Research Article

High GSK3β expression in *Pkd*1 cells

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ABSTRACT

Background: Polycystic kidney disease (PKD) is an inherited disease characterized by renal cysts and progressive decline in renal function. At the molecular level, PKD thought to be caused by defective primary cilia structure and/or function. Renal cells showed increased proliferation profile leading to progressively enlarged cysts. Primary cilia, as the mechanosensory organelles sensitize fluid flow and trigger intracellular events. Regulation of cell proliferation is one of the cellular functions regulated by ciliary signaling. GSK3β, a protein kinase that play a vital role in cell proliferation. **Methods:** In this study, analysis of the GSK3β and p-GSK3β protein expression by western blot in renal mouse *Pkd1* cells was performed as a prospected pathogenic pathway of renal cyst. **Results:** Data revealed that GSK3β expression in *Pkd1* cells significantly higher than wild type cells proposing a high proliferative state of cell. In the same manner, p-GSK3β, the inactive form, showed to be significantly low in *Pkd1* cells compared to wild type renal cells. **Conclusion:** *Pkd1* cells showed high GSK3β and low p-GSK3β protein expression, implying a high proliferative profile of cells and suggest a mechanism for renal cyst expansion.

KEY WORDS: Cilia bending, Primary cilia, Renal cyst

INTRODUCTION

Primary cilia are mechanosensory organelles extending from the apical surface of cells. Cilia bending in response to fluid flow trigger signals that regulate various cell functions. Many studies documented that the sensory entity of the primary cilia is polycystins 1 and 2 (PC1 and PC2). These proteins form complex at the ciliary membrane, where PC1 is the mechanosensory part.[1,2] When fluid flow induces cilium bending, PC1 responds by activates its partner PC2, a non-specification channel. As a result, calcium current generated in the cilioplasm. This calcium current propagated to the cytoplasm and regulated various cellular functions.[3,4] Polycystic kidney disease (PKD) is a ciliopathy characterized by progressively enlarged renal cysts accompanied by decline in renal function. Studies revealed that PKD resulted from defective primary cilia structure and/or function. Dysfunctional renal primary cilia play a crucial role in the pathogenic events of cyst formation and expansion. PKD1 resulted from defective PC1 structure/function. Studies showed that PKD cells have defective calcium signal, while experiment to increase cilia length and function showed improvement in calcium signal. Calcium ions have inhibitory effect on GSK3β-regulated pathways of cell proliferation.[5,6] GSK3β, a protein kinase, that modulates many cellular signaling pathways through phosphorylation of substrate. GSK3β increases cell proliferation rate in some types of cancer.[6,7] In addition, it was shown that GSK3β reduces cAMP concentration. In renal cyst, it was shown that there is reduced cAMP level and it suggested to be a mechanism for cyst expansion. P-GSK3β is the inactivated form resulted from serine 9 phosphorylation of GSK3β.[5,8,9]

In this study, mouse *Pkd1* cells known to have defective PC1 and primary cilia functions used to assess the protein expression level of GSK3β and p-GSK3β by western blot as a prospective mechanism of renal cyst expansion.

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Website: jprsolutions.info	ISSN: 0975-7619

METHODS

Cell Culture

Renal epithelial cell LLC-PK was maintained in Dulbecco's Modified Eagle Medium with 10% fetal bovine serum, penicillin 100 U/ml, and streptomycin 100 µg/ml. *Pkd1* cell generated and maintained in Nauli's lab as described previously.[2,10]

Western Blot

Cells maintained until 95% confluent then lysed using RIPA buffer, total cells protein collected and

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Received on: 25-08-2018; Revised on: 17-09-2018; Accepted on: 12-10-2018

blotted in SDS-PAGE gel. Primary antibodies for GSK3β, p-GSK3β, glyceraldehyde 3-phosphate dehydrogenase, and secondary antibodies (Cell Signaling Technology) were used. Western blot imaging performed using ChemiDoc (BioRad) and analysis by Image Lab software (BioRad).

Statistics

Values represented as mean ± standard error mean. All experiments repeated at least 3 times. Comparisons among means were done using Student's *t*-test. Statistically significant values represent $P < 0.05$. All data analyses were done using GraphPad Prism v.5.

RESULTS

GSK3β Expression in Cilia Defective Cells

Regarding PKD, it is known that *Pkd1* cells have defective primary cilia function, abnormally low flow-induced calcium signals, and aberrant proliferation profile.[11,12] To assess primary cilia-calcium axis-dependent cell effectors, protein expression of GSK3β in renal *Pkd1* and wild-type cells achieved. Western blot technique of whole cell lysate showed that expression of GSK3β in *Pkd1* cells is significantly ($P < 0.05$) greater than equimolar protein loading of wild-type LLC-PK cells [Figure 1]. Quantitative analysis of the blot after normalization and standardization relative to the control showed that expression of GSK3β significantly ($P < 0.05$) increased by about 1.9-fold compared to the wild-type cells (1.89 ± 0.11 vs. 1.0 ± 0.0) [Figure 2a]. To confirm our results, measurement of p-GSK3β protein expression - the inactive form of GSK3β - was also done. Western blot analysis showed that the p-GSK3β expression was significantly ($P < 0.05$) decreased in the *Pkd1* cells compared to wild-type renal cells [Figure 2b]. Quantitative analysis of the blot relative to control revealed that p-GSK3β expression in *Pkd1* cell was significantly ($P < 0.05$) lower by about 3.4-fold than the wild-type LLC-PK cells (0.29 ± 0.06 vs. 1.0 ± 0.0) [Figure 2b].

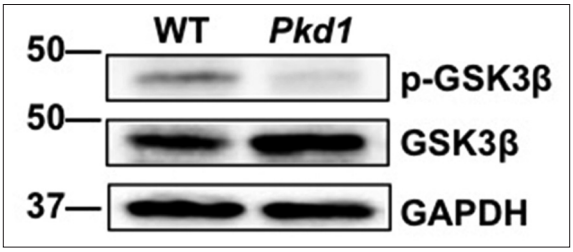

Figure 1: Images of western blot. Expression of GSK3β and p-GSK3β band in western blot membrane showed the various intensities in *Pkd1* and wild-type renal cells. Blot loaded in equimolar concentrations of total protein with glyceraldehyde 3-phosphate dehydrogenase as a control. Whole cell lysate of *Pkd1* and wild-type cells used, $n = 3$

DISCUSSION

Pkd1 cell generated in Nauli's lab and proved to have defective PC1 and consequently defective primary cilia mechanosensory function.[3] In this study, *Pkd1* cells used to evaluate the expression level of GSK3β and its inactive form p-GSK3β, as a suggestive pathogenic pathway for renal cyst expansion.

Renal cysts filled with fluid in PKD, a prominent feature that leads to renal failure was under thorough investigation to uncover the causative pathway of the disease. Primary cilia-induced calcium signal activates cAMP pathway in response to fluid flow. It was shown that *Pkd1* cells have defective response to fluid flow and have lower calcium current and cAMP level.[5,8,13] Renal cyst cells also shown to have high proliferative rate compared to normal cells.[14,15] In addition, GSK3β plays an important role in regulating cell proliferation has an inhibitory effect on cAMP pathway. In this study, GSK3β was measured in *Pkd1* cells proved to be significantly higher than wild-type renal cells. This result augments others who suggest a pathogenic role of GSK3β in renal cyst development.[5,8,13] In addition, this result considered compatible with others who suggest an inhibitory effect of cilia-induced calcium current on GSK3β pathway.[5,16] *Pkd1* cells known to have defective cilia-induced calcium signal.[17,18] Further support for this hypothesis provides in this study by measurement of p-GSK3β, the inactive

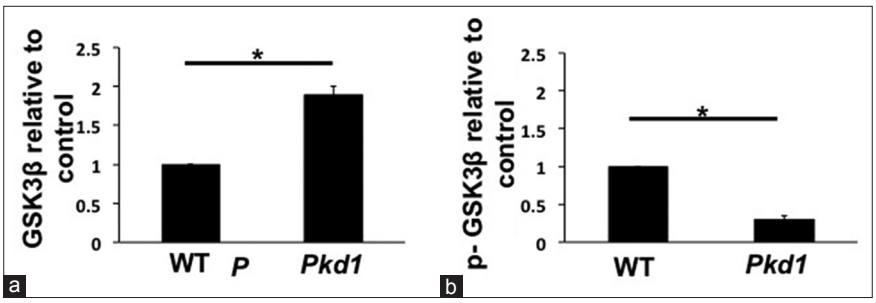

Figure 2: Representative graph from analysis of western blot. A, *Pkd1* cells showed higher expression of GSK3β compared to wild type. B, *Pkd1* cells showed lower expression of p-GSK3β compared to wild type. Results analyzed relative to control (glyceraldehyde 3-phosphate dehydrogenase). Asterisk denoted significant difference $p<0.05$, values are mean ± standard error mean, $n=3$.

form of GSK3β. Western blot analysis of p-GSK3β protein expression revealed a significantly lower level in Pkd1 cells compared to wild-type renal cells. The later result implied that GSK3β pathway is exaggerated in renal cyst and the regulatory pathway that phosphorylates and inactivates GSK3β is also downregulated in *Pkd1* cells. Altogether, the results obtained from this study support the hypothesis that suggests a pathogenic role of GSK3β in renal cyst development. This pathway activated by the defective cilia-induced calcium current pathway.[19] This pathway could be considered as a prospective therapeutic target for PKD. Intervention with GSK3β pathway by the use of GSK3β inhibitors and knockout models hindering renal cyst development and may be a promising target for new drugs.[5]

In summary, renal *Pkd1* protein expression of GSK3β is higher and p-GSK3β is lower compared to wild-type renal cells implying a pathogenic role of GSK3β in PKD.

ACKNOWLEDGMENT

The author would like to thank Dr. Surya Nauli (Chapman University, CA, USA) for his unlimited and generous support in this study.

REFERENCES

1. Kathem SH, Mohieldin AM, Nauli SM. The roles of primary cilia in polycystic kidney disease. AIMS Mol Sci 2014;1:27-46.
2. Nauli SM, Alenghat FJ, Luo Y, Williams E, Vassilev P, Li X, *et al*. Polycystins 1 and 2 mediate mechanosensation in the primary cilium of kidney cells. Nat Genet 2003;33:129-37.
3. Pala R, Alomari N, Nauli SM. Primary cilium-dependent signaling mechanisms. Int J Mol Sci 2017;18:E2272.
4. AbouAlaiwi W, Lo ST, Nauli SM. Primary cilia: Highly sophisticated biological sensors. Sensors 2009;9:7003-20.
5. Tao S, Kakade VR, Woodgett JR, Pandey P, Suderman ED, Rajagopal M, *et al*. Glycogen synthase kinase-3beta promotes cyst expansion in polycystic kidney disease. Kidney Int 2015; 87:1164-75.
6. Harwood AJ. Regulation of GSK-3: A cellular multiprocessor. Cell 2001;105:821-4.
7. Mishra R. Glycogen synthase kinase 3 beta: Can it be a target for oral cancer. Mol Cancer 2010;9:144.
8. Abdul-Majeed S, Nauli SM. Calcium-mediated mechanisms of cystic expansion. Biochim Biophys Acta 2011;1812:1281-90.
9. Calvet JP. In: Li X, editor. The Role of Calcium and Cyclic AMP in PKD. Brisbane (AU): Polycystic Kidney Disease; 2015.
10. AbouAlaiwi W, Takahashi M, Mell BR, Jones TJ, Ratnam S, Kolb RJ, *et al*. Ciliary polycystin-2 is a mechanosensitive calcium channel involved in nitric oxide signaling cascades. Circ Res 2009;104:860-9.
11. Mangolini A, de Stephanis L, Aguiari G. Role of calcium in polycystic kidney disease: From signaling to pathology. World J Nephrol 2016;5:76-83.
12. AbouAlaiwi W, Ratnam S, Booth RL, Shah JV, Nauli SM. Endothelial cells from humans and mice with polycystic kidney disease are characterized by polyploidy and chromosome segregation defects through survivin down-regulation. Hum Mol Genet 2011;20:354-67.
13. Kakade VR, Tao S, Rajagopal M, Zhou X, Li X, Yu AS, *et al*. A CAMP and CREB-mediated feed-forward mechanism regulates GSK3beta in polycystic kidney disease. J Mol Cell Biol 2016;8:464-76.
14. Belibi FA, Reif G, Wallace DP, Yamaguchi T, Olsen L, Li H, *et al*. Cyclic AMP promotes growth and secretion in human polycystic kidney epithelial cells. Kidney Int 2004;66:964-73.
15. Torres VE. Cyclic AMP, at the hub of the cystic cycle. Kidney Int 2004;66:1283-5.
16. Lu SY, Huang ZM, Huang WK, Liu XY, Chen YY, Shi T, *et al*. How calcium inhibits the magnesium-dependent kinase gsk3beta: A molecular simulation study. Proteins 2013; 81:740-53.
17. Upadhyay VS, Muntean BS, Kathem SH, Hwang JJ, Aboualaiwi WA, Nauli SM. Roles of dopamine receptor on chemosensory and mechanosensory primary cilia in renal epithelial cells. Front Physiol 2014;5:72.
18. Jin X, Muntean BS, Aal-Aaboda MS, Duan Q, Zhou J, Nauli SM. L-type calcium channel modulates cystic kidney phenotype. Biochim Biophys Acta 2014;1842:1518-26.
19. Atkinson KF, Kathem SH, Jin X, Muntean BS, Abou-Alaiwi WA, Nauli AM, *et al*. Dopaminergic signaling within the primary cilia in the renovascular system. Front Physiol 2015;6:103.

Source of support: Nil; Conflict of interest: None Declared