Detection of cholangiocarcinoma with protease activity probes

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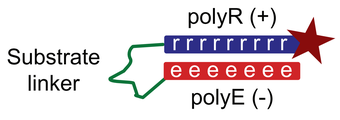
Introduction

- Cholangiocarcinoma (CCA) is a deadly malignancy of the bile ducts. Its poor prognosis is due in large part to inadequate diagnostic methods, which preclude early detection and hamper accurate disease staging.
- Proteases have been shown to be dysregulated in CCA and represent a promising diagnostic target.
- We have previously developed a novel class of diagnostic agents, activatable zymography probes (AZPs), to visualize tumor-associated protease dysregulation.
- We sought to develop probes to enable accurate, early detection of CCA.

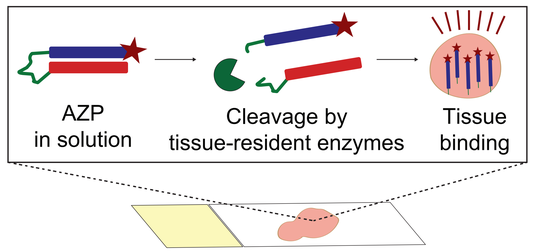
Methods

- We applied a library of 26 AZPs to tissue sections from mouse models of CCA and biliary fibrosis and performed image quantification.
- We then performed immunofluorescence staining to identify proteolytically active cell populations.
- Finally, we performed in vivo administration of AZPs in mouse models of CCA and biliary fibrosis.

Activatable zymography probes localize protease activity *in situ*



Activatable zymography probe (AZP)



Results

CCA tumors are induced in fibrotic livers

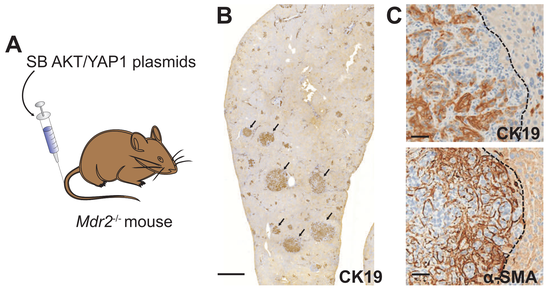


Fig. 1: A. Schematic of tumor induction **B.** CK19 staining of tumor-bearing livers. Scale: 500 μm. **C.** CK19 and α-SMA staining at the tumor boundary. Scale: 50 μm.

AZP6 distinguishes CCA from fibrosis

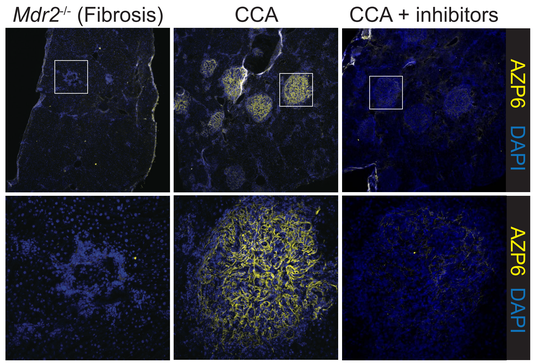


Fig. 2: Binding of AZP6 to CCA tumors from the AKT/YAP model and *Mdr2*$^{-/-}$ fibrotic portal tracts. Top is 4x, bottom is 20x.

Whole-slide quantification of AZP binding

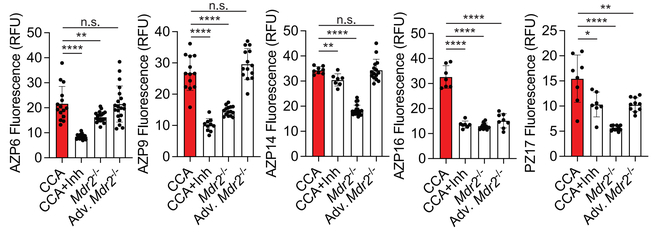


Fig. 3: Quantification of AZP binding in CCA tumors (+/- broad spectrum protease inhibitors), *Mdr2*$^{-/-}$ mice, and advance *Mdr2*$^{-/-}$ mice.

AZP6 binds to stromal cells in CCA tumors

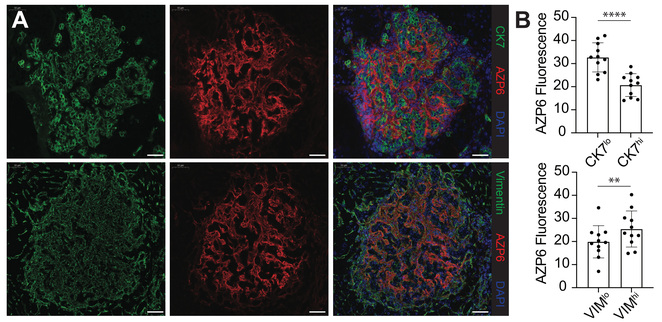


Fig. 4: A. Binding of AZP6 with co-immunostaining for cytokeratin 7 (top) or vimentin (bottom). Scale: 50 μm. **B.** Quantification of AZP6 fluorescence in cells with low or high expression of cytokeratin 7 (top) or vimentin (bottom). n = 11 tumors. **p < 0.01, ****p < 0.0001.

AZP6 is generalizable across subtypes of CCA

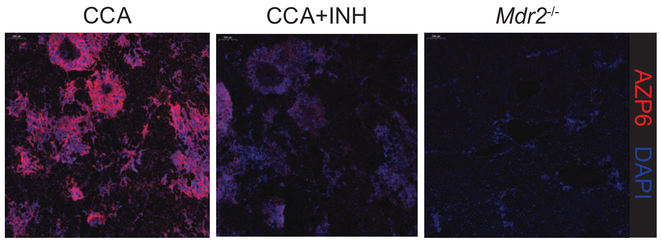


Fig. 5: Binding of AZP6 to CCA tumors from the FBXW7/AKT model (INH: broad-spectrum protease inhibitors) and *Mdr2*$^{-/-}$ fibrotic portal tracts. Top is 20x, bottom is 5x.

AZP6 preferentially accumulates in CCA *in vivo*

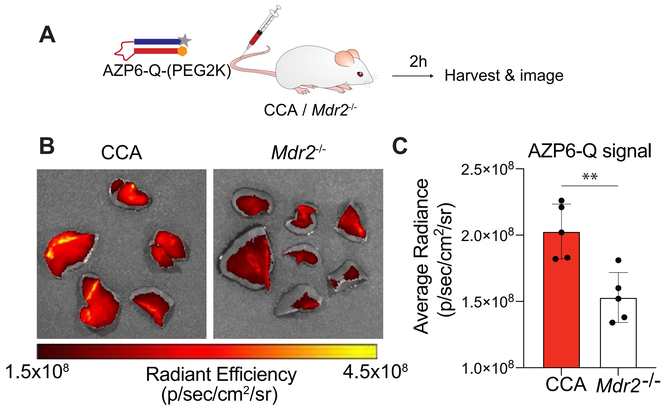


Fig. 6: A. Schematic of approach. **B.** Fluorescence images of explanted livers. **C.** Quantification of AZP6 fluorescence intensity in explanted livers (n = 5 per condition). **p < 0.01.

Conclusion

AZPs enable *ex vivo* identification of peptide substrates that are cleaved specifically by proteases in two distinct mouse models of CCA. AZP6 localizes to mesenchymal cells and enables tumor-selective labeling after *in vivo* administration. Protease-activated diagnostics may enable sensitive and specific detection of CCA *in vivo*.

Acknowledgments

We thank H. Fleming for editorial input. This study was supported in part by the Ludwig Fund for Cancer Research and the KI Marble Center for Cancer Nanomedicine. S.N.B. is a HHMI Investigator.