Pro-survival role of protein phosphatase-5 (PP5) in clear cell renal cell carcinoma through its post-translational regulation

Rebecca A. Sager[1,2], Elham Ahanin[1,2,3], Sarah J. Backe[1,2,3], Natela Dushukyan[1,2,3], Michael Daneshvar[4], Gennady Bratslavsky[1,2,3], Mark R. Woodford[1,2,3], Dimitra Bourboulia[1,2,3], Mehdi Mollapour[1,2,3*]

[1]Department of Urology, [2]Upstate Cancer Center, [3]Department of Biochemistry and Molecular Biology, SUNY Upstate Medical University, Syracuse, NY, USA; [4]Department of Urology, University of California Irvine, Orange, CA, USA
*Correspondence: mollapom@upstate.edu; Twitter: @MedMol

Background

- PP5 is a serine/threonine phosphatase that regulates hormone- and stress-induced signaling networks
- Substrates include GR, p53, and the kinase co-chaperone Cdc37, which assists in maturation of many oncogenic kinases
- PP5 plays a survival role in breast and colorectal cancer, among others
- PP5 levels and activity are increased in clear cell renal cell carcinoma

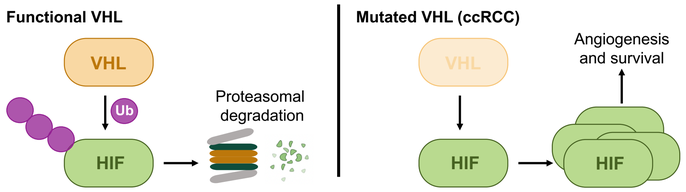
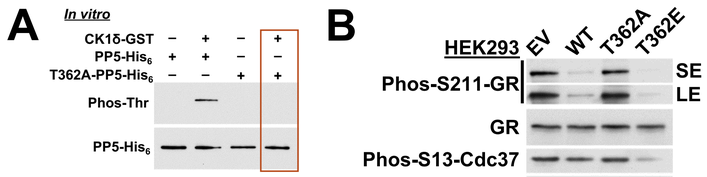
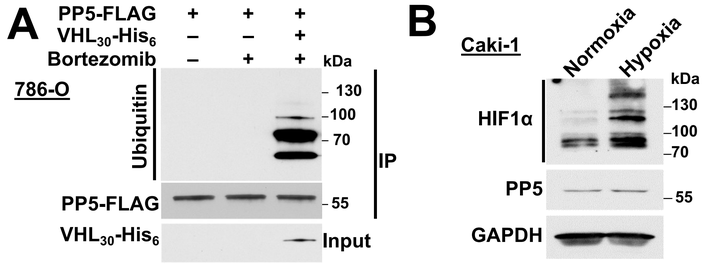


Fig 1. Phosphorylation of PP5 increases its activity



[A] Purified PP5-His and non-phosphomutant PP5-T362A were incubated with purified CK1δ-GST and phos-Thr examined. **[B]** PP5-FLAG and T362-PP5 phospho-mutants were transiently transfected into HEK293 cells, protein levels were examined by immunoblotting. (SE – short exposure, LE – long exposure)

Fig 2. VHL containing E3-ligase ubiquitinates and degrades PP5 independent of hypoxia



[A] 786-O cells transiently expressing PP5-FLAG with and without co-expression of VHL_{30}-His_6, were treated with 50 nM bortezomib (BZ) for 2 hr. PP5-FLAG was immunoprecipitated and ubiquitination was assessed by immunoblotting. **[B]** VHL-positive RCC, Caki-1 cultured in normoxia and hypoxia ($1\%O_2$, $5\%CO_2$, $94\%N_2$)

- **VHL** promotes **PP5 degradation** in a **hypoxia-independent manner**
- **PP5** is **phosphorylated** and has **increased activity in ccRCC**
- **Silencing or inhibition** of **PP5** leads to **apoptosis** in **VHL-null ccRCC**

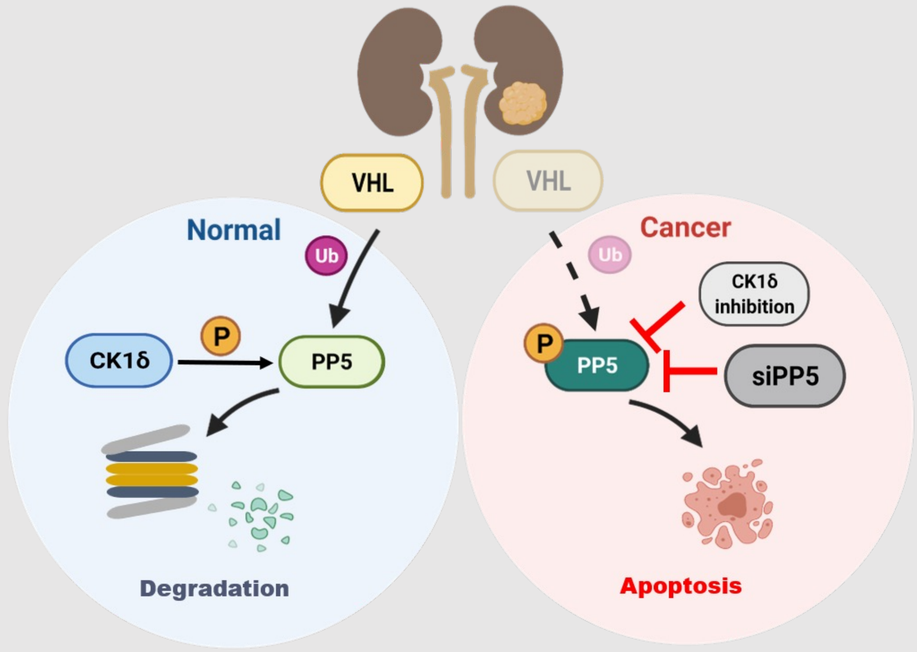
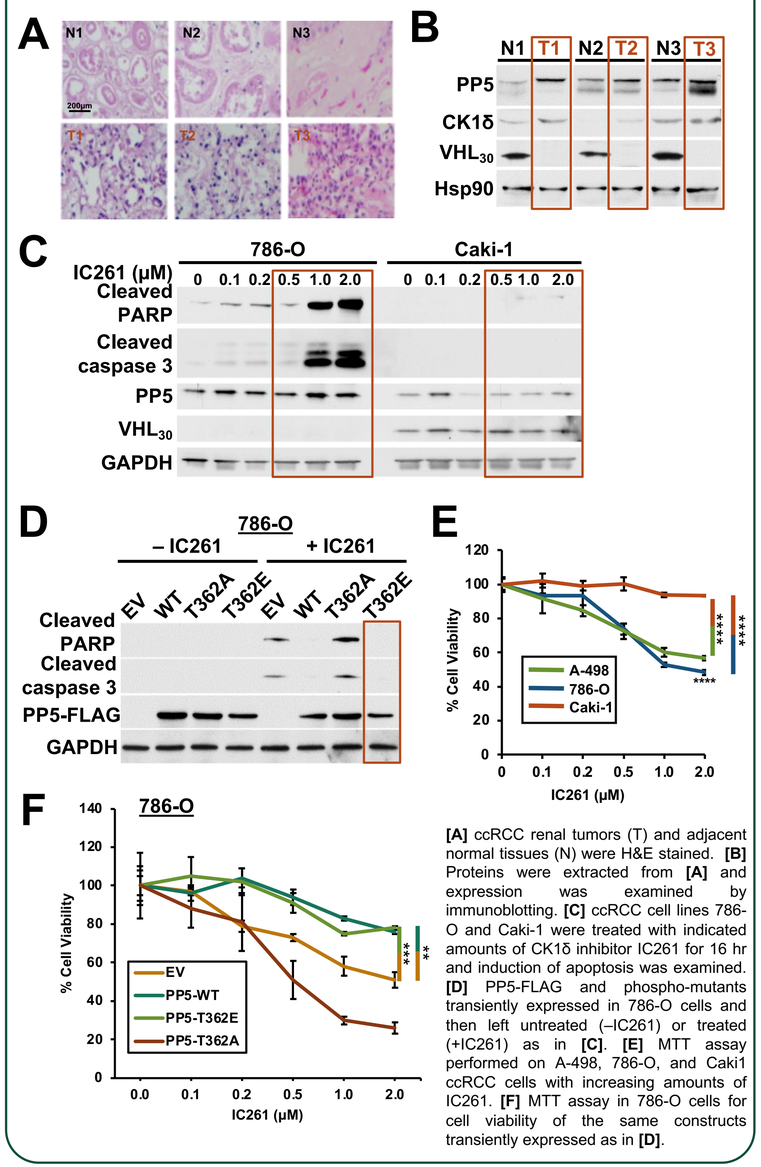


Fig 3. Inhibition of CK1δ induces apoptosis in VHL-null ccRCC cells through inhibition of PP5



[A] ccRCC renal tumors (T) and adjacent normal tissues (N) were H&E stained. **[B]** Proteins were extracted from **[A]** and expression was examined by immunoblotting. **[C]** ccRCC cell lines 786-O and Caki-1 were treated with indicated amounts of CK1δ inhibitor IC261 for 16 hr and induction of apoptosis was examined. **[D]** PP5-FLAG and phospho-mutants transiently expressed in 786-O cells and then left untreated (–IC261) or treated (+IC261) as in **[C]**. **[E]** MTT assay performed on A-498, 786-O, and Caki1 ccRCC cells with increasing amounts of IC261. **[F]** MTT assay in 786-O cells for cell viability of the same constructs transiently expressed as in **[D]**.

[1] Sager RA, Dushukyan N, Woodford M, Mollapour M. Structure and function of the co-chaperone protein phosphatase 5 in cancer. Cell Stress and Chaperones. 2020;25(3):383-394.
[2] Dushukyan N, Dunn DM, Sager RA, Woodford MR, Loiselle DR, Daneshvar M, Baker-Williams AJ, Chisholm JD, Truman AW, Vaughan CK, Haystead TA, Bratslavsky G, Bourboulia D, Mollapour M. CellRep. 2017 Nov 14;21(7):1883-1895.

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