Temporal and Spatial Dynamics of Hepatic Stellate Cell Activation in Intrahepatic Cholangiocarcinoma (Abstract P-01)

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INTRODUCTION

Cholangiocarcinoma (ICC) is the 2nd most common hepatic tumor after hepatocellular carcinoma (HCC) that is characterized by its highly desmoplastic stroma. This has led to investigations on cancer-associated fibroblasts as well as **hepatic stellate cells (HSCs)**, the major source of liver myofibroblasts, in ICC progression. Findings from these studies remain inconsistent on whether the fibrotic components function to promote or restrain ICC progression. One of the potential reasons accounting for this inconsistency is that these studies did not differentiate the fibrous components regarding their temporal and spatial relationship to ICC development. The liver is one of the few internal organs that are developmental equipped with a complex damage-response machinery to protect its vital function in metabolism. Considering that nearly every chronic liver condition eventually results in liver fibrosis, it is conceivable that a growing malignant mass will elicit various fibrotic responses in the liver during its progression, resulting in a dynamic tumor-liver interaction that dictates tumorigenesis.

AIM

We aim to track the spatial and temporal activation of HSCs elicited by ICC development as one of the first efforts to map liver host response to this rare but deadly cancer, providing new scientific insights into understanding its aggressive clinical behaviors.

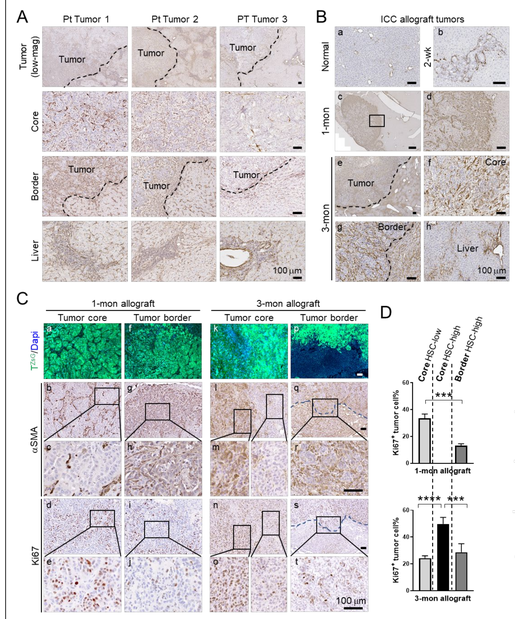
METHOD

- Immunohistochemical examination of HSC activation in ICC patient tumors
- Tracking of the spatial and temporal activation of **intratumoral HSCs (itHSCs)** and **peritumoral HSCs (ptHSCs)** in an orthotopic allograft of metastatic ICC established in our laboratory
- A "2.5D" ICC-liver coculture to determine the impact of itHSCs and ptHSCs on ICC cell growth and dissemination
- Orthotopic transplantation model of liver tumorigenesis and tail vein injection model of lung metastases to determine the effect of Vcam1 on ICC growth and dissemination
- Total RNA-sequencing of the liver tissues from control and ICC allograft model to determine liver host response to ICC development at the transcriptomic level.

RESULTS

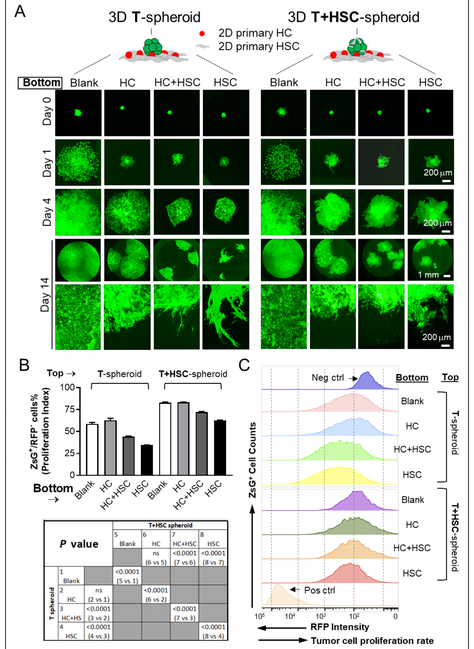
- There is a wide activation of HSCs in the tumor and surrounding liver tissues in ICC patients.
- HSCs are activated in a temporal- and spatial-specific manner during ICC development, starting at the tumor border and extending into both tumor core and tumor-surrounding liver at later stages.
- Intratumoral HSCs (HSCs mixed with tumor cells) promote ICC growth; however, ptHSCs (HSCs surrounding the tumor mass) exhibit a strong suppressive effect on ICC growth.
- Prolonged ICC-ptHSC interaction elicits tumor cell invasion and dissemination.
- Vcam1 is upregulated in the tumor cells in the early phase of ICC-ptHSC interaction and shows a dynamic regulation of the growth and dissemination of metastatic ICC in both liver and lung.
- ICC development elicits a broad range of biological changes in the peritumoral liver involving liver metabolism, oncogenic activation and immune response.

Figure 1. Temporal and spatial activation of HSCs in mouse and patient ICC tumors.



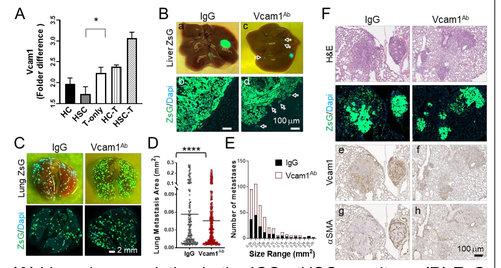
(A, B) IHC of αSMA in the ICC patient tumors **(A)** and mouse allograft tumors **(B)**. **(C)** ZsG (tumor cells, T^{ZsG})/Dapi fluorescence microscopy, αSMA and Ki67 IHC in the 1- and 3-mon ICC allograft tumors. **(D)** Quantification of $Ki67^+$ cells in the indicated areas of the 1- and 3-mon ICC allograft tumors. No HSC-high regions present in the 1-mon tumors. Student *t*-test, *P* value *** <0.001, ****<0.0001.

Figure 2. Peritumoral HSCs suppress, and intratumoral HSCs promote, ICC cell growth.



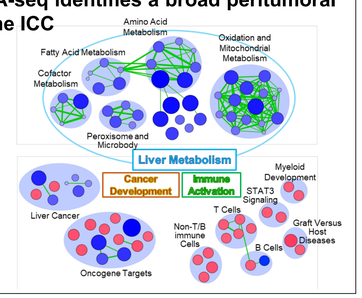
(A) ZsG^+ T- and T+HSC-spheroids in the 2.5D coculture. **(B)** Detection of tumor cell proliferation via CellTracker-RFP dye. Lower RFP indicates faster tumor cells proliferation. **(C)** RFP intensity histogram of ICC tumor cells in the indicated groups. Note that FRP intensity is reversely correlated with cell proliferation rate.

Figure 3. ICC-ptHSC interaction induces Vcam1 upregulation in tumor cells.



(A) Vcam1 upregulation in the ICC-ptHSC coculture. **(B)** ZsG images of the orthotopic tumors treated with IgG or $Vcam1^{Ab}$. Arrows in **(c)**: ZsG^+ micrometastases in the liver; arrows in **(d)**: small disseminating clones on tumor border. **(C)** ZsG images of the lung metastases of the tail vein injection model treated with IgG or $Vcam1^{Ab}$. **(D, E)** $Vcam1^{Ab}$ treatment reduces lung met size **(D)** and increases the number of small lung metastases **(E)**. **(H)** H&E, ZsG, Vcam1/αSMA IHC on the lung mets in **(C)** showing decreased Vcam1 level in DTCs and colocalization of $Vcam1^+$ and αSMA^+ regions.

Figure 4. RNA-seq identifies a broad peritumoral changes in the ICC allograft model.



CONCLUSIONS

- HSCs are rapidly activated at the tumor-liver border in early-stage ICC tumors. When tumor progresses, there is an increase in the activated itHSCs within the tumor as well as an expansion of ptHSCs extending from the tumor border into the liver.
- HSCs are beyond simple pro- or anti-tumorigenic to ICC development. Activated itHSCs are growth-promoting while ptHSCs have a strong growth-suppressive effect on ICC cell proliferation. However, prolonged ptHSC-ICC interaction induces tumor invasion and dissemination.
- Vcam1 is upregulation by ICC-ptHSC interaction and shows a dynamic regulation of ICC development by promoting ICC growth but negatively affect dissemination.
- We theorize that metastasis is an ongoing competitive process between the attempt of ptHSCs to block local tumor growth and that of tumor cells to break through the suppression. Future studies are in line to identify specific mechanisms underlying this complex tumor-host crosstalk with a goal to simultaneously address local tumor growth and early steps in metastatic progression.

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