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Decoding Polycystin-1's G-Proteolytic Site: Implications for Cell Interactions and Autosomal Dominent Polycystic Kidney Disease Pathogenesis

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Abstract Renal cysts are the outcome of ADPKD, which is characterised by abnormal cell proliferation, altered cellular polarity, and disordered extracellular matrix dynamics. Of ADPKD instances, the PKD1 gene mutation is responsible for about 85% of cases. The goal of this study is to better understand the function of polycystin-1, a membrane protein with unique peptide domains involved in interactions between cells and matrices, and the G-proteolytic site domain (GPS). Their importance in normal kidney development and the underlying mechanisms of ADPKD pathophysiology may be revealed by this interaction. In order to characterize MBP-GPS's function, its effect on the growth of human embryonic kidney epithelial cells (HEK-293) was investigated. The results showed that when the fusion protein was exposed to cells, the rates of cell proliferation significantly decreased in a dose-dependent manner. In addition, pull-down studies using MBP-GPS were carried out on HEK-293 cells in order to find potential interaction proteins connected to the GPS domain. Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry was utilised to identify the peptides that emerged from tryptic digestion after the cell lysate underwent sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Among the candidate proteins identified, laminin emerged as a potential interacting partner with the GPS domain. This research represents a significant step towards unraveling the nature of the putative interactions between the extracellular domains of ECM and the polycystin-1, shedding light on their potential roles in ADPKD development.

Key Words polycystin-1, ADPKD, G-proteolytic site (GPS) domain

1. Introduction

Polycystic Kidney Disease 1 gene (PKD1) is the most frequently mutated gene responsible for autosomal dominant polycystic kidney disease (ADPKD) in humans. ADPKD is an inherited and progressive renal disorder that affects a substantial number of individuals worldwide (Jing et al., 2023). Approximately 95% of individuals diagnosed with ADPKD can attribute their condition to mutations in the PKD1 and polycystic kidney disease 2 gene (PKD2) [1]. In Saudi Arabia, the prevalence of With a mortality rate of 5.44%, chronic kidney disease (CKD) is regarded as the fourth most common cause of death, accounting for approximately 5.7% of cases [2]. In Saudi Arabia, there were almost two million instances of chronic kidney disease (CKD) and 3818 CKD-related deaths in 2017 [3]. According to Mousa et al. [4], a recent study also revealed that 4.4% of Saudi citizens had CKD stages 3 to 5.

The PKD1 gene encodes polycystin-1 (PC1) protein. In human cells, PC1 has been reported to localize in various subcellular compartments, including the primary cilium and mitochondria [5]. The PC1 is expressed in many cells such as endothelial cells, brain, heart, bone, and muscle (Mbiakop and Jaggar, 2023). PC1 has eleven transmembrane helices, an extracellular N-terminus and an intracellular C-terminus [6], [7]. The N-terminus of PC1 encompasses various potential adhesion- and ligand- binding sites [7]. PC1 is suggested to function as a mechanical sensor and receptor [1]. The assembly of polycystin channels exhibit discrete localization within the cellular compartments. This is particularly evident in their sensory role within the primary cilia [8]. The extracellular N-terminal domain of PKD1 is responsible for interacting with the extracellular matrix (ECM) and neighboring cells [9]. The

categorization of PC1 as a mechanosensory is attributed to its capacity to engage with both the ECM and the cellular cytoskeleton via intermediate filaments. The prevailing idea is that PC1 and PC2 proteins combine to create a receptor-channel complex that participates in mechanosensation, possibly by means of the curvature of primary cilia on the luminal facade of renal epithelial cells. The ECM plays a crucial role in transmitting biomechanical signals to PC1, which are essential for maintaining proper cellular function. PC1 facilitates the cellular ability to notice the stiffness of the ECM, and that the absence of this signal in PKD cells is a contributing factor to the formation of cysts [9].

PC1 structure, include two leucine-rich repeats flanked by cysteine-rich regions, the cell wall integrity and stress response components region, a C-type lectin domain, and LDL-A region homologous to low-density lipoprotein [10]. It also includes repetitive PKD structures [11].

One key characteristic of PC1, is the modification after translation through cis-autoproteolytic cleavage at the juxt-membrane G-protein coupled receptor proteolytic site (GPS) motif, which is located at the bottom of the extracellular ectodomain N-terminal extracellular region. It is highly probable that defective cleavage plays a substantial role in the development of ADPKD [12]. The loss of GPS cleavage at PC1 delays its localization in the cilia, which has been proposed as a primary mechanism in the pathogenesis of PKD [13]. The cleavage at the GPS site of PC1 is essential in preventing the development of renal cysts [14], [15]. Since GPS cleavage is crucial for the proper function of PC1, it could serve as an excellent target for therapy specifically directed towards PC1. One potential approach could involve the use of chemical chaperones or other small molecules to overcome or by pass the impaired GPS cleavage of these mutated PC1 proteins. This concept has been successfully applied in the development of transformative drugs for other diseases such as cystic fibrosis, where similar strategies have yielded positive results [16]–[18].

Many proteins involved in the development of cell-matrix and cell-cell junctions interact with PC1 [1]. Different areas of PC1's extracellular N-terminal segment have been identified as locations for protein interaction with the extracellular matrix (ECM) within cell-matrix junctions. Collagen types I, II, and IV have been demonstrated to bind to PC1's C-type lectin domain [19]. It's interesting to note that ADPKD disrupts the expression of type I and type IV collagen. It has been proposed that this disruption of collagen-PC1 interactions may be a factor in the loss of epithelial cell polarity in ADPKD [20]–[22]. Malhas et al. [23] used blot overlay tests to show that the leucine-rich repeat region of PKD1 interacts with fibronectin, laminin, and laminin fragments extracted from cyst fluid in addition to collagen I. Two versions of PKD1 were used in these investigations: the full-length protein and a GST-fusion protein that included the cysteine-rich domains bordering the LRR region in addition to the LRR. They discovered that PKD1 and collagens II, III, and IV did not bond together. Upon closer inspection, laminin fragments produced from cyst fluid were discovered to promote cell proliferation in culture. Significantly, the addition of the LRR fusion protein reversed this stimulatory effect, indicating that the PKD1 LRR region regulates this cellular response [23]. The stability and signalling of the cell-matrix may be dependent on the interaction of PKD1's LRR and/or C-type lectin domain with ECM proteins. Any disruption of these connections may cause or worsen a number of the traits that define ADPKD. Interestingly, there is a region that coincides with the WNT-binding domain in the fusion construct used to illustrate the interactions between the LRRs and ECM components. This implies that within the N-terminal region of PKD1, interactions between ECM components and PKD1 may control the availability or activity of other domains involved in binding to various ligands [23].

This study aims to explore the involvement of PC1's extracellular domains in cell-matrix interactions. To assess the significance of the GPS domain in PC1's binding to ECM proteins and its potential relevance in the development of ADPKD, a fusion protein incorporating the GPS domain was employed in a sequence of GST pulldown experiments.

2. Materials and Methods

A. Chemicals

Dimethyl sulfoxide (DMSO), trypan blue solution, trypsin-EDTA, phosphate-buffered saline (PBS), penicillin-Streptomycin, and Dulbecco's modified eagle medium (DMEM) were acquired from Solarbio (China). Foetal bovine serum (FBS) that has been heat-inactivated was acquired from Gibco Thermo Fisher Scientific (KSA). Thiazolyl blue tetrazolium bromide powder (MTT) provided from Gold Biotechnology (USA). Mini gel electrophoresis system was obtained from Bio-Rad (UK), protein standards molecular weight marker was purchased from Gene Direx (Germany).

B. Cloning and Producing a Soluble Protein with the GPS Domain

As previously mentioned, the MBP-GPS domain was cloned, expressed, and fusion proteins were expressed and purified using PureCube His-Affinity agarose [24].

C. Culturing of the human embryo kidney epithelial cells

Human embryo kidney epithelial cells (HEK 293) were kindly provided by Jeddah, specialist center of King Faisal, Dr. Baghdadi, Kingdom of Saudi Arabia. HEK 293 cells from passage '9' were used to determine the effect of the MBP-GPS fusion proteins on cell proliferation. On tissue culture flasks without coating, HEK 293 cells were cultured as monolayers., using Dulbecco's Modified Eagle medium (DMEM) supplemented with 10% (v/v) FCS and 1% (v/v) penicillin-streptomycin solution. The cells were incubated in a humidified atmosphere at 37°C with 5% (v/v) CO2 and 95% air. The medium was changed every 2-3 days or as needed. All cell lines were routinely passaged and maintained at 37°C with 5% CO2. Cells were checked for

appropriate density and the lack of contamination using a phase-contrast microscope (Nikon, USA) once they had reached 70% confluency. Following trypsinization, they were centrifuged for 10 minutes at 1500 rpm, and the resultant cell pellets were saved for hemocytometer counting. Ultimately, before being employed in the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) experiment, cells were seeded at a density of 5.0×104 cells/μL and given an overnight to adhere.

D. Studying the effect of MBP-GPS fusion protein on cell viability and proliferation

Using the MTT assay, the impact of the MBP-GPS fusion protein on the proliferation of the HEK 293 cell line was ascertained. The mitochondrial dehydrogenase enzyme in living cells is measured by this calorimetric test, which can change pale yellow soluble MTT into an insoluble purple formazan result. The manufacturing instructions were followed in the preparation of the MTT solution.

Cells were grown in 96-well microtiter plates at a density of 5000 cells/100μl at 37°C and 5% (v/v) CO2. The cells were treated with varying amounts of MBP-GPS protein (0.5, 1, 1.5, and 2 μg/ml). The culture plates were incubated at 37 °C with 5% (v/v) CO2 for 24, 48, and 72 hours. Additionally, there were controls using cells and media. After 24, 48, and 72 hours of incubation, 10 μl of MTT (3 mg/ml) solution was added to each well. After that, the wells were left to incubate with 5% (v/v) CO2 at 37°C. Following a 4-hour incubation period, the supernatants were disposed of. The formazan crystals were then forcefully mixed in each well after 100 μl of dimethyl sulfoxide (DMSO) was added. The enzyme-linked immunosorbent assay ELIZA reader (BioTek Instruments, USA) was used to detect the absorbance at 570 nm. The formula from Husni et al. [25] was used to manually compute the percentage of cell viability. Choudhury et al. (2016) provided a formula that was manually used to compute the percentage of cell cytotoxicity. Cell morphological changes were monitored using a phase-contrast microscope (Nikon ECLIPSE ti-S, Japan) at 24, 48, and 72 hours after treatment.

E. MBP-GPS fusion protein's interactions with ECM proteins

Extracellular matrix alterations have been suggested to be part of the early symptoms occurring in ADPKD [26]. The possible interaction of a component of the extracellular matrix with polycystin-1 may be important in the initiation or progression of ADPKD [19]. In order to investigate protein-ECM interaction, a pulldown experiment was carried out in accordance with manufacturer instructions (Thermos Fisher, USA). Optimization experiments using different concentrations of MBP-GPS fusion protein (300 μl), beads volume (100 μl), incubation times (30 min), number of washes (4 X), volume of cell lysate (800 μl) were performed.

F. Pull-Down assays

HEK 293 cells were used for the pulldown test. On uncoated tissue culture flasks, HEK 293 cells were grown as monolayers to about 70% confluency using DMEM medium. The cells were washed twice with ice-cold PBS. One millilitre of RIPA lysis buffer (which contains 150 millilitres of sodium chloride, 1% Triton X100, 0.5% sodium deoxycholate, 0.1% SDS, 50 millimetres of tris, pH 8, Tris HCL, and 20 millimetres of tris-HCL, pH 7.5) was applied to the cells, followed by five minutes of cold treatment. Following the use of a cell scraper (Costar Corning integrated, USA), the cells were transferred to a sterile Eppendorf tube with a capacity of 0.5 ml and allowed to cool for an hour. During this time, the cell lysate was pipetted using small tips every 10 minutes to physically break down the cells. The harmed cells were centrifuged for half an hour at 13,000 rpm at 4°C. The supernatant was stored at -70 °C until it was needed.

Purified MBP-GPS fusion protein (300 μl) was incubated with 50% slurry of His-Pur Cobalt Resin (100 μl) for 30 min at 4oC with end-over mixing to avoid non-specific protein contact with the beads or His moiety. For one minute, the mixture was centrifuged at 3341 rpm. "Bait flow-through" was the label applied to the tube. The bait flow-through was subjected to a 15% reduced SDS-PAGE analysis. Using a 1:1 TBS washing buffer (pull dawn lysis buffer), His-Pur Cobalt Resin was rinsed five times.

The lysed cells were combined with pierce lysis buffer to prepare the prey fraction. The tube was then thoroughly mixed by repeatedly inverting it upward and downward. After around half an hour of cold incubation, the mixture was centrifuged for five minutes to extract the crude HEK 293 lysate. After being decanted into a fresh tube, the supernatant was kept on ice. The pellet of cells was supplemented with 10 mM imidazole. This tube was kept on ice and labelled "mammalian prey lysate." The prey protein (800 μl) was added to the microfuge tube and incubated at 4°C for 1 hour on a rotating platform with slight rocking motion in order to achieve a direct connection. After that, the samples were centrifuged for one minute at 3341 rpm. The description of this tube said "prey flow-through." The supernatants were removed and the tube was put on ice before being subjected to 15% reduced SDS-PAGE analysis. After lysing the cells, the beads were cleaned in ice-cold lysis buffer. After centrifuging the tubes for a minute, the washing process was carried out four times. The microfuge tube was filled with 250 μl of elution buffer and allowed to gently rock for five minutes on a revolving platform. The sample was centrifuged at 3341 rpm for 30 to 1 minute. This tube had the label "Elution 1" on it and was placed on ice. Five elution steps were performed. Untreated Among the samples that were electrophoresed on a 15% reduced SDS-PAGE were His-Pur Cobalt Resin, purified bait, and elution (1–5).

G. Characterization of interacting proteins by mass spectrometry

Mass spectrometry with matrix assistance for laser desorption ionisation and time of flight (MALDI-TOF MS) (Bruker Corporation, USA) was used to identify the proteins that have been pulled down with GPS, which has been subjected to 15% SDS-PAGE prior to characterization. After removing the pertinent dyed protein bands from the gel and storing them in 0.1% acetic acid, the proteins were subjected to MALDI-TOF MS analysis and database searching (Swiss Prot). The King Abdullah University of Science and Technology (KAUST) in Jeddah, the Kingdom of Saudi Arabia, was the location of the MALDI-TOF MS analysis procedure.

H. Statical analysis

With GraphPad Prism version 8.2.1, the data were examined (Prism 8). Dunnett's multiple comparisons test was used in one-way ANOVA to identify significant differences between the treated groups and the control. The findings were reported with a significance threshold of $P < 0.05$ and as mean ± standard deviation (SD).

3. Results

The cloning, subcloning, expression and affinity purification of the PET21-MBP(TEV)-GPS soluble fusion protein was demonstrated earlier [24].

A. The effect of MBP-GPS on cell viability and proliferation

Using the MTT assay, the impact of the MBP-GPS fusion protein on the HEK 293 cell line's cellular growth was assessed. The protein known as pure MBP-GPS fusion (0.5, 1, 1.5, and 2 μg/ml) was included. After being treated with MBP-GPS fusion protein over time, the quantity of viable cells in culture was observed. The initial cell density per well was 5x104. Following a 24-hour incubation period, the proportion of live cells in multi-well plates increased along with the amounts of the MBP-GPS fusion protein and the percentage of cells that were cytotoxic as compared to untreated cells. The control's mean±standard deviation was 0.339±0.022, and the calculation yielded a 100% viable cell percentage. The mean±SD lowers to 0.321±0.068, 0.294±0.034, 0.285±0.044, and 0.25±0.061μg/ml, respectively, when the concentrations increase from 0.5-2 μg/ml. Increased MBP-GPS fusion protein concentrations resulted in a lower percentage of viable cells as compared to the control. With a rise in concentration, the percentage of cell viability falls concurrently to 94.60, 86.63, 84.06, and 73.73 %, respectively. The association between concentration and cell cytotoxicity was inverse. With an increase in fusion protein concentration, the cell cytotoxicity rises to 5.40, 13.37, 15.94, and 26.27%, respectively (Table 1). The mean±SD of 0.5-2 μg/ml did not differ significantly from the control. The number of viable cells in the control well increased while the number of viable cells in the treated wells decreased as the incubation period was extended to 48 hours. To begin with, the control group's mean±standard deviation was 0.907±0.047, and its viable cell percentage was 100%. After administering 0.5-2 μg/ml to the cells, the mean±standard deviation drops to 0.828±0.095, 0.777±0.072, 0.572±0.078, and 0.513±0.069 μg/ml, in that order. Furthermore, compared to 100% for the untreated cells, 91.29, 85.67, 63.01, and 56.56% for the treated cells represent the percentage of viable cells. As the concentrations rise, cell cytotoxicity rises as well, rising from 8.71 % at 0.5 μg/ml to 14.33, 36.99, and 43.44 % (Table 1). Furthermore, the mean±SD of 0.5 and 1 μg/ml did not differ significantly from the control. Nonetheless, when compared to the control, there were notable variations in the mean±SD of the 1.5 and 2 μg/ml concentrations ($P < 0.0001$).

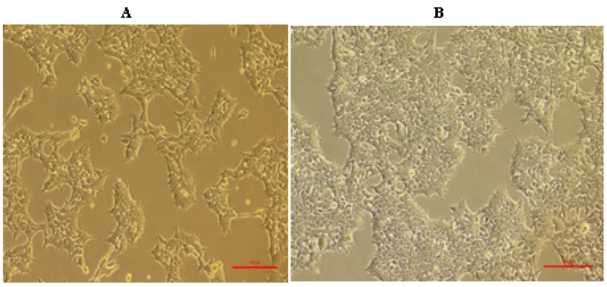

Figure 1: HEK293 monolayer (A) A 50% % confluency of viable HEK 293 cells. (B) 70% confluence of viable HEK 293 cells. Scale bar, 100 μm

The number of viable cells in the control group decreases as the incubation period is increased to 72 hours. At 100% viable cell count, the control group's mean±standard deviation was 0.428±0.151. In contrast, the number of viable cells in treated wells was much lower, with mean±SD values of 0.420±0.097, 0.347±0.100, 0.336±0.031, and 0.308±0.067 at the concentrations of 0.5-2 μg/ml. The correlation between concentration and cell viability was inverse. When cells are treated at dosages of 0.5-2 μg/ml, the percentage of viable cells drops from 100% for untreated cells to 97.99, 80.90, 80.00, and 71.87% for treated cells. Additionally, there was an inverse link between concentration and cell cytotoxicity. The corresponding increases in cell cytotoxicity are 2.01, 19.10, 20.00, and 26.27%. Table 1 shows the MBP-GPS fusion protein's cytotoxicity assessment over 24, 48, and 72 hours.

B. Pull-Down assays

Figure 1 displays images of HEK 293 cells at 50%-70% confluence, which were employed in the pulldown assay. The images illustrate diverse seeding numbers for these cells. Figure 2 displays the 15% SDS-PAGE results of the pull-down assay used to purify the MBP-GPS fusion protein and investigate potential interactions with the ECM. The MBP-GPS fusion protein, with an approximate molecular weight of 48 kDa, is visible in Lane 4-6.

Groups	Mean±SD	C (%)	Cell Cytotoxicity (%)	P value
24 hours				
Control	0.339±0.022	100	0	
C (0.5µg/ml)	0.321±0.068	94.60	5.40	0.991
D (1µg/ml)	0.294±0.034	86.63	13.37	0.702
E (1.5µg/ml)	0.285±0.044	84.06	15.94	0.569
F (2µg/ml)	0.250±0.061	73.73	26.27	0.096
48 hours				
Control	0.907±0.047	100	0	
C (0.5µg/ml)	0.828±0.095	91.29	8.71	0.578
D (1µg/ml)	0.777±0.072	85.67	14.33	0.120
E (1.5µg/ml)	0.572±0.078	63.01	36.99	0.000*
F (2µg/ml)	0.513±0.069	56.56	43.44	0.000*
72 h				
Control	0.428±.151	100	0	
C (0.5µg/ml)	0.420±.097	97.99	2.01	1.000
D (1µg/ml)	0.347±0.100	80.90	19.10	0.785
E (1.5µg/ml)	0.336±.031	80.00	20.00	0.690
F (2µg/ml)	0.308±.067	71.87	26.27	0.430

Table 1: Assessment of Cell Viability Using MTT Assay for Control and Various Concentrations (mg/mL) of MBP-GPS Fusion Protein

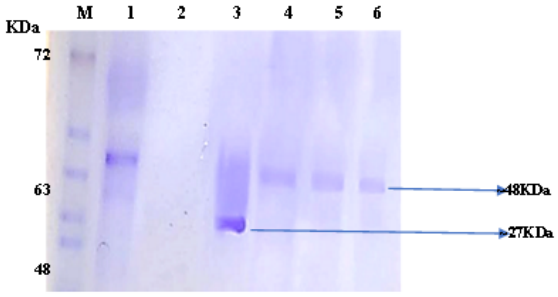

Figure 2: The purified MBP-GPS fusion protein was used as a bait in His-tag pull down assay. analysis of MBP-GPS fusion protein-cell lysate protein interaction on 15% reduced SDS-page. The purified MBP-GPS fusion protein was used as a bait in His-tag pull down assay. Lane M: is the molecular weight marker. Lane 1 purified MBP-GPS fusion protein (the bait). Lane 2: is the negative control (resin). Lane3: is the positive control (TEV protease). Lane4-6: elution of purified MBP-GPS fusion protein- proteins interaction

C. Characterization of interacting proteins by mass spectrometry

Analysis of the band in lane 6 presented in Figure 2, by mass spectrometry were achieved by using the MALDI-TOF MS. The proteins identified from the analysis of the band in lane 6 is presented in Table 2. The electrophoresis was carried out using 15% SDS-PAGE, which does not exclude the probability of the presence of a mixture of proteins in the gel band. Table 2 includes the predicted molecular weight, pI, protein name, and mass unit (KDa) of the proteins. On of the possible candidate suggested by uniport and expassy was human laminin subunit beta-2 fragment precursor (LAMB2). The database estimated molecular weight for this component of 195.98 kDa, (uniport) (expassy) LAMB2, pI is 6.07. The SDS PAGE image failed to represent any band with the expected molecular size of 195.98 kDa, however it was detected by MALDITOF analysis.

4. Discussion

The development of ADPKD is closely tied to mutations occurring in the PKD1 gene, responsible for encoding the PC1 protein. Within PC1, the GPS motif plays a crucial role in its biological functioning. The presence of mutations linked to PKD1 disrupts the functioning of a particular motif, thereby causing the progression of the associated disease [27]. Our research hypothesis aimed to predict and study a motif involved in PKD1-related disorders the GPS. This investigation is crucial for comprehending the underlying mechanisms of ADPKD.

This study confirmed an interaction between the GPS and laminin. The function of PC1 in the kidney may depend on its cellular localization as for example it is found in cilia [28] on the luminal surface of the tubules and in the basolateral membrane which interfaces with the extracellular matrix. PC1 may act by influencing cell function via controlling both the formation and activation of key signalling complexes [29].

In order to substantiate this, it was necessary to identify which extracellular ligand can initiate polycystin-mediated signalling pathways in order to understand the potential consequences for altered signal transduction in ADPKD. The generation of MBP-GPS fusion protein of PC1 enable functional aspects of GPS domain to be investigated in vitro. Another promising finding was the significant reduction in the viability of HEK293 cells treated by MBP-GPS fusion protein which expose the role of the GPS domain of PC1 as a receptor, regulating cell division by modulating cell signaling upon binding to specific ligands.

The expression of the GPS domain as an extracellular membrane protein offers several advantages compared to intracellular production. This expression strategy provides a more favorable oxidative environment for proper protein

protein	MW	UNI port Accession#	PI
Laminin subunit beta-2(Fragment)	195.98KDa	P55268-Humman	6.07

Table 2: MALDI TOF analysis of the predicted possible ECM- proteins-MBP-GPS protein interaction

folding, resulting in higher levels of recombinant protein expression. Additionally, it simplifies the purification process by eliminating the need for cell lysis and the removal of cellular debris. These benefits were highlighted in a study performed by Zhou et al. [30]. Understanding the expression characteristics and advantages of the GPS domain can aid in optimizing the production and purification processes, facilitating further investigations into its functional role in PKD1-related diseases.

By examining how the GPS motif impact the PC1 function, researchers can gain valuable insights into the molecular processes driving cyst formation and proliferation within the kidneys. Ultimately, this knowledge opens up possibilities for developing targeted therapies that specifically address the abnormal GPS motif, aiming to slow down or halt the progression of ADPKD [31].

It should be noted that the presence of the MBP-tags does not interfere with the function of the protein. Mouse leukemia inhibitory factor (mLIF) expressed in the cytoplasm of E. coli as an MBP-mLIF fusion exhibited bioactivity even without removing the MBP-tag [32]. These outcomes demonstrated that the inclusion of N-terminal MBP tags in MBP-protein fusions did not have any adverse effects on the bioactivity of the protein. This approach of generating recombinant fusion proteins was found to be a simple, convenient, cost-effective, and user-friendly protocol [32].

HEK 293 cells have become widely utilized in research as a human cell line. Due to their exceptional transfectivity, rapid growth rate, and capability to thrive in a serum-free, suspension culture [33]. In addition, HEK 293 have been extensively studied for their viability using various assays, including the MTT assay. Chumley et al. [34] conducted a study using MTT assay to investigate the extracellular acidification and glucose metabolism in HEK 293 cell lines carrying truncating defects in the polycystic kidney and hepatic disease 1 (PKHD1) and polycystic kidney disease 2 (PKD2) genes. These truncating defects were selected to represent various sites of truncating mutations identified in patients with PKD. It was observed that there was an elevation in the extracellular acidification rate in cell clones with homozygous PKHD1 and PKD2 truncating mutations, using a human HEK 293 cell line model [34].

When investigating the influence of GPS-MBP on kidney epithelial cells, it was noted that within the range of 0.5-2 μg/ml over 24 and 72 hours, the fusion protein did not induce any significant alterations in HEK293 cell proliferation. However, in contrast, when exposed to 1.5-2 μg/ml GPS-MBP for 48 hours, a decrease in cell growth was observed. This may be attributed to the role of the GPS domain of PC1, potentially acting as a receptor that modulates cell signaling and regulates cell division upon binding to specific ligands. Alternatively, the presence of GPS-MBP as an exogenous fusion protein introduced to normal HEK293 cells at a specific dosage could potentially compete for receptor binding sites, triggering a cessation signal for cell division.

The possible mechanisms by which GPS may reduce proliferation is demonstrated in Figure 3. The result may give an idea about the formation and the progression of ADPKD cyst. However, similar result obtained previously showed that LRR, of polycystin-1 reduces the baseline proliferation pf HEK 293 and it was dose dependent [23].

PC1-mutated kidney cystic cell lines exhibited elevated Ca2+ oscillations and increased cell proliferation, whereas these abnormal characteristics were attenuated in cells expressing exogenous PC1 protein. This study also proposed a molecular mechanism to explain the connection between abnormal Ca2+ homeostasis and increased cell proliferation in ADPKD. They observed that depleting endogenous PC1 in mutated HEK293 cells resulted in heightened Ca2+ oscillations when stimulated by serum, which subsequently activated the nuclear factor of activated T cells and promoted cell cycle progression [35].

In my opinion PC1 may regulate cell proliferation. This can be demonstrated by a study conducted by [36]. Upon the cleavage of the C-terminal of PC1, the fragment produced will be translocated to the mitochondria and interact with the mitochondrial enzyme, nicotinamide nucleotide transhydrogenase. This interaction influences tubular/cyst cell proliferation. These findings suggest that even a short fragment of PC1 protein is capable of suppressing the cystic phenotype, opening up possibilities for gene therapy strategies in ADPKD.

The ECM plays a vital role as a fundamental element in the biomechanical environment where cells operate. It serves critical functions in normal developmental processes as well as the advancement of different diseases [37]. To identify ECM proteins that interacted with the GPS domain of PC1, pulldown technique was employed in this study. The presence of laminin β-2 chain was detected through MALDI-TOF analysis, corresponding to a mass of 195.98 KDa (Table 2). This interaction may be important in maintaining cell-matrix stability and signaling pathway of GPS. If these interactions were disrupted, it may lead to or intensify several distinctive features associated with ADPKD. The findings of our study align closely with the research conducted by [23], which demonstrated the interaction between GST LRR a fusion protein with type I collagen, fibronectin and laminin fragments. Interestingly, our results also indicate the absence of binding between PC1 and collagens II, III, or IV.

In addition, in vitro study focused on investigating the function of C-type lectin domain and its capacity to bind to collagens type I, II, and IV. They successfully established the

interaction of the lectin domain to collagens type I, II, and IV proteins. These findings specified the potential for a direct communication between the product of the PKD1 gene and ECM proteins, as suggested in their research [19].

Changes in the composition, stiffness, and loading conditions of the ECM have a direct impact on cell behaviors. These altered cellular responses, in turn, contribute to the dysregulation of the ECM and the progression of various diseases [22], [37]. ADPKD, which is characterized by the formation of multiple bilateral renal cysts, the accumulation of ECM, and the development of tubulointerstitial fibrosis. Notably, cystic epithelial cells exhibit elevated levels of integrins, which are ECM receptors that regulate crucial cellular processes such as cell proliferation, migration, and survival.

The distinct alterations in integrins observed in cystic cells play a defining role in the pathophysiology of the disease [22]. In line with previous hypothesis, In PKD patients with fibrosis, inflammatory cells play a pivotal role in producing various pro-fibrotic growth factors. These growth factors, in turn, induce the proliferation of epithelial cells and activation of myofibroblasts, leading to the upregulation of genes associated with ECM production, such as collagen types 1 and 3, as well as fibronectin. Consequently, this process results in diminished glomerular function. It is noteworthy that approximately 50% of individuals with ADPKD eventually progress to ESRD [38].

PC1 and integrins have potential intersections at various levels. The extracellular region of PC1 contains multiple consensus motifs that are also found in cell adhesion molecules. This suggests that PC1 may possess adhesive properties and can bind to matrix components. Additionally, PC1 colocalizes with integrins at focal adhesions, implying that it may influence the interaction between integrins and their ECM ligands. Emerging evidence indicated that integrins expressed in cilia are involved in mechanical stimulation by ECM components, and this process may involve signal from PC1 [22].

In ADPKD, the increased expression of integrins results in changes to the adhesion and motility characteristics of cystic cells. This alteration in integrin levels can potentially initiate a positive feedback loop, which is further strengthened by the abnormal accumulation of ECM [39].

The ECM receptor integrin signaling pathway is crucial for the development of ADPKD in mice. Genetic evidence was generated indicating that a functional integrin-β1 is necessary for the initial stages of renal cyst formation in ADPKD. Moreover, it was suggested that targeting the integrin signaling pathway could be a promising therapeutic approach for slowing down the progression of the disease [39]. While previous research has established a significant interaction between PC1 and ECM , a direct connection was established in the current study between the GPS and ECM protein was novel and we also was hopping for therapeutic target to slow down or halt the formation of cysts [19], [23], [37].

It will take more research to define the functional connection between polycystin, integrin, and ECM. To track their interaction during the development of the kidney, to ascertain how extracellular events controlling polycystin conformation control the binding or release of extracellular matrix, and to investigate their expression and role in ADPKD cells and species without polycystin.

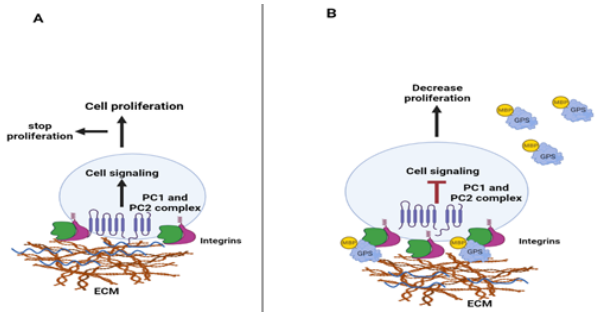

Figure 3: Predicated model to explaining the observed effect of GPS on HEK293 (A)Normal growth of cells. (B) MBP-GPS fusion protein may bind to the cell receptor or integrin, which triggers a cell signaling but don't completely stop proliferation. The extracellular material, PC1 and PC2 are represented

5. Conclusions

The period of work presented in this thesis has concurred with a period of active research on ADPKD and polycystins that has changed considerably since the localization of the PKD1 gene. Research moved from the study of minor events considered important for the pathogenesis of the disorder, alternatively concentrating on the major defect. The work presented here tried to link polycystin-1 to the basement membrane since mutation in the PKD1 gene lead to variety of TBM modification and the possible disturbance of any polycystin-1-ECM interactions could have important functional consequences for cell architecture and /or signalling. This interaction has confirmed previous prediction concerning polycystin-1-ECM interactions and contradicted others. Moreover, this could have a substantial effect on the area. The elucidation of the polycystin-1 GPS-ECM interactions and their possible role in cell proliferation also contribute to our potential importance for the identification of points of therapeutic invention.

The work presented in this study is novel and aimed to establish a connection between PC1 and the basement membrane.

Mutations in the PKD1 gene result in various modifications in the TBM, and any disruption of PC1 and basement membrane interactions could significantly impact cell structure and signalling. This interaction both confirmed previous predictions about PC1-ECM interactions and contradicted others. Furthermore, these findings could have significant implications for the affected areas. By unravelling the interaction between PC1 and the ECM in the context of cell proliferation, we can identify potential therapeutic targets.

Further studies can focus on characterizing the specific binding sites and the functional consequences of this interaction. Investigating the impact of the GPS domain on other cellular processes associated with ADPKD, such as cell-matrix adhesion, cell migration, and tissue remodeling. This can be explored using various in vitro and in vivo models, including cell culture systems, animal models, and three-dimensional tissue models.

Overall, these future research directions have the potential to advance our knowledge of ADPKD pathogenesis, contribute to the development of diagnostic tools, and open up avenues for the design of novel therapeutic interventions to mitigate the effects of this debilitating kidney disorder.

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Author Contributions

Study conceptualization, research question formulation, experimental framework design, hypothesis development, methodological outline, laboratory work supervision, paper authoring, and H.S.S. carrying out laboratory experiments, gathering and analysing data, carefully adhering to the experimental guidelines to guarantee accurate and trustworthy data collection, statistical analysis, and result interpretation, producing the report, M. A.

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Conflict of interest

The authors declare no conflict of interests. All authors read and approved final version of the paper.

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