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Hepatic Stellate Cell-Derived Cancer Associated Fibroblasts Sustain Tumor Growth in Intrahepatic Cholangiocarcinoma

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Background: Cholangiocarcinoma (CCA) is characterized by abundant cancer-associated fibroblasts (CAF). Clinical data suggest an inverse correlation between αSMA-positive CAF and survival in CCA. However, functional *in vivo* studies that determine whether CAF promote or restrict CCA are lacking. **Objectives**: To determine the cellular source and functions of CAF in intrahepatic CCA (ICC) *in vivo*. **Methods and Results:** Sleeping beauty-mediated overexpression of HA-tagged pT3-myr-AKT and pT3-EF1aH-YapS127A in the liver resulted in desmoplastic ICC with abundant αSMA-positive CAF and significant upregulation of fibrogenic genes *Acta2* (34-fold) and *Col1a1* (125-fold). Expression of hepatic stellate cell (HSC) markers *Des* and *Lrat* mRNA was significantly increased in ICC (11- and 3-fold, respectively). HSC origin of CAF was further supported by tracing studies in triple transgenic mice expressing LratCre and Cre reporter TdTom, which label >99% of HSCs in the liver, in combination with Col1a1-GFP, which labels collagen-producing fibroblasts. 92.95± 5.27% (n=10) of CAF were TdTom-positive, i.e. derived from LratCre+ HSC. To functionally manipulate HSC-derived CAF *in vivo*, we employed triple transgenic mice expressing LratCre, Cre-inducible iDTR and Cre reporter TdTom. By this approach, we depleted >90% CAF, as determined by TdTom fluorescence, qPCR for HSC markers *Lrat*, *Lhx2* and *Des*, as well as *Col1a1*, but not portal fibroblast marker *Msln*. Short-term CAF depletion reduced fibrosis, tumor proliferation, as determined by Ki67 staining and qPCR for *mKi67* and *Ccnb1*, and increased cell death in ICC. **Conclusion:** Our study suggest that the majority of CAF are HSC-derived, and HSC-derived CAF support the development of ICC by promoting tumor cell proliferation and survival.