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The Phosphorylation of Polycystin-2, the Product of the ADPKD2 Gene

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INTRODUCTION

Autosomal dominant polycystic kidney disease (ADPKD) consists of at least two genetically disorders characterized by bilateral cyst formation and progressive renal enlargement. It results in renal failure in approximately ~50% of affected individuals by the age of 60 years. Mutations in PKD1 and PKD2 account for ~85% and ~15% of ADPKD families, respectively. The function of polycystin-1 (PC-1) and polycystin-2 (PC-2), the gene products of PKD1 and PKD2, remain unknown. It has hypothesized that PC-2 functions as a cation channel subunit that is regulated by PC-1. PC-2 has been shown an ER membrane glycoprotein of 110 kDa and an intracellular calcium release channel. Ion channels, like so many other cellular proteins, are regulated by protein phosphorylation.

There are 4 strong putative phosphrylation sites in intracellar C-terminal of PC-2. We have examined (1) the phosphorylation status of PC-2 *in vivo* and determine the specific sites for phosphorylation (2) the role of phosphorylation of PC-2 a) on its cation channel function b) on its subcellular localization and trafficking, c) on its interaction with PC-1 and self-association with PC-2.

METHODS

1. *In vivo* ^{32}P-orthophosphate labeling of cultured cells and cultured kidney tissues

We have MDCK, M1 and LLC-PK1, stably expressing full length PKD-2 and PKD-2 phosphorylation site mutants. The cells and mouse kidney tissue were incubated with ^{32}Pi. The cell lysates were carried to immunoprecipitation (IP) by PC-2 Ab (E2). After autoradiography, IP was verified by immunoblot (IB) using PC-2 Ab (C2, B9).

2. The functional role of phosphorylation of PC-2

1) The cation channel function

LLC-PK1 cells stably expressing wild-type and mutant PC-2 were stimulated with vasopressin, to induce intracellular calcium release. Changes in the ratio of calcium-dependent fluorescence over prestimulus background fluorescence (F/F0) were plotted in LLC-PK1 cells.

2) The subcellular localization and trafficking

Several cells lines of PKD-2 and PKD-2 mutant were used. Subcellular localization were invested by IF cell staining, Endo H sensitivity test and cell surface biotinylation.

3) The interaction with PC-1 and self-association with PC-2 : Co-IP

Co-transfection of a HA-tagged wild type

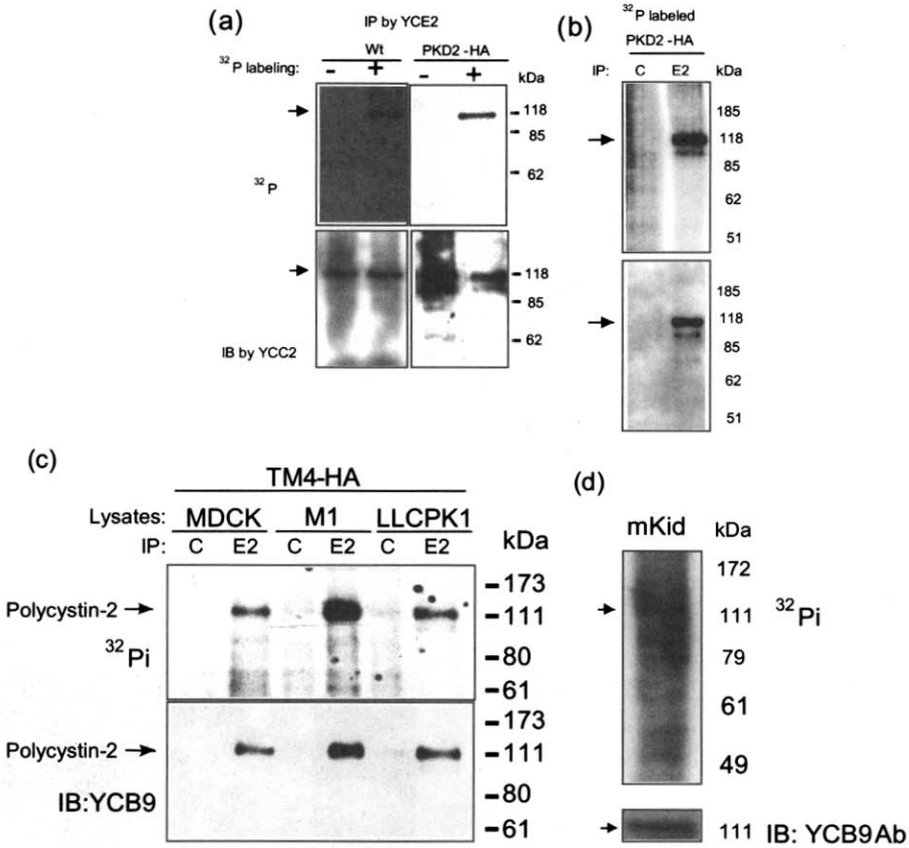

Fig. 1. *Polycystin-2 was constitutively phosphorylated in vivo.*

PKD-2 and mutant Myc-tagged PKD-2 were followed IP and IB with anti-HA and anti-Myc. Co-transfection of a HA-tagged PKD-1 and wild type or phosphrylation site-mutant Myc-tagged PKD-2 were followed by IP and IB with anti-HA and anti-Myc.

RESULTS

1. *In vivo* ^{32}P-orthophosphate labeling of cultured cells and cultured kidney tissues

We know there are 4 putative phosphorylation sites in PKD-2; T721, S801, S812 and S829. All cell lines and mouse kidney were constitutively phosphorylated in vivo (Fig. 1). PC-2 was phosphorylated at S812 (Fig. 2).

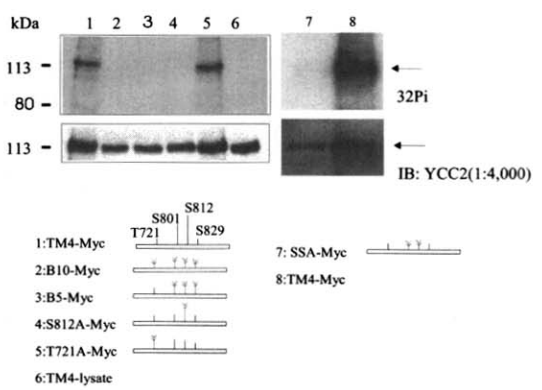

Fig. 2. *Polycystin-2 was phosphorylated at S812 site.*

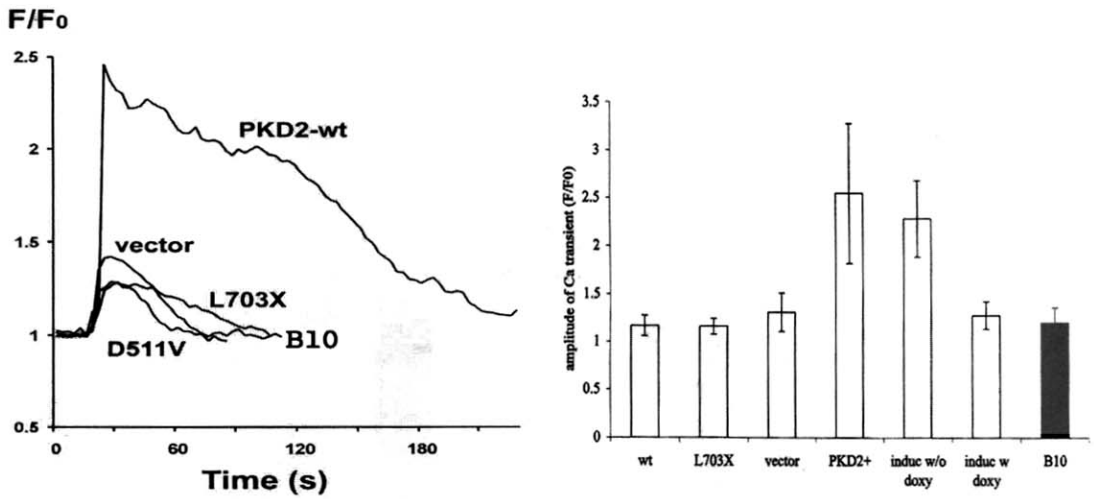

Fig. 3. *The vasopressin-induced calcium transients was measured in LLC-PK1 cells. B10 lost Ca^{++}-channel activity.*

2. The functional role of phosphorylation of PC-2

1) The cation channel function

Overexpressed wild type PKD-2 showed significant large calcium transients, compared to control cell. The phosphorylation site-mutant (B10) lost Ca^{++}-channel activity (Fig. 3).

2) The subcellular localization and trafficking

The B10 mutant, which was defect in phosphorylation, retained the same expression pattern (ER) as wild type PKD-2 (Fig. 4).

3) The interaction with PC-1 and self-association with PC-2 : Co-IP

Mutant PC-2 D511V (missense), B10 (phosphorylation mutant) as well as wild type, retained interaction with wild type PC-2 and L703 (truncated mutant) lost interaction with wild type PC-2 (Fig. 5). B10 probably showed loss of interaction capacity with PC-1 (Fig. 6).

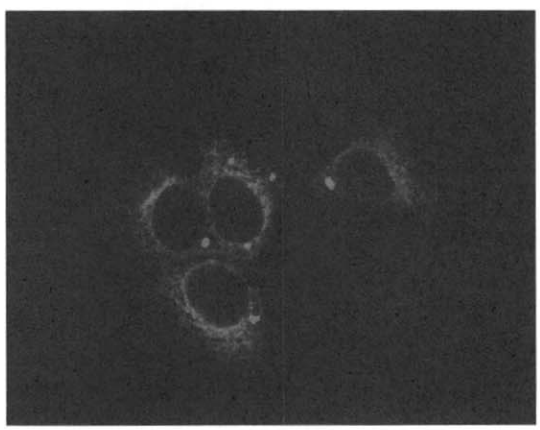

Fig. 4. *B10 mutant, which was defect in phosphoryaltion, was observed in a fine reticular cytoplasmic and perinuclear pattern consistant with expression in ER.*

DISCUSSION

In present study, we provide evidence that PC-2 expressed in the ER is a calcium channel. PC-2 is phosphorylated at S812 in vivo. The phosphorylation mutant (B10) loses the calcium channel activity. The phosphorylation of PC-2 has no effect in the subcellular localization and

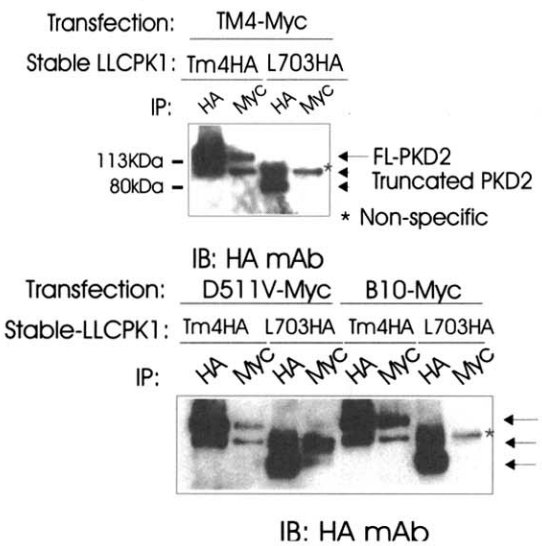

Fig. 5. *Mutant polycystin-2 D511V, B10 (phosphorylation-defective), as well as wild type, retained interaction with wild type polycystin-2.*

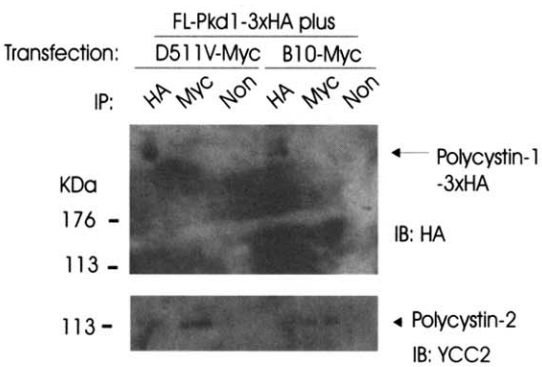

Fig. 6. *Phosphorylation-defective polycystin-2 (B10) probably showed loss of interaction capacity with polycystin-1.*

trafficking. But it probably influences the interaction capacity with PC-1. In conclusion, the phosphorylation of PC-2 is essential to its normal function.

In future study, we will determine which kinases are responsible for phosphorylation of PC-2. Using specific kinases and activators or inhibitors of these kinases will be utilized to identify important components of PKD2 pathway.

REFERENCES

1) Igarishi P, Somlo S : Genetics and pathogenesis of polycystic kidney disease. *J Am Soc Nephrol* **13**(9):2384-2398, 2002
2) Koulen P, Cai Y, Geng L, Maeda Y, Nishimura S, Witgall R, Ehrlich BE, Somlo S : Polycystin-2 is an intracellular calcium release channel. *Nat Cell Biol* **4**(3):191-197, 2002
3) Somlo S, Ehrlich B : Human disease : calcium signaling in polycystic kidney disease. *Curr Biol* **11**(9):R356-360, 2001
4) Somlo S, Markowitz GS : The pathogenesis of autosomal dominant polycystic kidney disease : an update. *Curr Opin Nephrol Hypertens* **9**(4): 385-394, 2000
5) Wu G, Somlo S : Molecular genetics and mechanism of autosomal dominant polycystic kidney disease. *Mol Genet Metab* **69**(1):1-15, 2000
6) Wu G, Markowitz, GS, Li L, D'Agati VD, Factor SM, Geng L, Tibra S, Tuchman J , Cai Y, Park JH, van Aelsberg J, Hou H Jr, Kucherlapati R, Edelmann W, Somlo S : Cardiac defects and renal failure in mice with targeted mutations in Pkd2. *Nat Genet* **24**(1):75-78, 2000
7) Obermuller N, Gallagher AR, Cai Y, Gassler N, Gretz N, Somlo S, Witzgall R : The rat pkd2 protein assumes distinct subcellular distributions in different organs. *Am J Physiol* **277**:F914-925, 1999
8) Emmons SW, Somlo S : Signal transduction. Mating, channels and kidney cysts. *Nature* **401** (6751):339-340, 1999
9) Cai Y, Maeda Y, Cedzich A, Torres VE, Wu G, Hayashi T, Mochizuki T, Park JH, Witzgall R, Somlo S : Identification and characterization of polycystin-2, the PKD2 gene product. *J Biol Chem* **274**(40):28557-28565, 1999
10) Markowitz GS, Cai Y, Li L, Wu G, Ward LC, Somlo S, D'Agati VD : Polycystin-2 expression is developmentally regulated. *Am J Physiol* **277**: F17-25, 1999
11) Wu G, D'Agati V, Cai Y, Markowitz G, Park JH, Reynolds DM, Maeda Y, Le TC, Hou H Jr, Kucherlapati R, Edelmann W, Somlo S : Somatic inactivation of Pkd2 results in polycystic kidney disease. *Cell* **93**(2):177-188, 1998
12) Wu G, Mochizuki T, Le TC, Cai Y, Hayashi T, Reynolds DM, Somlo S : Molecular cloning, cDNA sequence analysis, and chromosomal localization

of mouse Pkd2. *Genomics* **45**(1):220-3, 1997

13) Hayashi T, Mochizuki T, Reynolds DM, Wu G, Cai Y, Somlo S : Characterization of the exon structure of the polycystic kidney disease 2 gene (PKD2). *Genomics* **44**(1):131-136, 1997

14) Qian F, Germino FJ, Cai Y, Zhang X, Somlo S, Germino GG : PKD1 interacts with PKD2 through a probable coiled-coil domain. *Nat Genet* **16** (2):179-183, 1997

15) Mochizuki T, Wu G, Hayashi T, Xenophontos SL, Veldhuisen B, Saris JJ, Reynolds DM, Cai Y, Gabow PA, Pierides A, Kimberling WJ, Breuning MH, Deltas CC, Peters DJ, Somlo S : PKD2, a gene for polycystic kidney disease that encodes an integral membrane protein. *Science* **272**(5266):1339-1342, 1996