Detection of cholangiocarcinoma with Protease activity probes

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 in vivo administration.
- 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.

Results
- AZP6 distinguishes CCA from fibrosis
  - AZP6 binds to stromal cells in CCA tumors
  - AZP6 is generalizable across subtypes of CCA

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.

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Figures:
- 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
- Whole-slide quantification of AZP binding
- AZP6 is generalizable across subtypes of CCA
- AZP6 preferentially accumulates in CCA in vivo
- AZPs are effective in vivo diagnostic probes for CCA.