Post-translational regulation of Polycystin 2 (PC2) and its implication in biliary damage and repair

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Introduction

Polycystic liver diseases (PLD) represent important examples of genetic cholangiopathies: these are diseases of the intrahepatic biliary tree that lead to various degrees of chronic liver impairment and to a variety of complications, including cholangiocarcinoma. Among PLD, autosomal dominant polycystic kidney disease (ADPKD) is characterized by an abnormal development of intrahepatic biliary epithelia, which undergo a progressive cystic enlargement causing and massive hepatomegaly with mass effect or, more rarely, to cyst hemorrhage, infection, or rupture. ADPKD is due to mutations in the genes encoding for polycystin-1 (PC1) or -2 (PC2), membrane protein complexes localized at focal adhesions, cellcell junctions and cilia.

Recently in PC2-KO cholangiocytes we showed an altered cellular Ca2+ homeostasis and an increased intracellular cAMP/PKA/ERK-mediated stimulation of mTOR and HIF-1α-regulated VEGF secretion. Furthermore VEGF regulates cholangiocytes proliferation and peribiliary vascularization in several diseased conditions and experimental models and most likely represent a mechanism that drives the adaptive and reparative response after liver injury. The aim of our project is to study the mechanisms of protein degradation involved in PC2 down-regulation in liver damage.

Specific Background and Aims: a genetic defect of PC2 in cholangiocytes causes inappropriate production of cAMP, PKA-dependent activation of the ERK1/2 pathway, HIF1α-mediated VEGF production and stimulation of cyst growth and disease progression in in polycystic liver diseases (PLD). Based on these findings, we hypothesized that modulation of PC2 may represent a fundamental mechanism to stimulate VEGF secretion during repair from biliary damage.

Results

1. Is PC2 degradation Proteasome-mediated?

Proteasome inhibition (MG132) restores PC2 expression in inflammatory but not in ER stress conditions:

MG132 restore PC2 expression in mouse cholangiocytes treated with TNFa, IFNγ, IL-1β as a single agent or in combination (Mix), but not in cholangiocytes treated with DETAnonate or Thapsigargin (p<0.01)

2. Is PC2 degradation in ER-Stress conditions autophagy-mediated?

PI3K inhibitors and chloroquine restore PC2 expression in mouse cholangiocytes treated with ER-stressors or NO donors.

(I) and (II) PI3K inhibitors (LY and Wortmannin) and chloroquine restore PC2 expression in mouse cholangiocytes treated with Thapsigargin or DETAnonate. In cells treated with thapsigargin or DETAnonate, LC3B a well known marker of activated autophagy is increased (II) (p<0.01)

Proteasome inhibition (Bortezomib) in vitro reduces the biliary damage in mouse model of biliary cholesterol.

Treatment with Bortezomib in DDC treated (A) significantly reduce the ductular reaction as shown by the staining for the cholangiocyte-specific marker CK19 (p<0.01 vs Ctr) and restored the expression of PC2. Interestingly, the same treatment is able only to reduce the extent of ductular reaction (B), but also the deposition of biliary (Sirius Red, C) in Mx2 mouse

Conclusions

- PC2 protein expression is reduced in mice with biliary damage but not in mice with parenchymal damage;
- PC2 protein expression is reduced in cholangiocytes treated with pro-inflammatory cytokines, ER-stressors and NO-donors;
- Pro-inflammatory cytokines, NO-donors or ER-stressors increase HIF1α and VEGF in cholangiocytes;
- PC2 degradation is differentially mediated by proteasome during inflammation or by autophagy during ER-stress;
- In vivo proteasome inhibition reduces the ductular reaction and restores PC2 expression in mouse models of biliary damage.

Perspectives

Proteasome inhibitors and Autophagy regulators may represent a therapeutic option in cholangiopathies

Collaborations

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