Impact of poly(rC)-binding protein 2 (PCBP2) on the malignant phenotype of cholangiocarcinoma

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1. Background:

The altered expression of Poly(rC)-binding protein 2 (PCBP2), belonging to the hnRNP family of regulatory splicing factors, has been associated with oncogenesis and chemoresistance in several cancer types [1, 2]. However, its relevance in cholangiocarcinoma (CCA) is yet unknown.

The **aim** of this work was to elucidate the role of PCBP2 in CCA malignant phenotype and refractoriness to antitumor drugs.

2. Methods:

- PCBP2 gene expression was analyzed in CCA and adjacent non-tumor tissues (NT) in silico using data from TCGA database (n=36/9, CCA/NT) and by TLDA in samples obtained at the Salamanca University Hospital (n=23/9, CCA/NT) and in human primary cholangiocytes MMNK-1 (n=2).
- PCBP2 protein expression and localization was determined by IHC in both intrahepatic (iCCA) and extrahepatic (eCCA) CCA and NT samples (n=13/10/9, iCCA/eCCA/NT) .
- EGI-1 and Huh7 cell lines were used as in vitro models. PCBP2 was silenced in these cell lines by transducing them with lentiviral vectors either empty (MOCK) or carrying shRNA against PCBP2 (sh-PCBP2). Silencing was confirmed by RT-qPCR and WB.
- Transcriptomic analysis of sh-PCBP2 cells was carried out by RNASeq.
- Migration and proliferation assays were performed by holographic microscopy.
- The cell cycle was analyzed by flow cytometry, while the expression of apoptosis-related genes was determined by RT-qPCR to study the induction of apoptosis.
- MTT assay was performed to determine the effect of antitumor drugs on cell viability.

3. Results:

PCBP2 expression in CCA

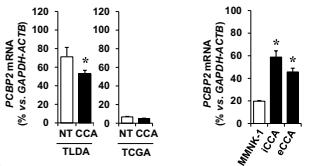

Figure 1. mRNA abundance of PCBP2 in unpaired samples of cholangiocarcinoma (CCA) and in adjacent non-tumor tissue (NT) determined experimentally (TLDA) and in silico (TCGA). Values are mean ± SEM of expression normalized by that of GAPDH and ACTB. *, p<0.05 comparing CCA vs. NT.

Figure 2. mRNA abundance of PCBP2 in the immortalized cholangiocyte cell line MMNK-1, in iCCA and eCCA samples determined by TLDAs. Values are mean ± SEM of expression normalized by that of ACTB. *, p<0.05 compared with MMNK-1.

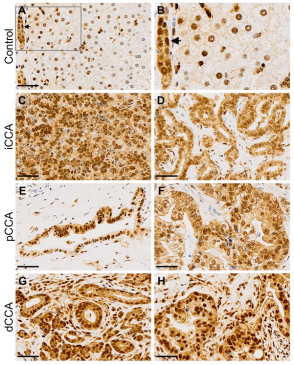

Figure 3. Representative images of histological sections of adjacent non-tumor (control) tissue (A,B) and tumors from patients with intrahepatic cholangiocarcinoma (iCCA) (C,D), perihilar cholangiocarcinoma (pCCA) (E,F), and distal cholangiocarcinoma (dCCA) (G,H) analyzed by immunohistochemistry for PCBP2. The slides were counterstained with hematoxylin. Scale bar = 50 µm. The arrows in A and B point to cholangiocytes.

Cellular models of *PCBP2* silencing

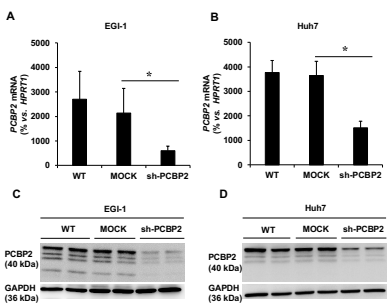

Figure 4. *PCBP2* expression determined by RT-qPCR in EGI-1 (A) and Huh7 (B) cells: wild-type (WT), MOCK or sh-PCBP2. Values are the mean ± SD of expression normalized by that of HPRT1 (n=4). Representative western blots of PCBP2 in EGI-1 (C) and Huh7 (D) cell lines for WT, MOCK, and sh-PCBP2 conditions (30 µg protein/ lane). *, p<0.05 comparing sh-PCBP2 vs. MOCK.

Effect of *PCBP2* silencing on cell cycle and apoptosis

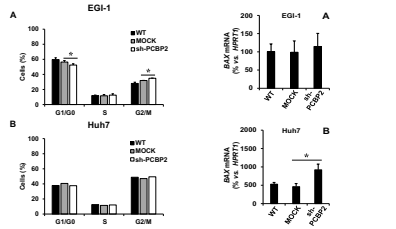

Figure 5. Percentage of cells in each cell cycle phase measured by flow cytometry in EGI-1 (A) and Huh7 (B) cells: wild-type (WT), MOCK or sh-PCBP2. Values are the mean ± SD of % cells (n=2). *, p<0.05 comparing sh-PCBP2 vs. MOCK.

Figure 6. *BAX* expression determined by RT-qPCR in EGI-1 (A) and Huh7 (B) cells: wild-type (WT), MOCK or sh-PCBP2. Values are the mean ± SD of normalized expression by that of HPRT1 gene (n=4). *, p<0.05.

Effect of PCBP2 silencing on cell proliferation and response to antitumor drugs

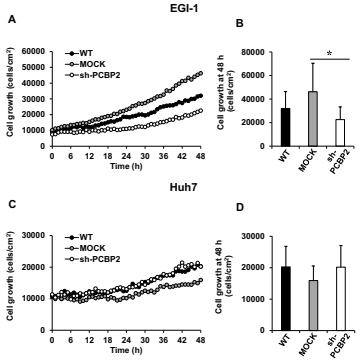

Figure 7. Cell proliferation determined by real-time digital holographic microscopy for 48 h in EGI-1 (A) and Huh7 (C) cells: wild-type (WT), MOCK or sh-PCBP2. Number of cells detected by holographic microscopy at 48 h from the start of the experiment in EGI-1 (B) and Huh7 (D) cells Values are the mean ± SD of the number of cells/well at different points (n=4). *, p<0.05 comparing sh-PCBP2 vs. MOCK.

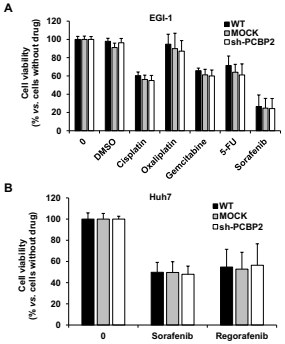

Figure 8. Effect of PCBP2 silencing on cell proliferation in EGI-1 (A) and Huh7 (B) cells in wild-type (WT), MOCK or sh-PCBP2 cells treated or not (Control?) with cisplatin, oxaliplatin, gemcitabine, 5-fluorouracil (5-FU), sorafenib, regorafenib or with vehicle (DMSO). Values are mean ± SD determined in triplicate (n=4).

4. Conclusions

PCBP2 is involved in regulating CCA cell migration and proliferation. Therefore, this splicing factor can be considered a potential target for developing novel pharmacological strategies to treat this cancer.

BIBLIOGRAPHY

1. 1 Zhang X et al. Overexpression of PCBP2 contributes to poor prognosis and enhanced cell growth in human hepatocellular carcinoma. Oncol Rep 2016; 36: 3456-3464.
2. 2 Chen C et al. Poly(rC) binding protein 2 (PCBP2) promotes the viability of human gastric cancer cells by regulating CDK2. FEBS Open Bio 2018; 8: 764-773.

Acknowledgements

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Patient Friendly Lay Summary Panel

Aim:

To understand whether a protein called PCBP2 helps cholangiocarcinoma (bile duct cancer) grow and spread, and to see if it could become a future treatment target.

Methods:

We studied tumor samples from patients with cholangiocarcinoma and compared them with nearby non-cancer tissue. We also used bile duct cancer cells in the laboratory and reduced the amount of PCBP2 in these cells. Then we measured how well the cancer cells survived, multiplied, formed colonies, moved, and responded to common chemotherapy drugs. We also analyzed which genes changed after lowering PCBP2.

Results:

Higher levels of PCBP2 were linked to cancer spread (metastasis) and faster disease progression in patients. In lab-grown cancer cells, lowering PCBP2 reduced cell growth, survival, colony formation, and movement, meaning the cells became less aggressive. However, reducing PCBP2 did not clearly improve the effect of standard chemotherapy drugs. The study also found that PCBP2 may control other genes that help tumors grow.

Conclusion:

PCBP2 appears to support the aggressive behavior of bile duct cancer. Although it may not strongly affect chemotherapy resistance, blocking PCBP2 could be a promising new strategy to slow tumor growth and spread in the future.