Original
ISSN 2413-0516

Unveiling the Mediating Role of the REJ Region: PC1 and Extracellular Matrix Dynamics in ADPKD

Aljazi Abdullah Alrashidi[1], Hala Salim Sonbol[2*]

[1]Department of Chemistry, Faculty of Sciences, University of Hail, Hail 2440, Saudi Arabia.
[2]Department of Biochemistry, Faculty of Sciences, King Abdulaziz University, Jeddah 80200, Saudi Arabia.
*Correspondence to: Hala Salim Sonbol (E-mail: hsonbol@kau.edu.sa)
(Submitted: 24 November 2023 – Revised version received: 10 December 2023 – Accepted: 15 January 2024 – Published online: 26 February 2024)

Abstract
Objective: This study aimed to investigate the interactions of PC1 with extracellular matrix proteins (ECM) to gain insights into its role in ADPKD onset. This exploration objective is to unravel the significance of these interactions in both the healthy development of kidneys and the underlying mechanisms of ADPKD, potentially identifying therapeutic targets.
Methods: We cloned and expressed the receptor of the egg jelly (REJ) domain as a maltose-binding protein-fusion protein (PET21-MBP(TEV)-REJ). Subsequently, we utilized this construct in a pulldown assay involving HEK 293 cells. In vitro, pull-down assays were conducted to evaluate the binding of the REJ fusion to various ECM components.
Results: The REJ fusion protein effectively binds to vitronectin, Fibulin-1, and actin filament-associated protein (AFAP) 1. These findings indicate that the REJ region acts as a mediator for the interaction between polycystin-1 and the ECM, shedding light on the functional role of polycystin-1 in both cell-matrix and cell-cell interactions.
Conclusion: The detailed characterization of REJ-ECM interactions offers a valuable foundation for future research aimed at systematically studying the effects of disease-causing mutations within the REJ module of human PC-1.
Keywords: Polycystin-1, receptor of the egg jelly (REJ) domain, pull-down technique, MALDI-TOF MS, extracellular matrix (ECM)

Introduction

Polycystic kidney disease gene 1 (PKD1), accounts for approximately 85% of instances.[1] Mutations in the PKD1 genes are responsible for the formation of cysts in ADPKD.[2] The expression of the PKD1 gene is prevalent across diverse cell types, encompassing renal epithelial cells, vascular smooth muscle cells, and endothelial cells.[1] The structural and functional attributes of PC1 include various domains located in both the N-terminal extracellular region and the membrane-associated segment. Its functions as a transmembrane entity are crucial for promoting the proper development of kidneys, preventing cyst formation[3] and indicating its participation in interactions between cells and/or the extracellular matrix.

PC1 protein domains include GPCR proteolysis site (GPS), GPCR autoproteolysis inducing, transmembrane segments, polycystin/lipoxygenase/α toxin, leucine-rich repeats, C-type lectin, cell-wall integrity and stress-response component, and fibronectin-like regions.[4] Among the complex structures of PC1, the receptor of the egg jelly (REJ) domain[5] stands out and may hold the potential to unlock the mysteries of cellular interactions and disease onset.

The story unfolds as we zoom into the REJ domain. Questions arise about the REJ domain's role in cellular dynamics, particularly in the context of the extracellular matrix (ECM), a dynamic network of proteins that shapes the microenvironment surrounding cells.[6]

REJ constitutes a vital component of the PC-1 ectodomain, spanning roughly 1000 amino acids. Within this module, several missense mutations have been identified, attributing to diverse diseases. Nevertheless, the structure and function of this particular region remain poorly understood.[7] The REJ proteins play a role in facilitating the influx of calcium ions (Ca^{2+}). There are approximately 230 mutations identified, encompassing 80 missense mutations within the REJ region. Among these missense mutations, 65 are causative factors for the disease. The encoding of the REJ region occurs within PKD-1 exons 15–23.[8]

Our research team aimed to investigate the REJ protein due to its strategically located domains within the extracellular region of the PC-1 protein, facilitating interactions with ECM proteins.[9] This selection serves as the foundation for delving into the functionality of REJ in ADPKD. Subsequently, we cloned the REJ protein and utilized the resulting fusion protein in a pulldown assay to examine its interaction with ECM proteins. The PC-1 protein possesses a single REJ domain, representing the most extensive domain in the N-terminal segment of the PC-1 protein.[10] Positioned in proximity to the GPS domain at the GPSG-protein-coupled proteolytic site, the PC-1 protein undergoes cleavage, with this process encompassing the entirety of the REJ region.[11]

Polycystin 1's interesting connection with the ECM and the protein-protein interactions observed by several researchers underscore its functional significance. The ECM, comprising a complex assembly of proteins such as collagen, fibronectin, and integrins, plays a pivotal role in cellular adhesion, signaling, and overall tissue integrity.[12] This prompts our research focus: to unravel the interactions between the REJ domain, situated on the extracellular side of PC1, and various components within the ECM.

There is a connection between the signaling pathway of polycystin and ECM in various organs, including the kidneys, even though ADPKD is characterized by progressive cystogenesis.[13] The REJ domain's mediation between PC1 and the ECM emerges as a central theme in our exploration.

Cells orchestrate a sophisticated network to ensure optimal tissue functionality, engaging in dynamic interactions with a diverse array of proteins within the ECM. This intricate interplay facilitates the exchange of signals among cells,[14] fostering a harmonious environment for tissue integrity and function. The basement membrane is a specialized ECM component. The binding of ECM proteins to receptors on the basement

membrane (BM) is orchestrated through integrin and hemidesmosomes strategically positioned on the cell surface.[12] This relationship ensures structural integrity and contributes significantly to the dynamic equilibrium that governs cellular behaviour and tissue architecture. Examples of proteins that reside in the BM are laminins, type IV collagen (collagen IV), vitronectin, nidogens and heparan sulfate proteoglycans (HSPGs). Vitronectin (Vtn) was identified as one of the significantly upregulated ECM proteins in the tissue scaffold of a fibrotic kidney.[14] This cooperative assembly forms a sheet that enables cells to adhere and carry out essential physiological functions. Also, the BM plays a vital role in overseeing various aspects of cell biology, including cell polarity, proliferation, apoptosis, survival, migration, differentiation, and signaling.[15]

Recent study has made a notable contribution to the understanding of the molecular mechanisms underlying inherited ADPKD by successfully expressing a REJ fusion protein in vitro., resulting in the production of a 13 kDa REJ protein that corresponds to the human REJ gene in exon 15. This novel research provides valuable insights into the molecular mechanisms involved in ADPKD and represents a significant advancement in this field.[16] Based on the details presented, the main aim of this investigation is to demonstrate the expression of a soluble REJ fusion protein. The focus is on investigating how this protein interacts with ECM proteins using a pulldown assay. This specific step is crucial for gaining insights into the protein's function and discerning pertinent biological pathways.

In an about-to-be-published study (in press), a Pull-down assay was performed and the results were analysed with MALDI-TOF MS, it was shown that there is an interaction between REJ fusion protein and ECM proteins including collagen, integrin, and fibronectin. And this study is a continuation of the analysis, we found that there is also interaction with vitronectin as well. Our results demonstrated a powerful experimental approach to further studying the function, and REJ-ECM interactions of proteins and should pave the way to systematically characterizing the effects of disease-causing mutations in the REJ module of human PC-1.

Materials and Methods

Materials

Most of the chemicals were purchased from Sigma Aldrich (UK). Human embryonic kidney (HEK 293) was kindly provided by Dr. Baghdadi, Research Centre, King Faisal Specialist Center, Jeddah, Kingdom of Saudi Arabia.

Methods

Cloning and expression of the GPS domain as soluble protein

Cloning and expression of the MBP-REJ domain and fusion protein expression and purification with PureCube His-Affinity agarose were made according to manufacturer instructions.

Human embryo kidney epithelial cell culture and proliferation assay

HEK 293 cells were cultured as monolayers in tissue culture flasks without coating. Dulbecco's Modified Eagle medium (DMEM) was employed for cell cultivation. The medium was enriched with 10% fetal calf serum (FCS) and 1% penicillin-streptomycin solution. The cells were nurtured in a regulated setting at 37°C with 5% carbon dioxide (CO_2) and 95% air. Refreshment of the culture medium occurred every 2–3 days or as deemed necessary. Regular cell line subculturing occurred in an incubation environment set at 37°C with 5% CO_2. Once reaching approximately 70% confluency, visual inspection under a phase-contrast microscope (Nikon, USA) was performed to confirm optimal density and absence of contamination. Following this, trypsin was employed to detach the cells, which were then centrifuged at 1500 rpm for 10 minutes, and the resulting cell pellets were preserved for cell counting using a hemocytometer. The cells were then seeded at a density of 5.0×10^4 cells/µL and allowed to adhere overnight.

Interaction of MBP-REJ fusion protein with extracellular matrix protein

Pull-down assay

A pull-down assay to study protein-ECM interaction was performed according to manufacturer instructions (Pull-Down Kit, Thermos Fisher, UAS). HEK 293 cells were used in the pulldown assay. HEK 293 cells were grown as monolayers to confluency (1×10^7) on an uncoated tissue culture flask using a DMEME medium. The cells were washed 2X with ice-cold PBS. One millilitre of RIPA lysis buffer (150 mM Nacl, 1% Triton X100, 0.5% of Sodium Deoxycholate, 0.1% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), 50 mM Tris, pH 8, Tris HCL, 20 mM Tris-HCL, pH 7.5), phenylmethylsulphonyl fluoride Protease inhibitors PMFS (one tablet/10 ml RIPA buffer) was added (1 ml) to the cells and left on ice for 5 min. The cells were then scraped with a cell scraper transferred to a sterile 0.5 ml Eppendorf and kept on ice for 1 h. During this period, the cell lysate was pipetted using small tips every 10 min to physically break down the cells. The disrupted cells were centrifuged down in a bench centrifuge at full speed (13000 rpm) for 30 min at 4°C. The supernatant was either used immediately or stored at –70°C until further use.

During the pulldown assay, a 300 µl aliquot of purified MBP-REJ fusion protein was incubated with a 50% slurry of HisPur Cobalt Resin (100 µl) for one hour at 4°C, employing gentle end-over mixing. Subsequently, the mixture underwent centrifugation in a bench centrifuge, operating at 3341 rpm for 30 seconds to one minute. The resulting tube, designated as the "bait flow-through," underwent analysis by subjecting it to a 15% reduced SDS-PAGE. Next, the HisPur Cobalt Resin was washed five times using a washing buffer comprised of a 1:1 mixture of TBS and pull-down lysis buffer. For the preparation of the prey fraction, 2.5 ml of Pierce lysis buffer (w/v) was added to the lysed cells. The tube underwent thorough mixing by repeated inversion both upward and downward. Subsequently, the mixture was placed on ice for approximately 30 minutes with periodic inversions. After this incubation, the mixture was centrifuged at 3341 rpm for 5 minutes to achieve clarification of the crude mammalian lysate. The supernatant obtained was moved to a new tube and kept chilled. Simultaneously, 10 mM Imidazole was added to the residual cell pellet. This specific tube was labelled as the "mammalian prey lysate" and, once more, stored on ice. In the experimental process,

800 µl of the target protein was incubated at 4°C for 2 hours on a rotating platform with gentle rocking. After the incubation period, the samples were subjected to centrifugation at 3341 rpm for 1 minute, and the resulting tube was identified as the "prey flow-through" and kept on ice. Afterwards, the supernatants were gathered for examination through a 15% SDS-PAGE with a reduced gel percentage. The beads capturing the proteins were washed with 1 ml of lysis buffer, and kept at an ice-cold temperature, the same buffer used for cell lysis initially. Next, the tubes were centrifuged at their maximum speed of 1500 rpm for 1 minute. The washing process was reiterated 3 to 4 times. Afterwards, a volume of 250 µl Elution Buffer was added to the microfuge tube, followed by a 5-minute incubation with gentle rocking on a rotating platform. The sample was then centrifuged at 3341 rpm for 1 minute. This specific tube was identified as "Elution 1" and placed on ice. The elution steps were replicated five times. To enhance the protein pull-down experiment, adjustments were made in the concentration of the MBP-REJ fusion protein, the bead quantity, incubation durations, the number of washes, and incubation periods. Various samples, such as untreated His-Pur Cobalt Resin, purified bait, cell lysate, bait flow-through, prey flow-through, and bait-prey elution, underwent electrophoresis using a 15% reduced SDS-PAGE. Characterization of protein-protein interaction by mass spectrometry.

Proteins captured with MBP-REJ, previously subjected to SDS-PAGE for characterization, were identified using Matrix-Assisted Laser Desorption Ionization-Time of Flight-Mass Spectrometry (MALDI-TOF MS) with the Ultra-flex III instrument manufactured by Bruker Corporation, USA. The relevant protein bands, stained appropriately, were carefully excised from the gel and preserved in 0.1% acetic acid. Subsequently, these proteins underwent analysis through MALDI-TOF MS, followed by a database search using Swiss Prot.[17] The collaborative analysis was conducted in partnership with King Abdullah University of Science and Technology (KAUST) in Jeddah, Saudi Arabia.

Results

Interaction of MBP-REJ Fusion Protein with ECM Proteins

The HEK 293 cells used in the pull-down assay are presented in Figure 1.

The pull-down experiment was analysed via 15% SDS-PAGE. In Figure 2, lane 1 shows the HEK 293 cell lysate, while lanes 2, 4, 7, 8 and 9 serve as negative controls with untreated resin. In lanes 3 and 6, the bait protein MBP-REJ fusion is visible as a narrow band that has a size of 53 kDa. Lanes 5 and 10 show the prey proteins that interacted with MBP-REJ, and a protein band of approximately 100–127 kDa was detected in those lanes.

Characterization of Protein-Protein Interaction by Mass Spectrometry

MALDI-TOF MS was employed to analyze the bands in lane 10 of Figure 2. The proteins identified in the analysis of the protein bands in lane 10 were recorded in Table 1. The UniProt database was then utilized to determine the protein masses associated with the MBP-REJ fusion protein. Several

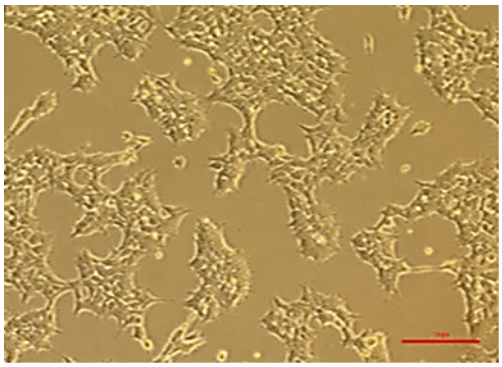

Fig. 1 **The HEK293 monolayer cells. Scale bar, 100 µm.**

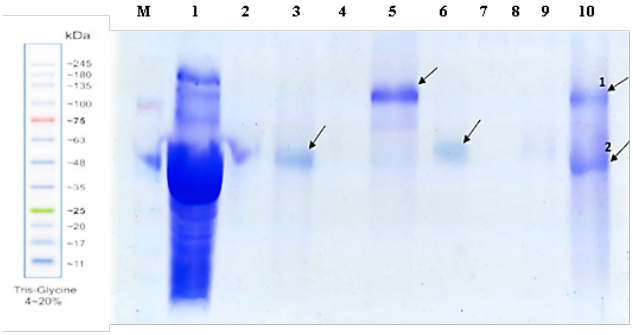

Fig. 2 **This figure represents the pull-down experiment. The electrophoresis was carried out in 15% SDS-PAGE. Lane 1, HEK 293-cell lysate. Lanes 2, 4, 7, 8 and 9 are untreated resins as a control. Lanes 3 and 6 are the MBP-REJ fusion protein (bait) (~53 kDa as the arrow indicated). Lane 5 was prey flow through. Lane 10 is the bait-prey elution that has two protein bands prey (~ 100-127 kDa as arrow 1 indicated), and MBP-REJ fusion protein (bait) (~53 kDa as arrow 2 indicated). Lane M is the molecular weight protein marker.**

Table 1. **ECM proteins that interacted with MBP-REJ fusion protein detected by MALDI-TOF MS using a pull-down assay**

Protein name	UNI port accession #	MW (kDa)	PI
Vitronectin	P04004_HUMAN	5.4	5.5
Fibulin-1	P23142_HUMAN	7.7	5.0
AFAP	A0A087X177_HUMAN	9.7	9.9

candidates' proteins were identified in band lane 10, (Figure 2). Three proteins were pulled down using MBP-REJ fusion protein (Figure 2).

The potential for detecting a protein mixture within the gel band is possible. The proteins identified in lane 10, Figure 2, exhibited estimated molecular weights of approximately 100 and 127 kDa (Table 1). Table 1 includes information such as the predicted molecular weight, isoelectric point (pI), protein name, and mass for each identified protein. Among the potential protein candidates suggested by UniProt and EXPASY were vitronectin, with estimated molecular weights of 5.4 kDa, a Fibulin-1 protein with a molecular weight of 7.7 kDa, and an AFAP protein with a molecular weight of 9.7 kDa. All three proteins were identified within a single, large, transparent band.

Discussion

Our study delved into the interactions of the REJ module of polycystin 1, shedding light on its binding partners and potential implications for therapeutic interventions in ADPKD. The results of the pull-down assay showed interactions between the REJ fusion protein and various ECM proteins, such as vitronectin, fibulin-1, and AFAP 1. These discoveries stemmed from the analysis of the SDS-PAGE bands, which were subsequently cut and examined using MALDI-TOF MS data. This process ensured a comprehensive validation of the observed interactions. The identification of proteins was carried out using MALDI-TOF-MS, utilizing the corresponding accession numbers in the UniProt database for the identification process.[18] This approach aligns with our chosen method, further substantiating the credibility of our results. The MALDI technique proves advantageous for the rapid identification of proteins isolated through gel electrophoresis and heightened sensitivity, facilitating the accurate detection of proteins[18] showcased that the combination of pull-down assays with liquid chromatography-tandem mass spectrometry (LC-MS/MS) allowed for the identification of protein interactions.

HEK 293 cells were chosen in the pulldown assay since it is a frequently utilized cell line in scientific investigations, owing to the multitude of advantages it provides. A major strength lies in its capacity to undergo post-translational modifications mirroring those observed in human cells. HEK 293 is favoured for its exceptional efficiency in integrating external genetic material (transfectivity), rapid proliferation, and the ability to thrive in a serum-free culture medium in a suspension setting.[19]

A case illustrating the utilization of fusion proteins for investigating protein-protein interactions involved studying the association of the mitochondrial ribosomal small subunit (MRPS) protein and its role in carcinogenesis.[20] Fusion proteins of MRPS and glutathione S-Transferase (GST) were synthesized for their study. The successful generation of GST/MRPS fusion proteins was confirmed through MALDI-TOF analysis. The source of anchor proteins was the lysate of HEK293 cells. Subsequently, protein interactions associated with MRPS were identified and examined using nano-liquid chromatography/tandem mass spectrometry (nLC/MS/MS) analysis. They used mass spectrometry techniques to gain valuable insights into the protein-protein interactions related to MRPS and their potential implications in tumorigenesis.[20] The results of their study aligned with our findings. Another study found a similar interaction between the PC-1 C-type lectin domain and various ECM proteins, including collagen types I, II, and IV, in vitro. They also discovered an interaction between unidentified components of cyst fluid from ADPKD patients and the PC-1 C-type lectin domain, implying its involvement in cell-matrix interactions.[21]

The identification of ECM proteins as binding partners of the REJ module broadens our understanding of the functional roles played by this domain. One of the pulled-down proteins was vitronectin. It is one of the most highly expressed ECM proteins in the kidney tissue scaffold of the fibrotic kidney. Multifunctional glycoprotein vitronectin is found in blood and the ECM compartment. It is involved in many biological processes, including cell adhesion, invasion, migration, proliferation, and tissue remodeling.[14] Upon release into the extracellular space, vitronectin can engage with numerous proteins, including integrin receptors on the cell membrane, and subsequently convey its signal to the target cells.[22] An earlier study revealed that increased urinary vitronectin was noted in patients who had undergone kidney transplantation.[23] Additionally, elevated levels of vitronectin have been documented in models associated with tissue fibrosis across various organs such as the liver as well as in conditions involving degenerative skin or nervous system diseases.[24] This ECM protein is involved in kidney cell adhesion, invasion, migration, proliferation, tissue remodeling and interaction with integrin receptors on the cell membrane. The discovery of vitronectin as a binding partner of the REJ module hints at a potentially crucial role for this versatile glycoprotein in the development of fibrotic kidneys and, on a broader scale, in the dynamics of the ECM. Given the substantial expression of vitronectin in the fibrotic kidney tissue scaffold,[23,24] we propose that the interaction between the REJ module of polycystin-1 and vitronectin may play a role in governing various biological processes. Furthermore, the observed association between vitronectin and the REJ module may be indicative of its involvement in signaling pathways that impact cellular responses, potentially influencing the progression of fibrosis.[24] Therefore, we suggest that the interaction between the REJ module and vitronectin may hold therapeutic relevance, offering a potential target for interventions aimed at modulating ECM dynamics and mitigating fibrotic processes in the kidney and other organs.

PKDREJ was co-localized with acetylated tubulin, a marker of primary cilia. These results open a new direction for the further investigation of PKDREJ that will provide deeper insight into its function and physiological relevance.[25]

The second pulled-down protein fibulin-1, is a member of the fibulin family of extracellular glycoproteins and is expressed in the vessel wall, offering protection against vascular damage.[26] It emerges as a potential contributor to the development of cardiovascular disease observed in individuals with chronic kidney disease and diabetes. Its involvement in the pathogenesis of cardiovascular issues in chronic kidney disease has been established in previous research.[27] Fibulin-1 actively participates in the formation of elastic fibers since it is closely associated with basement membranes, elastic fibers, and other extracellular matrix components,.[28] Furthermore, it has been known to be associated with ADPKD. Researchers conducted a study to examine the levels of fibulin-1 in different stages of individuals with ADPKD and to explore potential connections between fibulin-1 and arterial stiffness. The findings indicated a significant increase in fibulin-1 levels among the ADPKD patient group. A notable positive correlation was observed between fibulin-1 and creatinine levels. However, there were no significant correlations found between fibulin-1 levels and factors such as age, pulse pressure used for assessing arterial stiffness, and systolic and diastolic blood pressures, as well as mean arterial pressure.[29] The discovery of fibulin-1 in conjunction with REJPC1 suggests a potential connection between fibulin-1 and the polycystin-1 complex, prompting exploration of its functional roles beyond safeguarding blood vessels. Expanding on this finding, our hypothesis posits that fibulin-1, in partnership with REJPC1, may play a crucial role in influencing the dynamics of the extracellular matrix within the vascular context. Considering fibulin-1's established role in cardiovascular disease within the realm of chronic kidney disease and diabetes, we suggest that its interaction with REJPC1 could be involved in regulating vascular health and

potentially contribute to cardiovascular issues in these conditions. Investigating deeper into the nature of this interaction and its effects on vascular and cardiac physiology through further research may yield valuable insights, offering potential novel therapeutic targets for addressing cardiovascular complications linked to chronic kidney disease, diabetes, and related disorders.

The AFAP family genes consist of AFAP1/AFAP-110, AFAP1L1, and AFAP1L2/XB130. While there is growing evidence suggesting the involvement of these three AFAP family members in tumour progression, their clinical significance and the underlying molecular mechanisms in gastric cancer (GC) are not yet fully understood.[30] The actin cytoskeleton is involved in regulating cell migration and organizing cellular structures in the kidney. Dysregulation of the actin cytoskeleton has been connected to the formation of cystic kidneys in PKD.[31] Moreover, cystic cells derived from PKD1-null cells exhibit a highly disorganized actin cytoskeleton, suggesting a potential link between actin dynamics, AFAP 1, and the development of PKD.[32] The search results suggest that AFAP1 may be indirectly associated with polycystic kidney disease (PKD) through its involvement in the regulation of the actin cytoskeleton.[32]

From the previous information, we suggest a potential indirect association between AFAP1 and PC1. The dysregulation of the actin cytoskeleton observed in cystic cells derived from PKD1-null cells, coupled with the identified role of AFAP1 in actin dynamics,[32] implies that AFAP1 may play a role in the development of PKD through its influence on the actin cytoskeleton. Further investigations into the molecular mechanisms connecting AFAP1, actin dynamics, and the pathogenesis of PKD may provide valuable insights into potential therapeutic targets for this renal disorder. The REJ domain of PC1 has been found to interact with various proteins. The AFAP1, implicated in actin dynamics, provides additional layers to the complex network of REJ interactions. The presence of a diverse protein population may explain the identification of various proteins in the current study.

Conclusion

In conclusion, our study has demonstrated the pivotal role of the REJ module in mediating interactions with key ECM proteins and its potential impact on cell proliferation. These findings not only contribute to the fundamental understanding of polycystin 1 but also pave the way for the development of targeted therapies for ADPKD. Future research should focus on decoding the details of REJ module interactions and their relevance to disease progression, offering new insights into the molecular basis of ADPKD.

The strength of our experimental approach lies in the combination of pull-down assays and MALDI-TOF MS, providing a robust and reliable validation of protein-protein interactions. The detailed characterization of REJ-ECM interactions offers a valuable foundation for future research aimed at systematically studying the effects of disease-causing mutations within the REJ module of human PC-1.

While our study advances our understanding of REJ module interactions, there are limitations to consider. The observed effects were specific to the conditions of our experiments, and further investigations under varied cellular contexts and physiological conditions are warranted.

Author Contributions

Conceptualizing the study, formulating the research question, designing the experimental framework, developing the hypothesis, outlining the methodology supervising the laboratory work, writing the paper, Hala Salim Sonbol. Execution of laboratory experiments, data collection, data analysis, diligently following the experimental protocols and ensuring the accurate and reliable collection of data, statistical analysis, and the interpretation of results, writing the paper, Aljazi Abdullah Alrashidi.

Funding

This research received no external funding.

Informed Consent Statement

Not applicable.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Lea WA, Winklhofer T, Zelenchuk L, Sharma M, Rossol-Allison J, Fields TA, Reif G, Calvet JP, Bakeberg JL, Wallace DP, Ward CJ. Polycystin-1 Interacting Protein-1 (CU062) Interacts with the Ectodomain of Polycystin-1 (PC1). Cells. 2023 Aug 29;12(17):2166. doi: 10.3390/cells12172166. PMID: 37681898; PMCID: PMC10487028.
2. Douguet, D., Patel, A. & Honoré, E. Structure and function of polycystins: insights into polycystic kidney disease. Nat Rev Nephrol 15, 412–422 (2019). https://doi.org/10.1038/s41581-019-0143-6
3. Orhi Esarte Palomero, Megan Larmore, and Paul G. De Caen. Polycystin Channel Complexes. Annu Rev Physiol 2023.85:425–48. https://doi.org/10.1146/annurev-physiol-031522084334
4. Maser RL, Calvet JP, Parnell SC. The GPCR properties of polycystin-1- A new paradigm. Front Mol Biosci. 2022 Nov 4;9:1035507. doi: 10.3389/fmolb.2022.1035507. PMID: 36406261; PMCID: PMC9672506.
5. Schröder, S., Fraternali, F., Quan, X., Scott, D., Qian, F., & Pfuhl, M. (2011). When a module is not a domain: the case of the REJ module and the redefinition of the architecture of polycystin-1. Biochemical Journal, 435(3), 651–660.
6. Hu, M., Ling, Z. & Ren, X. Extracellular matrix dynamics: tracking in biological systems and their implications. J Biol Eng 16, 13 (2022). https://doi.org/10.1186/s13036-022-00292-x
7. Xu, M., Ma, L., Bujalowski, P. J., Qian, F., Sutton, R. B., & Oberhauser, A. F. (2013). Analysis of the REJ module of polycystin-1 using molecular modeling and force-spectroscopy techniques. Journal of Biophysics, 2013: 525231.
8. Kurbegovic, A., Kim, H., Xu, H., Yu, S., Cruanès, J., Maser, R. L., & Qian, F. (2014). Novel functional complexity of polycystin-1 by GPS cleavage in vivo: role in polycystic kidney disease. Molecular and Cellular Biology, 34(17), 3341–3353.
9. Weston, B. S., Malhas, A. N., & Price, R. G. (2003). Structure–function relationships of the extracellular domain of the autosomal dominant polycystic kidney disease-associated protein, polycystin-1. FEBS Letters, 538(1-3), 8–13.

10. Hardy E, Tsiokas L. Polycystins as components of large multiprotein complexes of polycystin interactors. Cell Signal. 2020 Aug;72:109640. doi: 10.1016/j.cellsig.2020.109640. Epub 2020 Apr 17. PMID: 32305669; PMCID: PMC7269800.
11. Babich, V., Zeng, W. Z., Yeh, B. I., Ibraghimov-Beskrovnaya, O., Cai, Y., Somlo, S., & Huang, C. L. (2004). The N-terminal extracellular domain is required for polycystin-1-dependent channel activity. Journal of Biological Chemistry, 279(24), 25582–25589.
12. Pastor-Pareja, J. C. (2020). Atypical basement membranes and basement membrane diversity–what is normal anyway. Journal of Cell Science, 133(8), jcs241794.
13. Nigro EA, Boletta A. Role of the polycystins as mechanosensors of extracellular stiffness. Am J Physiol Renal Physiol. 2021 May 1;320(5):F693–F705. doi: 10.1152/ajprenal.00545.2020. Epub 2021 Feb 22. PMID: 33615892.
14. Yiling Peng, Li Li, Jingyue Shang, Haili Zhu, Jinlin Liao, Xue Hong, Fan Fan Hou, Haiyan Fu and Youhua Liu. Macrophage promotes fibroblast activation and kidney fibrosis by assembling a vitronectin-enriched microenvironment. Theranostics 2023;13(11):3897–3913.
15. Dzobo, K.; Dandara, C. The Extracellular Matrix: Its Composition, Function, Remodeling, and Role in Tumorigenesis. Biomimetics 2023, 8, 146. https://doi.org/10.3390/biomimetics8020146
16. Sonbol, H. S., & AlRashidi, A. A. (2022). Cloning and expression of receptor of egg jelly protein of polycystic kidney disease 1 gene in human receptor of egg jelly protein. Pharmacophore, 13(6), 97–105.
17. Marvin, L. F., Roberts, M. A., & Fay, L. B. (2003). Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry in clinical chemistry. Clinica Chimica Acta, 337(1-2), 11–21.
18. Suvarna, K., Honda, K., Muroi, M., Kondoh, Y., Watanabe, N., & Osada, H. (2020). Identification of target protein for bio-active small molecule using photo-cross linked beads and MALDI-TOF mass spectrometry. Bio-protocol, 10(3), e3517–e3517.
19. Tan, E., Chin, C. S. H., Lim, Z. F. S., & Ng, S. K. (2021). HEK293 cell line as a platform to produce recombinant proteins and viral vectors. Frontiers in Bioengineering and Biotechnology, 1288.
20. Revathi Paramasivam, O., Gopisetty, G., Subramani, J., & Thangarajan, R. (2021). Expression and affinity purification of recombinant mammalian mitochondrial ribosomal small subunit (MRPS) proteins and protein–protein interaction analysis indicate putative role in tumourigenic cellular processes. The Journal of Biochemistry, 169(6), 675–692.
21. Weston, B. S., Bagnéris, C., Price, R. G., & Stirling, J. L. (2001). The polycystin-1 C-type lectin domain binds carbohydrate in a calcium-dependent manner, and interacts with extracellular matrix proteins in vitro. Biochimica Et Biophysica Acta (BBA)-Molecular Basis of Disease, 1536(2-3), 161–176.
22. Leavesley DI, Kashyap AS, Croll T, Sivaramakrishnan M, Shokoohmand A, Hollier BG, et al. Vitronectin--master controller or micromanager? IUBMB Life. 2013; 65: 807–818.
23. Carreras-Planella L, Cucchiari D, Canas L, Juega J, Franquesa M, Bonet J, et al. Urinary vitronectin identifies patients with high levels of fibrosis in kidney grafts. J Nephrol. 2021; 34: 861–874.
24. Hayashida M, Hashimoto K, Ishikawa T, Miyamoto Y. Vitronectin deficiency attenuates hepatic fibrosis in a non-alcoholic steatohepatitis-induced mouse model. Int J Exp Pathol. 2019; 100: 72–82.
25. Butscheid, Y. (2006). Characterization of mouse polycystic kidney disease and receptor for egg jelly gene and protein in heterologous and native system. Philipps-Universität Marburg. https://doi.org/10.17192/z2006.0239
26. Zencir C, Sivri F, Gulasti S, Turkdogan AK, Yilmaz M, Gungor H. Fibulin-1 and fibulin-5 as rule-out tests for non-ST-elevation myocardial infarction in the emergency setting. Kardiol Pol. 2019 Dec 19;77(12):1170–1175. doi: 10.33963/KP.15041. Epub 2019 Oct 30. PMID: 31663514.
27. Scholze Alexandra, Bladbjerg Else-Marie, Sidelmann Johannes J, et al. Plasma concentrations of extracellular matrix protein fibulin-1 are related to cardiovascular risk markers in chronic kidney disease and diabetes. Cardiovascular Diabetology. 2013;12(1):6. doi:10.1186/1475-2840-12-6
28. Halper, J. (2021). Basic Components of Connective Tissues and Extracellular Matrix: Fibronectin, Fibrinogen, Laminin, Elastin, Fibrillins, Fibulins, Matrilins, Tenascins and Thrombospondins. In: Halper, J. (eds) Progress in Heritable Soft Connective Tissue Diseases. Advances in Experimental Medicine and Biology, vol 1348. Springer, Cham. https://doi.org/10.1007/978-3-030-80614-9_4
29. Inci A, Akin O, Ellidag HY, Eren E, Dolu S, Sari F. Fibulin Levels in Autosomal Dominant Polycystic Kidney Disease and Its Relationship to Arterial Stiffness. Clin Lab. 2017 Nov 1;63(11):1869–1874. doi: 10.7754/Clin.Lab.2017.170617. PMID: 29226648.
30. Sun, B., Ding, B., Chen, Y. Chuang Peng and Xu Chen. AFAP1L1 promotes gastric cancer progression by interacting with VAV2 to facilitate the CDC42-mediated activation of ITGA5 signaling pathway. J Transl Med 21, 18 (2023). https://doi-org.sdl.idm.oclc.org/10.1186/s12967-023-03871-8
31. Yao G, Su X, Nguyen V, Roberts K, Li X, Takakura A, Plomann M, Zhou J. Polycystin-1 regulates actin cytoskeleton organization and directional cell migration through a novel PC1-Pacsin 2-N-Wasp complex. Hum Mol Genet. 2014 May 15;23(10):2769–79. doi: 10.1093/hmg/ddt672. Epub 2014 Jan 2. PMID: 24385601; PMCID: PMC3990174.
32. Streets AJ, Prosseda PP, Ong AC. Polycystin-1 regulates ARHGAP35-dependent centrosomal RhoA activation and ROCK signaling. JCI Insight. 2020 Aug 20;5(16):e135385. doi: 10.1172/jci.insight.135385. PMID: 32663194; PMCID: PMC7455122.