* **6**, 101-119.
Gluba-Sagr, A., Franczyk, B., Rysz-Gorzynska, M., Lawinski, J. and Rysz, J. (2023) The role of miRNA in renal fibrosis leading to chronic kidney disease. *Biomedicines* **11**, 2358.
Grantham, J. J., Cook, L. T., Torres, V. E., Bost, J. E., Chapman, A. B., Harris, P. C., Guay-Woodford, L. M. and Bae, K. T. (2008) Determinants of renal volume in autosomal-dominant polycystic kidney disease. *Kidney Int.* **73**, 108-116.
Hao, Y., Guo, F., Huang, Z., Feng, Y., Xia, Z., Liu, J., Li, L., Huang, R., Lin, L., Ma, L. and Fu, P. (2020) 2-Methylquinazoline derivative 23BB as a highly selective histone deacetylase 6 inhibitor alleviated cisplatin-induced acute kidney injury. *Biosci. Rep.* **40**, BSR20191538.
Holditch, S. J., Brown, C. N., Atwood, D. J., Lombardi, A. M., Nguyen, K. N., Toll, H. W., Hopp, K. and Edelstein, C. L. (2019) A study of sirolimus and mTOR kinase inhibitor in a hypomorphic Pkd1 mouse model of autosomal dominant polycystic kidney disease. *Am. J. Physiol. Renal Physiol.* **317**, F187-F196.
Hori, M. (2013) Tolvaptan for the treatment of hyponatremia and hypervolemia in patients with congestive heart failure. *Future Cardiol.* **9**, 163-176.
Hye Khan, M. A., Schmidt, J., Stavniichuk, A., Imig, J. D. and Merk, D. (2019) A dual farnesoid X receptor/soluble epoxide hydrolase modulator treats non-alcoholic steatohepatitis in mice. *Biochem. Pharmacol.* **166**, 212-221.
Imig, J. D., Merk, D. and Proschak, E. (2021) Multi-target drugs for kidney diseases. *Kidney360* **2**, 1645-1653.
Jamadar, A., Suma, S. M., Mathew, S., Fields, T. A., Wallace, D. P., Calvet, J. P. and Rao, R. (2021) The tyrosine-kinase inhibitor Nintedanib ameliorates autosomal-dominant polycystic kidney disease. *Cell Death Dis.* **12**, 947.
Karner, C., Wharton, K. A., Jr. and Carroll, T. J. (2006) Planar cell polarity and vertebrate organogenesis. *Semin. Cell Dev. Biol.* **17**, 194-203.
Kato, T., Hagiyama, M. and Ito, A. (2018) Renal ADAM10 and 17: their physiological and medical meanings. *Front. Cell Dev. Biol.* **6**, 153.
Ke, B., Chen, Y., Tu, W., Ye, T., Fang, X. and Yang, L. (2018) Inhibition of HDAC6 activity in kidney diseases: a new perspective. *Mol. Med.* **24**, 33.
Kim, J., Yoon, S. P., Toews, M. L., Imig, J. D., Hwang, S. H., Hammock, B. D. and Padanilam, B. J. (2015) Pharmacological inhibition of soluble epoxide hydrolase prevents renal interstitial fibrogenesis in obstructive nephropathy. *Am. J. Physiol. Renal Physiol.* **308**, F131-F139.
Kong, T., Xu, D., Yu, W., Takakura, A., Boucher, I., Tran, M., Kreidberg, J. A., Shah, J., Zhou, J. and Denker, B. M. (2009) G alpha 12 inhibits alpha2 beta1 integrin-mediated Madin-Darby canine kidney cell attachment and migration on collagen-I and blocks tubulogenesis. *Mol. Biol. Cell* **20**, 4596-4610.
Kunimoto, K., Bayly, R. D., Vladar, E. K., Vonderfecht, T., Gallagher, A. R. and Axelrod, J. D. (2017) Disruption of core planar cell polarity signaling regulates renal tubule morphogenesis but is not cystogenic. *Curr. Biol.* **27**, 3120-3131.e4.
Lanke, S. and Shoaf, S. E. (2019) Population pharmacokinetic analyses and model validation of tolvaptan in subjects with autosomal dominant polycystic kidney disease. *J. Clin. Pharmacol.* **59**, 763-770.
Le Corre, S., Viau, A., Burtin, M., El-Karoui, K., Cnops, Y., Terryn, S., Debaix, H., Berissi, S., Gubler, M. C., Devuyst, O. and Terzi, F. (2015) Cystic gene dosage influences kidney lesions after nephron reduction. *Nephron* **129**, 42-51.
Lee, M., Katerelos, M., Gleich, K., Galic, S., Kemp, B. E., Mount, P. F. and Power, D. A. (2018) Phosphorylation of acetyl-CoA carboxylase by AMPK reduces renal fibrosis and is essential for the anti-fibrotic effect of metformin. *J. Am. Soc. Nephrol.* **29**, 2326-2336.
Liang, D., Song, Z., Liang, W., Li, Y. and Liu, S. (2019) Metformin inhibits TGF-beta 1-induced MCP-1 expression through BAMBI-mediated suppression of MEK/ERK1/2 signalling. *Nephrology (Carlton)* **24**, 481-488.
Lichtenthaler, S. F., Lemberg, M. K. and Fluhrer, R. (2018) Proteolytic ectodomain shedding of membrane proteins in mammals-hardware, concepts, and recent developments. *EMBO J.* **37**, e99456.
Liu, F., Wang, L., Qi, H., Wang, J., Wang, Y., Jiang, W., Xu, L., Liu, N. and Zhuang, S. (2017) Nintedanib, a triple tyrosine kinase inhibitor, attenuates renal fibrosis in chronic kidney disease. *Clin. Sci. (Lond.)* **131**, 2125-2143.
Liu, F. and Zhuang, S. (2016) Role of receptor tyrosine kinase signaling in renal fibrosis. *Int. J. Mol. Sci.* **17**, 972.
Liu, W., Fan, L. X., Zhou, X., Sweeney, W. E., Jr., Avner, E. D. and Li, X. (2012) HDAC6 regulates epidermal growth factor receptor (EGFR) endocytic trafficking and degradation in renal epithelial cells. *PLoS One* **7**, e49418.
Liu, Y., Pejchinovski, M., Wang, X., Fu, X., Castelletti, D., Watnick, T. J., Arcaro, A., Siwy, J., Mullen, W., Mischak, H. and Serra, A. L. (2018) Dual mTOR/PI3K inhibition limits PI3K-dependent pathways activated upon mTOR inhibition in autosomal dominant polycystic kidney disease. *Sci. Rep.* **8**, 5584.
Lorenz, M. C. and Heitman, J. (1995) TOR mutations confer rapamycin resistance by preventing interaction with FKBP12-rapamycin. *J. Biol. Chem.* **270**, 27531-27537.
Lorenzo Pisarello, M., Masyuk, T. V., Gradilone, S. A., Masyuk, A. I., Ding, J. F., Lee, P. Y. and LaRusso, N. F. (2018) Combination of a histone deacetylase 6 inhibitor and a somatostatin receptor agonist synergistically reduces hepatorenal cystogenesis in an animal model of polycystic liver disease. *Am. J. Pathol.* **188**, 981-994.
Luyten, A., Su, X., Gondela, S., Chen, Y., Rompani, S., Takakura, A. and Zhou, J. (2010) Aberrant regulation of planar cell polarity in polycystic kidney disease. *J. Am. Soc. Nephrol.* **21**, 1521-1532.
Mangolini, A., de Stephanis, L. and Aguiari, G. (2016) Role of calcium in polycystic kidney disease: from signaling to pathology. *World J. Nephrol.* **5**, 76-83.
Maretzky, T., Reiss, K., Ludwig, A., Buchholz, J., Scholz, F., Proksch, E., de Strooper, B., Hartmann, D. and Saftig, P. (2005) ADAM10 mediates E-cadherin shedding and regulates epithelial cell-cell adhesion, migration, and beta-catenin translocation. *Proc. Natl. Acad. Sci. U. S. A.* **102**, 9182-9187.
McCarty, M. F., Barroso-Aranda, J. and Contreras, F. (2009) Activation of AMP-activated kinase as a strategy for managing autosomal dominant polycystic kidney disease. *Med. Hypotheses* **73**, 1008-1010.

Mekahli, D., Decuypere, J. P., Sammels, E., Welkenhuyzen, K., Schoeber, J., Audrezet, M. P., Corvelyn, A., Dechenes, G., Ong, A. C., Wilmer, M. J., van den Heuvel, L., Bultynck, G., Parys, J. B., Missiaen, L., Levtchenko, E. and De Smedt, H. (2014) Polycystin-1 but not polycystin-2 deficiency causes upregulation of the mTOR pathway and can be synergistically targeted with rapamycin and metformin. *Pflugers Arch.* **466**, 1591-1604.

Meyer-Schwesinger, C., Seipold, L. and Saftig, P. (2022) Ectodomain shedding by ADAM proteases as a central regulator in kidney physiology and disease. *Biochim. Biophys. Acta Mol. Cell Res.* **1869**, 119165.

Milazzo, G., Mercatelli, D., Di Muzio, G., Triboli, L., De Rosa, P., Perini, G. and Giorgi, F. M. (2020) Histone deacetylases (HDACs): evolution, specificity, role in transcriptional complexes, and pharmacological actionability. *Genes (Basel)* **11**, 556.

Nakamura, M., Sunagawa, O. and Kinugawa, K. (2018) Tolvaptan improves prognosis in responders with acute decompensated heart failure by reducing the dose of loop diuretics. *Int. Heart J.* **59**, 87-93.

Nigro, E. A., Castelli, M. and Boletta, A. (2015) Role of the polycystins in cell migration, polarity, and tissue morphogenesis. *Cells* **4**, 687-705.

Norman, J. (2011) Fibrosis and progression of autosomal dominant polycystic kidney disease (ADPKD). *Biochim. Biophys.* **1812**, 1327-1336.

Oh, Y. K., Park, H. C., Ryu, H., Kim, Y. C. and Oh, K. H. (2021) Clinical and genetic characteristics of Korean autosomal dominant polycystic kidney disease patients. *Korean J. Intern. Med.* **36**, 767-779.

Papakrivopoulou, E., Jafree, D. J., Dean, C. H. and Long, D. A. (2021) The biological significance and implications of planar cell polarity for nephrology. *Front. Physiol.* **12**, 599529.

Pastor-Soler, N. M., Li, H., Pham, J., Rivera, D., Ho, P. Y., Mancino, V., Saitta, B. and Hallows, K. R. (2022) Metformin improves relevant disease parameters in an autosomal dominant polycystic kidney disease mouse model. *Am. J. Physiol. Renal Physiol.* **322**, F27-F41.

Pathomthongtaweechai, N., Soodvilai, S., Chatsudthipong, V. and Muanprasat, C. (2014) Pranlukast inhibits renal epithelial cyst progression via activation of AMP-activated protein kinase. *Eur. J. Pharmacol.* **724**, 67-76.

Perico, N., Antiga, L., Caroli, A., Ruggenenti, P., Fasolini, G., Cafaro, M., Ondei, P., Rubis, N., Diadei, O., Gherardi, G., Prandini, S., Panozo, A., Bravo, R. F., Carminati, S., De Leon, F. R., Gaspari, F., Cortinovis, M., Motterlini, N., Ene-Iordache, B., Remuzzi, A. and Remuzzi, G. (2010) Sirolimus therapy to halt the progression of ADPKD. *J. Am. Soc. Nephrol.* **21**, 1031-1040.

Pulya, S., Amin, S. A., Adhikari, N., Biswas, S., Jha, T. and Ghosh, B. (2021) HDAC6 as privileged target in drug discovery: a perspective. *Pharmacol. Res.* **163**, 105274.

Raina, R., Chakraborty, R., DeCoy, M. E. and Kline, T. (2021) Autosomal-dominant polycystic kidney disease: tolvaptan use in adolescents and young adults with rapid progression. *Pediatr. Res.* **89**, 894-899.

Riga, A., Castiglioni, V. G. and Boxem, M. (2020) New insights into apical-basal polarization in epithelia. *Curr. Opin. Cell Biol.* **62**, 1-8.

Rockey, D. C., Bell, P. D. and Hill, J. A. (2015) Fibrosis--a common pathway to organ injury and failure. *N. Engl. J. Med.* **372**, 1138-1149.

Roitbak, T., Ward, C. J., Harris, P. C., Bacallao, R., Ness, S. A. and Wandinger-Ness, A. (2004) A polycystin-1 multiprotein complex is disrupted in polycystic kidney disease cells. *Mol. Biol. Cell* **15**, 1334-1346.

Sabers, C. J., Martin, M. M., Brunn, G. J., Williams, J. M., Dumont, F. J., Wiederrecht, G. and Abraham, R. T. (1995) Isolation of a protein target of the FKBP12-rapamycin complex in mammalian cells. *J. Biol. Chem.* **270**, 815-822.

Satriano, J., Sharma, K., Blantz, R. C. and Deng, A. (2013) Induction of AMPK activity corrects early pathophysiological alterations in the subtotal nephrectomy model of chronic kidney disease. *Am. J. Physiol. Renal Physiol.* **305**, F727-F733.

Schonauer, R., Baatz, S., Nemitz-Kliemchen, M., Frank, V., Petzold, F., Sewerin, S., Popp, B., Munch, J., Neuber, S., Bergmann, C. and Halbritter, J. (2020) Matching clinical and genetic diagnoses in autosomal dominant polycystic kidney disease reveals novel phenocopies and potential candidate genes. *Genet. Med.* **22**, 1374-1383.

Seliger, S. L., Abebe, K. Z., Hallows, K. R., Miskulin, D. C., Perrone, R. D., Watnick, T. and Bae, K. T. (2018) A randomized clinical trial of metformin to treat autosomal dominant polycystic kidney disease. *Am. J. Nephrol.* **47**, 352-360.

Serra, A. L., Poster, D., Kistler, A. D., Krauer, F., Raina, S., Young, J., Rentsch, K. M., Spanaus, K. S., Senn, O., Kristanto, P., Scheffel, H., Weishaupt, D. and Wuthrich, R. P. (2010) Sirolimus and kidney growth in autosomal dominant polycystic kidney disease. *N. Engl. J. Med.* **363**, 820-829.

Seto, E. and Yoshida, M. (2014) Erasers of histone acetylation: the histone deacetylase enzymes. *Cold Spring Harb. Perspect. Biol.* **6**, a018713.

Sharma, M., Reif, G. A. and Wallace, D. P. (2019) *In vitro* cyst formation of ADPKD cells. *Methods Cell Biol.* **153**, 93-111.

Sharma, S. and Smyth, B. (2021) From proteinuria to fibrosis: an update on pathophysiology and treatment options. *Kidney Blood Press. Res.* **46**, 411-420.

Shillingford, J. M., Leamon, C. P., Vlahov, I. R. and Weimbs, T. (2012) Folate-conjugated rapamycin slows progression of polycystic kidney disease. *J. Am. Soc. Nephrol.* **23**, 1674-1681.

Shillingford, J. M., Murcia, N. S., Larson, C. H., Low, S. H., Hedgepeth, R., Brown, N., Flask, C. A., Novick, A. C., Goldfarb, D. A., Kramer-Zucker, A., Walz, G., Piontek, K. B., Germino, G. G. and Weimbs, T. (2006) The mTOR pathway is regulated by polycystin-1, and its inhibition reverses renal cystogenesis in polycystic kidney disease. *Proc. Natl. Acad. Sci. U. S. A.* **103**, 5466-5471.

Shillingford, J. M., Piontek, K. B., Germino, G. G. and Weimbs, T. (2010) Rapamycin ameliorates PKD resulting from conditional inactivation of Pkd1. *J. Am. Soc. Nephrol.* **21**, 489-497.

Shiu, J. S., Hsieh, M. J., Chiou, H. L., Wang, H. L., Yeh, C. B., Yang, S. F. and Chou, Y. E. (2018) Impact of ADAM10 gene polymorphisms on hepatocellular carcinoma development and clinical characteristics. *Int. J. Med. Sci.* **15**, 1334-1340.

Shum, M. G., Shao, Q., Lajoie, P. and Laird, D. W. (2019) Destination and consequences of Panx1 and mutant expression in polarized MDCK cells. *Exp. Cell Res.* **381**, 235-247.

Silberberg, M., Charron, A. J., Bacallao, R. and Wandinger-Ness, A. (2005) Mispolarization of desmosomal proteins and altered intercellular adhesion in autosomal dominant polycystic kidney disease. *Am. J. Physiol. Renal Physiol.* **288**, F1153-F1163.

Silverman, W., Locovei, S. and Dahl, G. (2008) Probenecid, a gout remedy, inhibits pannexin 1 channels. *Am. J. Physiol. Cell Physiol.* **295**, C761-C767.

Solanas, G., Cortina, C., Sevillano, M. and Batlle, E. (2011) Cleavage of E-cadherin by ADAM10 mediates epithelial cell sorting downstream of EphB signalling. *Nat. Cell Biol.* **13**, 1100-1107.

Song, A., Zhang, C. and Meng, X. (2021) Mechanism and application of metformin in kidney diseases: an update. *Biomed. Pharmacother.* **138**, 111454.

Staruschenko, A., Pochynyuk, O., Vandewalle, A., Bugaj, V. and Stockand, J. D. (2007) Acute regulation of the epithelial Na+ channel by phosphatidylinositide 3-OH kinase signaling in native collecting duct principal cells. *J. Am. Soc. Nephrol.* **18**, 1652-1661.

Stavniichuk, A., Savchuk, O., Khan, A. H., Jankiewicz, W. K., Imig, J. D. and Merk, D. (2020) The effect of compound DM509 on kidney fibrosis in the conditions of the experimental model. *Visnyk Kyivskoho Natsionalnoho Universytetu Imeni Tarasa Shevchenka Biolohiia* **80**, 10-15.

Su, L., Yuan, H., Zhang, H., Wang, R., Fu, K., Yin, L., Ren, Y., Liu, H., Fang, Q., Wang, J. and Guo, D. (2022) PF-06409577 inhibits renal cyst progression by concurrently inhibiting the mTOR pathway and CFTR channel activity. *FEBS Open Bio* **12**, 1761-1770.

Sudarikova, A. V., Vasileva, V. Y., Sultanova, R. F. and Ilatovskaya, D. V. (2021) Recent advances in understanding ion transport mechanisms in polycystic kidney disease. *Clin. Sci. (Lond.)* **135**, 2521-2540.

Takiar, V., Nishio, S., Seo-Mayer, P., King, J. D., Jr., Li, H., Zhang, L., Karihaloo, A., Hallows, K. R., Somlo, S. and Caplan, M. J. (2011) Activating AMP-activated protein kinase (AMPK) slows renal cysto-

genesis. *Proc. Natl. Acad. Sci. U. S. A.* **108**, 2462-2467.
Tamma, G., Di Mise, A., Ranieri, M., Geller, A., Tamma, R., Zallone, A. and Valenti, G. (2017) The V2 receptor antagonist tolvaptan raises cytosolic calcium and prevents AQP2 trafficking and function: an *in vitro* and *in vivo* assessment. *J. Cell. Mol. Med.* **21**, 1767-1780.
Tan, Y. C., Blumenfeld, J. and Rennert, H. (2011) Autosomal dominant polycystic kidney disease: genetics, mutations and microRNAs. *Biochim. Biophys. Acta* **1812**, 1202-1212.
Torres, V. E., Chapman, A. B., Devuyst, O., Gansevoort, R. T., Grantham, J. J., Higashihara, E., Perrone, R. D., Krasa, H. B., Ouyang, J. and Czerwiec, F. S.; TEMPO 3:4 Trial Investigators (2012) Tolvaptan in patients with autosomal dominant polycystic kidney disease. *N. Engl. J. Med.* **367**, 2407-2418.
Torres, V. E., Devuyst, O., Chapman, A. B., Gansevoort, R. T., Perrone, R. D., Ouyang, J., Blais, J. D., Czerwiec, F. S. and Sergeyeva, O.; REPRISE Trial Investigators (2017) Rationale and design of a clinical trial investigating tolvaptan safety and efficacy in autosomal dominant polycystic kidney disease. *Am. J. Nephrol.* **45**, 257-266.
Torres, V. E. and Harris, P. C. (2014) Strategies targeting cAMP signaling in the treatment of polycystic kidney disease. *J. Am. Soc. Nephrol.* **25**, 18-32.
Tran Nguyen Truc, L., Matsuda, S., Takenouchi, A., Tran Thuy Huong, Q., Kotani, Y., Miyazaki, T., Kanda, H., Yoshizawa, K. and Tsukaguchi, H. (2023) Mechanism of cystogenesis by Cd79a-driven, conditional mTOR activation in developing mouse nephrons. *Sci. Rep.* **13**, 508.
Valenzuela-Fernandez, A., Cabrero, J. R., Serrador, J. M. and Sanchez-Madrid, F. (2008) HDAC6: a key regulator of cytoskeleton, cell migration and cell-cell interactions. *Trends Cell Biol.* **18**, 291-297.
Vinciguerra, M., Mordasini, D., Vandewalle, A. and Feraille, E. (2005) Hormonal and nonhormonal mechanisms of regulation of the NA,K-pump in collecting duct principal cells. *Semin. Nephrol.* **25**, 312-321.
Wahl, P. R., Serra, A. L., Le Hir, M., Molle, K. D., Hall, M. N. and Wuthrich, R. P. (2006) Inhibition of mTOR with sirolimus slows disease progression in Han:SPRD rats with autosomal dominant polycystic kidney disease (ADPKD). *Nephrol. Dial. Transplant.* **21**, 598-604.
Walz, G., Budde, K., Mannaa, M., Nurnberger, J., Wanner, C., Sommerer, C., Kunzendorf, U., Banas, B., Horl, W. H., Obermuller, N., Arns, W., Pavenstadt, H., Gaedeke, J., Buchert, M., May, C., Gschaidmeier, H., Kramer, S. and Eckardt, K. U. (2010) Everolimus in patients with autosomal dominant polycystic kidney disease. *N. Engl. J. Med.* **363**, 830-840.
Wang, J. N. and Cao, X. J. (2023) Targeting ADAM10 in renal diseases. *Curr. Mol. Med.* **23**, 1037-1045.
Wang, X. X., Jiang, T., Shen, Y., Caldas, Y., Miyazaki-Anzai, S., Santamaria, H., Urbanek, C., Solis, N., Scherzer, P., Lewis, L., Gonzalez, F. J., Adorini, L., Pruzanski, M., Kopp, J. B., Verlander, J. W. and Levi, M. (2010) Diabetic nephropathy is accelerated by farnesoid X receptor deficiency and inhibited by farnesoid X receptor activation in a type 1 diabetes model. *Diabetes* **59**, 2916-2927.
Watkins, P. B., Lewis, J. H., Kaplowitz, N., Alpers, D. H., Blais, J. D., Smotzer, D. M., Krasa, H., Ouyang, J., Torres, V. E., Czerwiec, F. S. and Zimmer, C. A. (2015) Clinical pattern of tolvaptan-associated liver injury in subjects with autosomal dominant polycystic kidney disease: analysis of clinical trials database. *Drug Saf.* **38**, 1103-1113.
Wei, Z. Y., Qu, H. L., Dai, Y. J., Wang, Q., Ling, Z. M., Su, W. F., Zhao, Y. Y., Shen, W. X. and Chen, G. (2021) Pannexin 1, a large-pore membrane channel, contributes to hypotonicity-induced ATP release in Schwann cells. *Neural Regen. Res.* **16**, 899-904.
Whyte-Fagundes, P. and Zoidl, G. (2018) Mechanisms of pannexin1 channel gating and regulation. *Biochim. Biophys. Acta Biomembr.* **1860**, 65-71.
Wicki-Stordeur, L. E. and Swayne, L. A. (2013) Panx1 regulates neural stem and progenitor cell behaviours associated with cytoskeletal dynamics and interacts with multiple cytoskeletal elements. *Cell Commun. Signal.* **11**, 62.
Wilson, P. D. (2011) Apico-basal polarity in polycystic kidney disease epithelia. *Biochim. Biophys. Acta* **1812**, 1239-1248.
Wu, Y., Xu, J. X., El-Jouni, W., Lu, T., Li, S., Wang, Q., Tran, M., Yu, W., Wu, M., Barrera, I. E., Bonventre, J. V., Zhou, J., Denker, B. M. and Kong, T. (2016) Galpha12 is required for renal cystogenesis induced by Pkd1 inactivation. *J. Cell Sci.* **129**, 3675-3684.
Xiao, W., Pinilla-Baquero, A., Faulkner, J., Song, X., Prabhakar, P., Qiu, H., Moremen, K. W., Ludwig, A., Dempsey, P. J., Azadi, P. and Wang, L. (2022) Robo4 is constitutively shed by ADAMs from endothelial cells and the shed Robo4 functions to inhibit Slit3-induced angiogenesis. *Sci. Rep.* **12**, 4352.
Xu, D., Lv, J., He, L., Fu, L., Hu, R., Cao, Y. and Mei, C. (2018a) Scribble influences cyst formation in autosomal-dominant polycystic kidney disease by regulating Hippo signaling pathway. *FASEB J.* **32**, 4394-4407.
Xu, J. X., Lu, T. S., Li, S., Wu, Y., Ding, L., Denker, B. M., Bonventre, J. V. and Kong, T. (2015) Polycystin-1 and Galpha12 regulate the cleavage of E-cadherin in kidney epithelial cells. *Physiol. Genomics* **47**, 24-32.
Xu, X., Wicki-Stordeur, L. E., Sanchez-Arias, J. C., Liu, M., Weaver, M. S., Choi, C. S. W. and Swayne, L. A. (2018b) Probenecid disrupts a novel pannexin 1-collapsin response mediator protein 2 interaction and increases microtubule stability. *Front. Cell. Neurosci.* **12**, 124.
Yanda, M. K., Liu, Q. and Cebotaru, L. (2017a) An inhibitor of histone deacetylase 6 activity, ACY-1215, reduces cAMP and cyst growth in polycystic kidney disease. *Am. J. Physiol. Renal Physiol.* **313**, F997-F1004.
Yanda, M. K., Liu, Q., Cebotaru, V., Guggino, W. B. and Cebotaru, L. (2017b) Histone deacetylase 6 inhibition reduces cysts by decreasing cAMP and Ca(2+) in knock-out mouse models of polycystic kidney disease. *J. Biol. Chem.* **292**, 17897-17908.
Yu, W., Ritchie, B. J., Su, X., Zhou, J., Meigs, T. E. and Denker, B. M. (2011) Identification of polycystin-1 and Galpha12 binding regions necessary for regulation of apoptosis. *Cell. Signal.* **23**, 213-221.
Yuan, Q., Tan, R. J. and Liu, Y. (2019) Myofibroblast in kidney fibrosis: origin, activation, and regulation. *Adv. Exp. Med. Biol.* **1165**, 253-283.
Yuan, Q., Yu, H., Chen, J., Song, X. and Sun, L. (2020) ADAM10 promotes cell growth, migration, and invasion in osteosarcoma via regulating E-cadherin/beta-catenin signaling pathway and is regulated by miR-122-5p. *Cancer Cell Int.* **20**, 99.
Zafar, I., Ravichandran, K., Belibi, F. A., Doctor, R. B. and Edelstein, C. L. (2010) Sirolimus attenuates disease progression in an orthologous mouse model of human autosomal dominant polycystic kidney disease. *Kidney Int.* **78**, 754-761.
Zhang, Y., Daniel, E. A., Metcalf, J., Dai, Y., Reif, G. A. and Wallace, D. P. (2022) CaMK4 overexpression in polycystic kidney disease promotes mTOR-mediated cell proliferation. *J. Mol. Cell Biol.* **14**, mjac050.
Zheng, W., Song, J., Zhang, Y., Chen, S., Ruan, H. and Fan, C. (2017) Metformin prevents peritendinous fibrosis by inhibiting transforming growth factor-beta signaling. *Oncotarget* **8**, 101784-101794.
Zschiedrich, S., Budde, K. and Walz, G. (2015) Effect of everolimus on polycystic liver volume in autosomal dominant polycystic kidney disease. *Clin. Exp. Nephrol.* **19**, 757-758.