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Elucidation of Structural Determinants of Polycystin-1 Interaction with Gα12

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Abstract

Autosomal dominant polycystic kidney disease (ADPKD) is a widespread genetic disease with a mortality rate of approximately 50%. Mutations in the cell surface protein polycystin-1 are found in nearly 80% of cases of this malady, and its interaction with the heterotrimeric G protein Gα12 has been implicated as an important event in disease progression. Heterotrimeric G proteins are involved in a myriad of cell signaling processes, including transcriptional activation and apoptosis. Polycystin-1 is important in down regulating Gα12-mediated JNK/Bcl-2 activation of programmed cell death (apoptosis), and abnormal apoptotic activity is a hallmark of ADPKD. Previous work has revealed a 20 amino acid G protein binding domain (GPS domain) within the cytosolic C-terminal tail of polycystin-1. Utilizing PCR directed mutagenesis several single and multiple charge swap mutations of charged amino acid residues within the first half of the GPS domain were developed. Co-precipitation assays of these mutant polycystin-1 constructs reveal the effect on the binding affinity for Gα12. In an attempt to mimic the genetics of the disease by engineering the same polycystin-1 variant found in some disease samples, revealed that a naturally occurring nonsense mutation resulting in a truncated protein severely impaired Gα12 binding. In identifying key amino acids within polycystin-1 that mediate Gα12 binding, the hope is to characterize the structural interface between these two proteins.

1. Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is attributed to mutations in the *PKD1* or *PKD2* genes. *PKD1* encodes for the protein polycystin-1 (Pc1), with mutations in this gene contributing to 70-85% of all cases of ADPKD. *PKD2* encodes for the protein polycystin-2 (Pc2), with mutations in this gene contributing to 15-30% of all cases of ADPKD.[1,3] Pc1 is a large (4302 amino acid), 11 trans-membrane spanning cell surface protein, with a short (~225 amino acid) carboxy-terminal cytoplasmic domain that has been characterized to interact with heterotrimeric G proteins.[1-4] G proteins are known to regulate many cell signaling functions, including the activation of the intrinsic pathway of apoptotic signaling.[2] Abnormal apoptotic behavior is an important hallmark of ADPKD; previous evidence shows that loss of Pc1 leads to cyst development.[1] Over-expression of Pc1 has also been shown to play a role in the development of cystic disease.[1,3]

Characteristic heterotrimeric G protein signaling results from a ligand binding to a G protein coupled receptor (GPCR) inducing a conformational change in the Gα subunit triggering dissociation of GDP and a loss of binding affinity for the Gβγ heterodimer.[1] Gα then rapidly binds to GTP, resulting in a conformational change to the active form, which allows for downstream signaling before the Gα subunit elicits it's intrinsic GTPase activity, causing hydrolysis of the GTP to GDP, thus returning the Gα subunit to its inactive conformation.[1]

Pc1 has been described as an atypical GPCR by mediating JNK/AP-1 activation of apoptosis through the down regulation of Gα12, a member of the Gα12/13 family of the Gα subunits.[1,3,5] Gα12 activates apoptosis by signaling PP2A-mediated activation of JNK1, which leads to the proteasomal degradation of Bcl-2, an anti-apoptotic protein.[2,3] Pc1 over-expression has been shown to decrease Gα12 activation of apoptosis, indicating that Pc1 inhibits Gα12-stimulated apoptosis.[3]

Previous research consisted of examining Pc1 for sequences within the cytoplasmic C-terminal tail necessary in binding

Gα12, which lead to the identification of a 74 amino acid sequence (4110-4183) necessary for binding Gα12.[1] This 74 amino acid sequence contained a 20 amino acid sequence RRLRLWMGFSKVKEFRHKVR (4134-4153) that has been described as a putative G protein binding domain (GPS domain).[1,5] A naturally occurring disease phenotype that is present in some patients with ADPKD is characterized by a single nucleotide change that changes the Arg-4227 to a stop codon (R4227X).[6,7] This nonsense mutation creates a loss of the extreme C-terminal 76 amino acids.[6,7] This mutation is in the cytoplasmic domain of Pc1, which is our region of interest for its interaction with Gα12.

Further research consisted of utilizing PCR directed mutagenesis in developing single and multiple charge swap mutations within the GPS domain. Pulldown assays were utilized to determine the ability of mutant GST-Pc1 fusion proteins to bind with $G\alpha12^{QL}$, the GTP deficient constitutively active form of Gα12, as previously described.[1] Serum response element assays and apoptotic reporter assays were used to determine the ability of $G\alpha12^{QL}$ to perform its normal signaling of transcriptional activation or activation of apoptosis when subjected to an interaction with wild-type Pc1 (Pc1-WT) or various mutant constructs of Pc1. Herein, we describe the interaction between several mutant forms of Pc1 (including the disease phenotype Pc1-R4227X) and Gα12, further elucidating the role of Pc1 interaction with Gα12-mediated transcription activation and Gα12-mediated apoptosis.

2. Materials and Methods

2.1 Creation of GST-Pc1 fusion mutants

GST-Pc1 fusion constructs were engineered using PCR directed mutagenesis to develop single and multiple charge swap mutations, C-terminal deletion mutations, and truncation mutations. Oligonucleotides were designed to make the single charge swap mutations K4144D, S4143D, R4135E, R4137E, and R4134E. Oligonucleotides were also designed to make the triple charge swap mutation RRLR4134-37EELE, termed Pc1-EELE. These single and multiple charge swap mutations are all within the GPS domain. Dr. Meigs had designed previously the C-terminal deletion mutant R4227X, which introduced a stop codon after the amino acid sequence LLTQFD, resulting in a deletion of the extreme C-terminal 76 amino acids. Oligonucleotides were also designed to create a deletion mutation of the sequence RLNQATEDVYQ, termed Δ4227-37. The Δ4227-37 construct required the use of PCR sewing techniques. The single and multiple charge swap mutations required quick-change PCR techniques. Each amplimer was then digested with NotI and BamHI, in order to create sticky ends, and then ligated into pGEX-4T-1, the GST-Pc1 fusion plasmid. Constructs were verified through sequencing.

2.2 Co-precipitation assays of GST-Pc1 C-terminal mutants and $G\alpha12^{QL}$

GST-Pc1 wild-type and mutant fusion constructs were expressed in BL21-Gold(DE3) *E. coli*, and immobilized on glutathione-sepharose beads. $G\alpha12^{QL}$ and Blank lysates were prepared from HEK293 cell supernatant as previously described.[1] Lysates were diluted with 1.6mL $H_{50}E_1D_3M_{10}$ (50mM Hepes, 1mM EDTA, 3mM DTT, 10mM $MgSO_4$) buffer. GST-Pc1 fusion beads and GST beads were re-suspended in 440µL $H_{50}E_1D_3M_{10}$ buffer with 200µL transferred to both 300µL of $G\alpha12^{QL}$ and Blank lysates. 30µL of cell lysates were kept as "loads" to quantify base levels of $G\alpha12^{QL}$. Interaction tubes were then gently rocked at 4ºC for 90 minutes. Interaction tubes were then spun in a refrigerated Beckman bench top centrifuge for 3 minutes at 1300 x g at 4ºC. Tubes were then washed with HEDLM ($H_{50}E_1D_3M_{10}$ with added 10% LPX at $1/200^{th}$ volume). Tubes were spun and washed again for a total of three spins and three washes. After the last spin supernatant was decanted, leaving 25µL left with sepharose bead pellet. 12µL of 4X/DTT was added to each interaction tube, and 15µL added to "load" tubes. Tubes were then vortexed and subjected to a 72ºC water bath for 10 minutes. Pulldown tubes were then stored at -20ºC to be used in polyacrylamide gel electrophoresis.

2.3 Polyacrylamide gel electrophoresis of GST-Pc1 pulldown of $G\alpha12^{QL}$

GST-Pc1 C-terminal pulldowns of $G\alpha12^{QL}$ and "load" tubes were separated using sodium-dodecylsulfate 12% polyacrylamide gel electrophoresis (SDS-PAGE). 5µL of interaction tubes were separated with 12% SDS-PAGE and stained with Coomassie blue staining solution. Gels not intended for Coomassie blue staining were then subjected to immunoblotting for $G\alpha12^{QL}$.

2.4 Immunoblot of Pulldowns

Proteins subjected to SDS-PAGE were then transferred to nitrocellulose paper using Western blot techniques as described previously.[1] The nitrocellulose paper with immobilized proteins were washed with a primary rabbit antibody specific to Gα12 at a 1:1000 dilution in 5% w/v Milk/TBST solution and then washed with a secondary anti-rabbit AP-conjugated antibody at a 1:7500 dilution in 5% w/v Milk/TBST. Immunoblots were developed using an AP/NBT/BCIP solution. Developed immunoblots and Coomassie blue stained gels had their bands quantified using a Gaussian fit curve to compare intensity of bands to the background in a Kodak Gel-Logic 100 gel documentation apparatus.

2.5 Co-transfection of Pc1 and Gα12 to determine effect on Serum Response

HEK293 eukaryotic cells were transfected with 500ng of $G\alpha12^{QL}$ or G228A plasmids (in pcDNA3.1(-) mammalian expression vector) together with 500ng of FLM, FLM-R4227X, pcDNA4ΔFLM, or FLM(-74) plasmids. Plasmids were allowed to complex with 1.5µL PEI mix for at least 15 minutes before adding drop-wise for the transfection of HEK293 cells. After 36-48 hours of incubation at 37ºC cells were prepared for Serum Response Element Luciferase assay by using passive lysis techniques. FLM is fused to Pc1 to act as a surrogate trans-membrane segment in order to localize Pc1 in the cell membrane.

2.6 Serum Response Element Luciferase Assay

HEK293 cells that had been co-transfected, as described above, had their medium discarded and replaced with 1mL 1X PBS buffer. 1X PBS was then removed and replaced with 250µL 1X Passive Lysis Buffer (Promega) containing protease inhibitors. Plates containing cells were then shaken on the New Brunswick platform shaker at 140 RPM for 25 minutes. After 25 minutes cell extracts were retrieved and transferred to microfuge tubes to be centrifuged for 60 seconds at 16,000 x g. 75µL of supernatant was transferred to 25µL 4X-DTT and subjected to a 72ºC water bath for 10 minutes. These tubes were then subjected to SDS-PAGE and immunoblotting for Gα12 as described previously.[1] Luciferase assay (Promega) was used, following manufacturer's instructions, to describe the effect of wild-type and mutant forms of Pc1 on $G\alpha12^{QL}$ transcriptional activation of Serum Response Element.

2.7 Detection of Bcl-2 degradation as an apoptotic reporter

Co-transfection techniques of HEK293 cells were used as described above; however, 1µg of either pcDNA3.1(-) or $G\alpha12^{QL}$ were co-transfected with 1µg of FLM, FLM-R4227X, FLM(-74), or pcDNA4ΔFLM and allowed to incubate with 3µL of PEI mix, before transient transfection of HEK293 cells. Cells were then lysed and prepared for SDS-PAGE and immunoblotting. Cells and lysis buffer were kept on ice throughout this process. Media from cells was removed and replaced with 5mL cold 1X PBS. 1X PBS was removed and replaced with 250µL lysis buffer (50mM HEPES (pH 7.5), 1mM EDTA, 3mM DTT, 2mM $MgSO_4$, 1% $C_{12}E_{10}$, and protease inhibitor mix). Cells were then scraped and the cell extract was transferred to microfuge tubes and subjected to gentle rocking for 20 minutes at 4ºC. Tubes were then spun at 4ºC at 18,000 x g for 30 minutes. Supernatant was transferred to fresh tubes and 60µL of the supernatant were transferred to 25µL 4X-DTT and subject to a 72ºC water bath for 10 minutes. These tubes were then subjected to SDS-PAGE and immunoblotting as described previously[1]; however, rather than a primary Gα12 antibody a primary, monoclonal Bcl-2 mouse antibody (BD Transduction Laboratories) was used at a 1:1000 dilution, and an appropriate secondary antibody (1:7500 dilution) was used.

2.8 Detection of phosphorylated JNK1 as an apoptotic reporter

Detection of total-JNK and phospho-JNK in HEK293 cells was used as an apoptotic reporter assay. HEK293 cells were co-transfected with 1µg of either pcDNA3.1(-) or $G\alpha12^{QL}$ was co-transfected with 1µg of FLM, FLM-R4227X, FLM(-74), or pcDNA4ΔFLM (as described above). After 36-48 hours of incubation cell lysates were obtained by removing nutrient media and replacing with 2mL 1X PBS buffer. 1X PBS buffer was then removed and replaced with 500µL 1X Passive Lysis buffer (Promega). Plates were then shaken at 140 rpm on a New Brunswick platform shaker for 25 minutes. Lysates were

transferred to 4X/DTT solution (¼ total volume) and subjected to a 72ºC water bath for 10 minutes. SDS- Pc1PAGE and immunoblotting were performed as described previously.[1] However, samples were probed separately with primary total-JNK and phospho-JNK T183/Y185 polyclonal rabbit antibodies at 1:200 dilution (SA Biosciences), and the appropriate AP-conjugated secondary antibody at 1:7500 dilution.

3. Results

3.1 Pc1 C-terminal mutant pulldowns of Gα12^{QL}

Several GST fusion Pc1 C-terminal mutant forms have been developed in order to characterize the interaction with Gα12: Pc1- K4144D, S4143D, R4135E, R4137E, R4134E (within the GPS domain, described above); Pc1- R4227X, Δ4227-37 (Fig. 1). Pulldown assays were performed to test the ability of GST-Pc1 C-terminal mutants (Pc1-R4137E, Pc1-RRLR4134-37EELE termed Pc1-EELE, Pc1-R4227X, Pc1-Δ4227-37) in binding with Gα12^{QL} (Fig. 2-5). GST-Pc1 C-terminal mutants K4144D, S4143D, R4135E, and R4134E were tested in pulldown experiments but are not shown as immunoblots did not develop. SDS-PAGE gels intended for Coomassie blue staining and immunoblotting was performed to analyze GST-Pc1 pulldowns of Gα12^{QL} (Fig. 2-5).

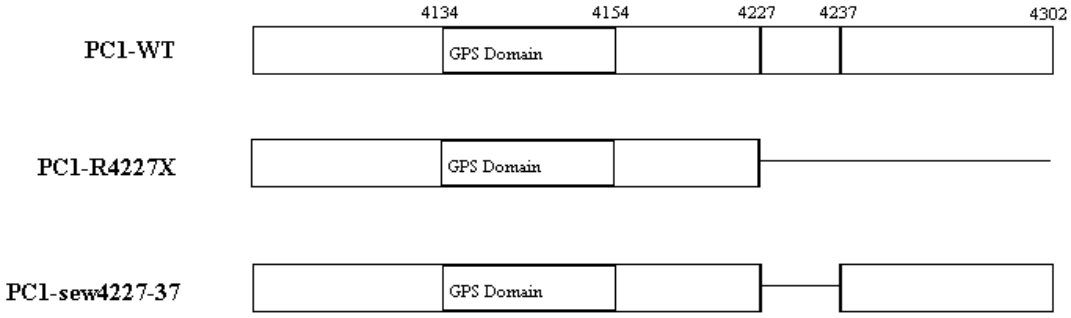

Figure 1. Polycystin-1 (Pc1) C-terminal forms: wild-type (WT) and mutants (R4227X, Δ4227-37). Pc1-R4227X has a stop codon introduced at Arg-4227, creating a nonsense mutation in proteins expressed in BL21-Gold (DE3) *Escherichia coli* and HEK293 cells. Pc1-Δ4227-37 was engineered using sewing PCR techniques to remove the DNA sequence encoding for amino acids 4227-37, resulting in a truncation mutation when expressed in BL21-Gold (DE3) *E. coli* with those 11 missing amino acids.

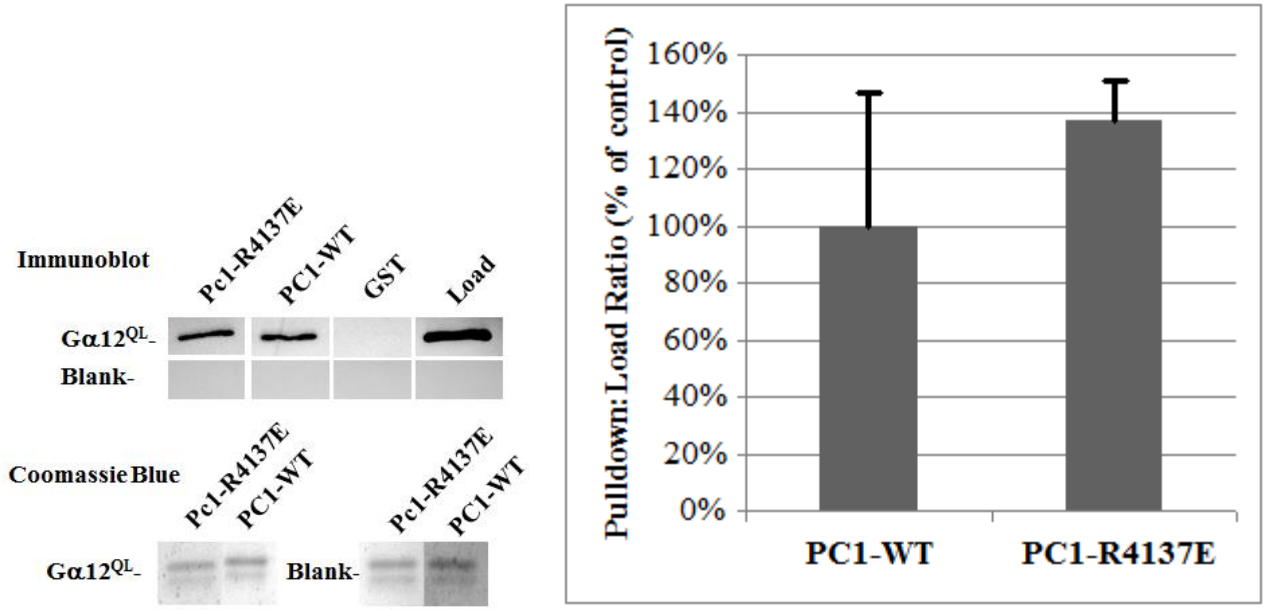

Figure 2. GST-Pc1 C-terminal wild-type and mutant form pulldowns of Gα12^{QL} (constitutively active form of Gα12). Band intensities for immunoblots were adjusted for concentration of GST beads determined by SDS-PAGE and Coomassie blue staining. (A) Representative immunoblot and Coomassie blue gel of pulldown. (B) Adjusted intensities (±SE) of Pc1-WT pulldowns that have been performed in conjunction with Pc1-R4137E.

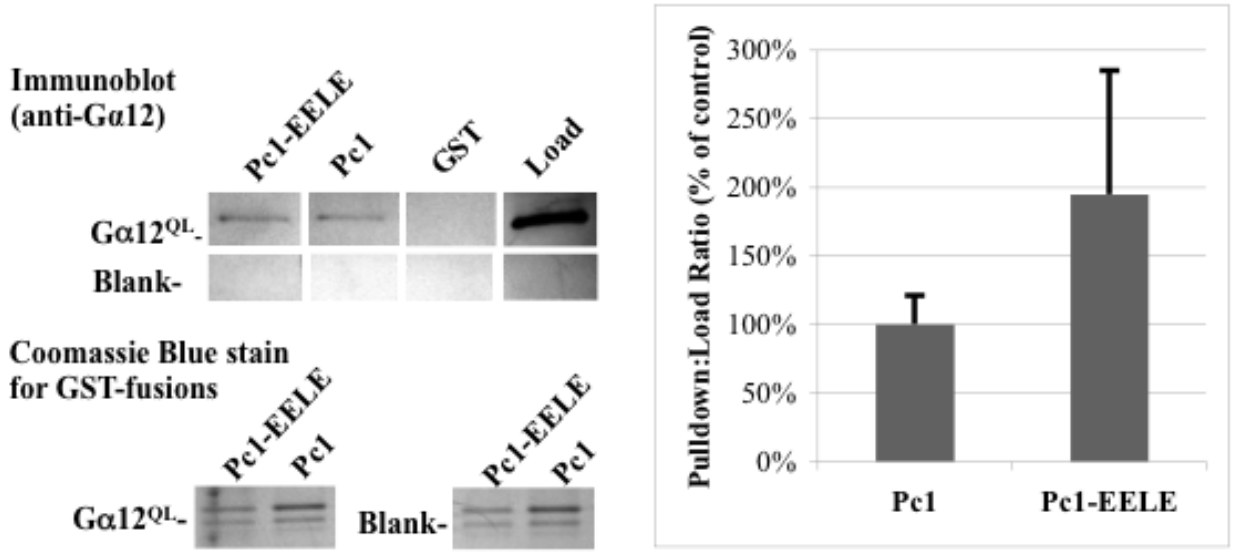

Figure 3. GST-Pc1 C-terminal wild-type and mutant form pulldowns of Gα12^{QL} (constitutively active form of Gα12). Band intensities for immunoblots were adjusted for concentration of GST beads determined by SDS-PAGE and Coomassie blue staining. (Above Left) Representative immunoblot and Coomassie blue gel of pulldown. (Above Right) Adjusted intensities (±SE) of Pc1-WT pulldowns that have been performed in conjunction with Pc1-EELE.

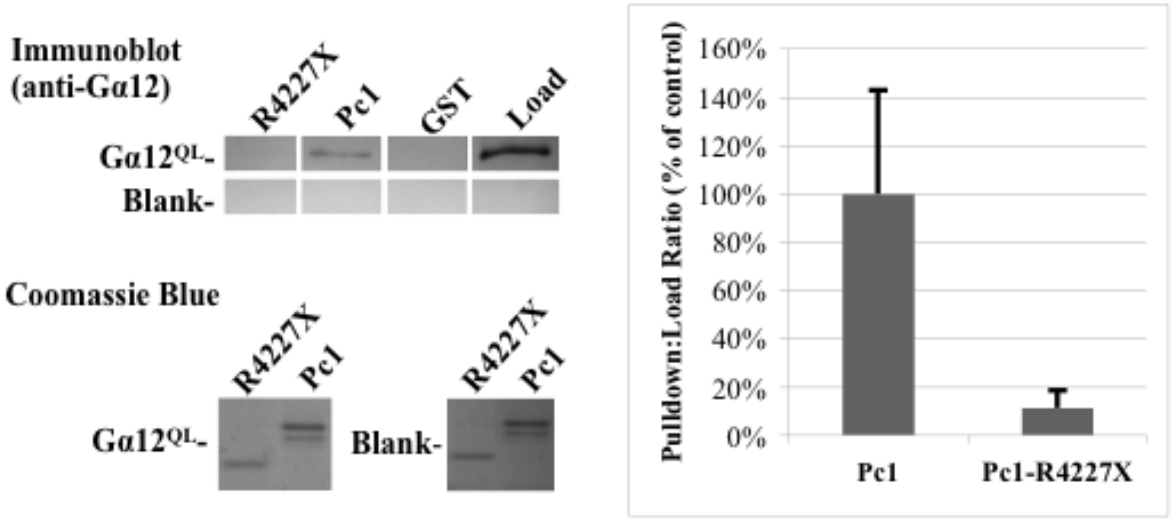

Figure 4. GST-Pc1 C-terminal wild-type and mutant form pulldowns of Gα12^{QL} (constitutively active form of Gα12). Band intensities for immunoblots were adjusted for concentration of GST beads determined by SDS-PAGE and Coomassie blue

staining. (Above Left) Representative immunoblot and Coomassie blue gel of pulldown. (Above Right) Adjusted intensities (±SE) of Pc1-WT pulldowns that have been performed in conjunction with Pc1-R4227X.

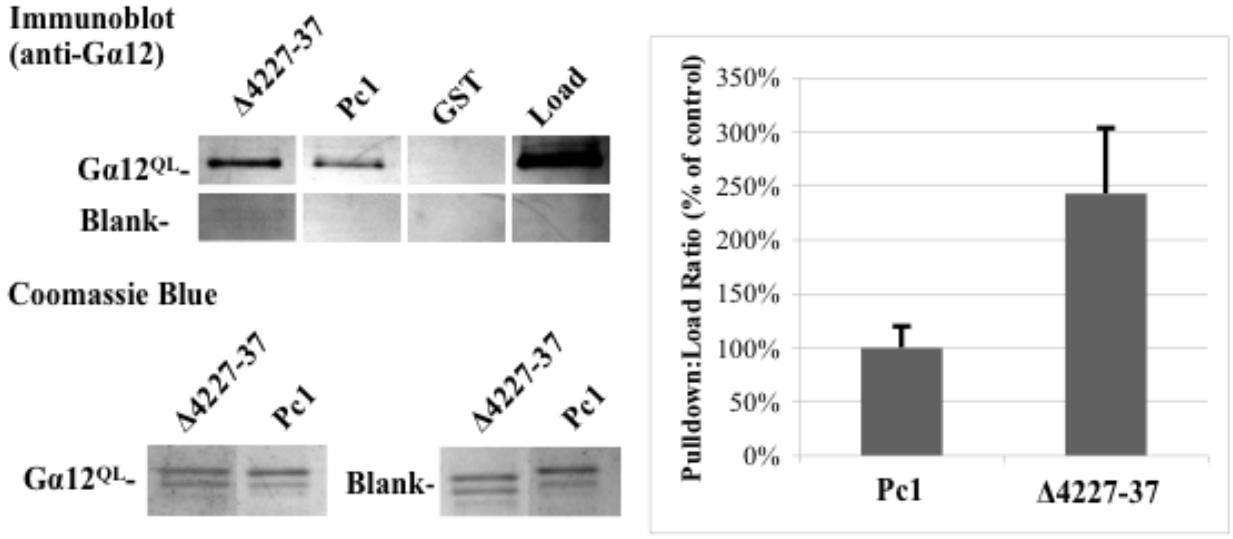

Figure 5. GST-Pc1 C-terminal wild-type and mutant form pulldowns of $G\alpha12^{QL}$ (constitutively active form of Gα12). Band intensities for immunoblots were adjusted for concentration of GST beads determined by SDS-PAGE and Coomassie blue staining. (Above Left) Representative immunoblot and Coomassie blue gel of pulldown. (Above Right) Adjusted intensities (±SE) of Pc1-WT pulldowns that have been performed in conjunction with Pc1-Δ4227-37.

Qualitatively, disruption of charged residues within the first 10 amino acids of the GPS domain showed not to reduce binding of the Pc1 C-terminal tail with $G\alpha12^{QL}$. Interestingly, the multiple charge swap mutation, Pc1-EELE, seemed to increase binding, with no apparent decrease. Surprisingly, Pc1-R4227X, the naturally occurring disease phenotype, showed nearly complete interruption in binding with $G\alpha12^{QL}$. Pc1-Δ4227-37 did not interrupt binding with $G\alpha12^{QL}$, rather, binding activity seemed to increase.

3.2 Serum Response Element assay of HEK293 cells co-transfected with $G\alpha12^{QL}$ and FLM-Pc1 C-terminal wild type and mutant forms

Serum Response Element (SRE) assays allow us to determine the ability of $G\alpha12^{QL}$ to perform transcriptional activation of when co-transfected with FLM-Pc1 C-terminal forms. When $G\alpha12^{QL}$ signals for transcriptional activation, the serum response factor will localize to the nucleus and bind to the SRE and promote the transcription of the downstream sequence. HEK293 cells will express luciferase proteins, which allow for us to determine luminometry and therefore, how well Gα12 is activated SRE-mediated transcriptional activation. Relative luciferase expression levels in HEK293 cells co-transfected with $G\alpha12^{QL}$ and FLM, FLM-R4227X, pcDNAΔFLM (empty vector), or FLM(-74) (Fig. 6). FLM is a surrogate of Pc1-CTT that allows Pc1 to be easily manipulated. FLM is a trans-membrane spanning sequence that acts to localize Pc1-WT and C-terminal mutant forms in the cell membrane.

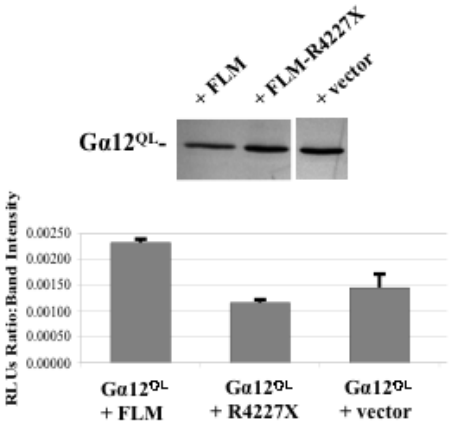

Figure 6. Serum response element assays performed on HEK293 cell lysates co-transfected with $G\alpha12^{QL}$ and FLM, FLM-R4227X, pcDNA4ΔFLM, or FLM(-74). (Top panel) Representative immunoblots for presence and relative amounts of $G\alpha12^{QL}$. (Lower panel) RLUs ratio determined by luciferase assay adjusted for immunoblot band intensity (±SE).

Serum response element assays revealed an increase in $G\alpha12^{QL}$-mediated transcriptional activation when co-transfected in HEK293 cells with FLM when compared to transcription activation by $G\alpha12^{QL}$ co-transfected with FLM-R4227X, pcDNA4ΔFLM, or FLM(-74).

3.3 Detection of Bcl-2 degradation as an apoptotic reporter

In order to determine the effect of wild-type and mutant forms of Pc1 C-terminus on Gα12 activation of apoptosis an apoptotic reporter assay was used. Assay based on Yanamadala et al. (2007).[2] Preliminary results indicate no marked differences in degradation of Bcl-2 in HEK293 cells (Figure 7), which is unexpected.

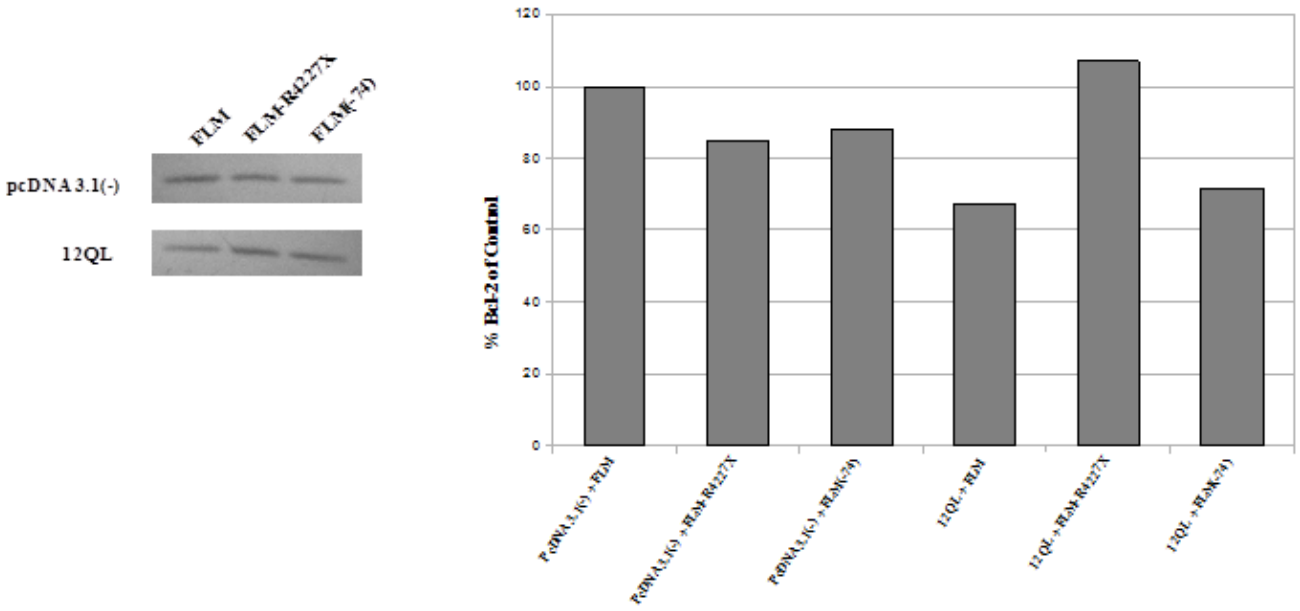

Figure 7. Apoptotic assay using detection of Bcl-2 degradation in HEK293 cell lysates co-transfected with pcDNA3.1(-) or $G\alpha12^{QL}$ and FLM, FLM-R4227X, pcDNA4ΔFLM, or FLM(-74). (A) Representative immunoblots for presence and relative amounts of $G\alpha12^{QL}$. (B) Bcl-2 degradation as percent Bcl-2 in controls (cells co-transfected with pcDNA3.1(-) and FLM). Assay adjusted for immunoblot band intensity.

3.4 Detection of phosphorylated JNK as an apoptotic reporter

Gα12-mediated signaling of apoptosis is facilitated through the phosphorylation of JNK1, which leads to proteasomal degradation of Bcl-2.[2] Apoptotic reporter assay based on Yanamadala et al. (2007)[2]. Results indicated no marked increase in cells co-transfected with $G\alpha12^{QL}$ (Figure 8), which is unexpected. There was a marked decrease in the percentage of phosphorylated-JNK in cells expressing $G\alpha12^{QL}$ and pcDNA4ΔFLM compared to cells expressing pcDNA3.1(-) and pcDNA4ΔFLM.

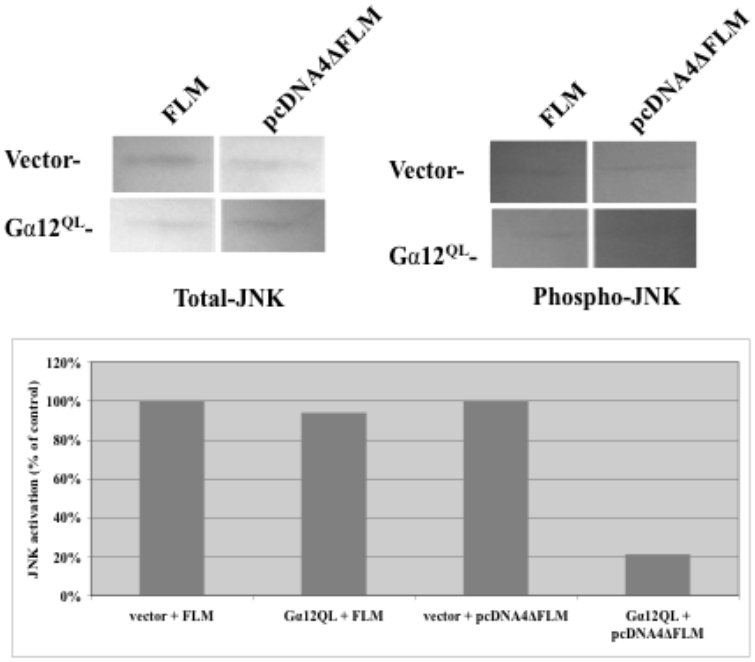

Figure 8. Apoptotic assay through immunoblotting of total and phosphorylated JNK in HEK293 cell lysates co-transfected with pcDNA3.1(-) or $G\alpha12^{QL}$ and FLM, FLM-R4227X, pcDNA4ΔFLM, or FLM(-74). (A) Representative immunoblots for presence and relative amounts of $G\alpha12^{QL}$. (B) Phosphorylated JNK as percentage of total-JNK. Assay adjusted for immunoblot band intensity.

4. Discussion

4.1 Polycystin-1 C-terminal interaction with Gα12

ADPKD is a complex disease characterized by abnormal apoptotic behavior, with the majority of cases attributed to mutations in *PKD1*, the gene encoding for the protein product Pc1; however, the role of Pc1 is not fully understood. By engineering mutations within the cytosolic C-terminal tail, a region known to interact with Gα12, we hope to better elucidate the structural interface between Pc1 and Gα12, and further describe the role of Pc1 in regulating Gα12-mediated apoptosis. Several mutations within the GPS domain appeared not to interrupt Gα12 binding. These included the single charge swap R4137E, and the multiple charge swap Pc1-EELE. Other single charge swap mutations tested were no longer pursued, as immunoblots failed to develop. These mutants included K4144D, S4143D, R4135E, and R4134E. Pc1-EELE actually seemed to increase binding to Gα12. Failure to disrupt binding shows that the Arg-4134, Arg-4135, and Arg-4137 do not play a role in binding Gα12.

In an attempt to mimic the genetics of a phenotype appearing in some disease samples, characterized by a nonsense mutation at Arg-4227, we revealed that the nonsense mutation R4227X resulted in a near complete loss in Gα12 binding. This prompted the development of the Δ4227-37 mutation, in which the eleven amino acid sequence RLNQATEDVYQ (4227-37) was removed, as previous research described a truncation after the sequence QATEDVYQ resulted in loss of

binding to Gα12.[1] With these findings in mind it appeared that the region 4227-37 may play a role in Gα12 binding; however, with the deletion of 4227-37 we revealed that Gα12 binding increased rather than decreased. This may be due to a change in conformation of the Pc1 C-terminal tail that allowed for more exposed binding sites elsewhere with the C-terminus.

The disease phenotype, R4227X, having near complete loss of Gα12 binding, was further pursued in order to gain a better understanding of the role of Pc1 in regulating Gα12-mediated apoptosis and transcriptional activation. Driving transcriptional activation through serum response element (SRE) is a downstream process of Gα12. In an attempt to determine the uncoupling effects of the Pc1-R4227X mutant on the regulation of Gα12, HEK293 cells were co-transfected with pcDNA3.1(-) or $G\alpha12^{QL}$ and FLM, FLM-R4227X, pcDNA4ΔFLM, or FLM(-74). These results reveal an increase in $G\alpha12^{QL}$-mediated transcriptional activation in cells co-transfected with FLM, the FLM-Pc1-WT fusion protein, when compared to cells co-transfected with FLM-R4227X, pcDNA4ΔFLM, or FLM(-74). These findings were unexpected, as Pc1 is known to down-regulate $G\alpha12^{QL}$-mediated apoptosis. Further research is necessary in order to determine if Pc1 is down-regulating Gα12-mediated apoptosis by up-regulating Gα12-mediated transcriptional activation.

Further investigation into the interaction of Pc1 and Gα12 consisted of attempting to characterize the uncoupling effects of Pc1 on Gα12-mediated signaling of apoptosis through the phosphorylation of JNK1 and proteasomal degradation of Bcl-2. Co-transfection assays of HEK293 cells with pcDNA.31(-) or $G\alpha12^{QL}$ and FLM, FLM-R4227X, pcDNA4ΔFLM, or FLM(-74). In the detection of either Bcl-2 or total/phospho-JNK, there were no marked increases in phosphorylation of JNK1 or degradation of Bcl-2. These results were unexpected, as literature indicates under the experimental conditions there is an increase in the degradation of Bcl-2 and an increase in phosphorylation activity of JNK1 when Madin Darbin Canine Kidney cells (MDCK) are transfected with $G\alpha12^{QL}$.[2] The literature also indicates that over-expression of Pc1 in MDCK cells results in the down-regulation of Gα12-mediated apoptosis.[3] The experiments we performed utilized HEK293 cells, which may account for the differences in findings reported in the literature.[2,3] These findings are all preliminary results from pilot experiments that must be further replicated and optimized.

Future research should consist of further investigation into the precise amino acids playing a role in Pc1 interaction with Gα12 within the GPS-domain spanning the sequence RRLRLWMGFSKVKEFRHKVR (4134-4153), by identifying the amino acids resulting in an uncoupling of Gα12. Future research may also consist of further analysis of the uncoupling effects of the disease phenotype, Pc1-R4227X, and how this uncoupling of Gα12 affects Gα12-mediated transcriptional activation and apoptosis. Subsequent apoptotic reporter assays will need to be performed in co-transfection experiments of HEK293 cells in order to determine the effects of Pc1 regulation of Gα12 in this cell line.

5. Acknowledgments

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