Activation of PI3K pathway contributes to anticancer treatment-mediated hepatic stellate cell activation in liver cancer

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Introduction

Hepatocellular carcinoma (HCC) accounts for 75% of primary liver cancer. Over 80% HCC patients are discovered in advanced stage.[1] Transarterial chemoembolization (TACE) is one of the standard treatment for unresectable HCC by using high dose of cisplatin or doxorubicin.[2] However, chemotherapy is considered as a "double-edged sword" through activating hepatic stellate cells (HSCs) which can promote tumour growth and chemoresistance.[3] With a better understanding of the molecular mechanism mediated HSC activation in response to chemotherapy, novel potential targets could be revealed for improving the treatment efficacy of liver cancer.

Aim

To investigate the molecular mechanism of HSC activation in the tumour microenvironment with the treatment of chemotherapeutic drugs.

Methods

- Cancer associated fibroblasts (CAFs) subpopulations in HCC patients with or without TACE treatment were analysed using a single cell RNA sequencing dataset.
- Two *in vitro* models were used to investigate the effect of chemotherapeutic drugs on HSC activation:
  - Mixed-cell spheroids of LX2 cells and cisplatin pretreated Huh7 cells;
  - Incubation of HSCs in conditioned medium (CM) collected from cisplatin- or doxorubicin-pretreated Huh7 cells
- LX2 cells were stably transfected by a pFRET HSP33 plasmid for real-time monitoring ROS levels of LX2 cells cultured in different CM.
- Transcriptional profile of human primary HSCs cultured in Huh7 CM and cisplatin CM were investigated by RNA sequencing.
- Three *in vivo* models were used to validate the results:
  - Orthotopic HCC mouse model;
  - Orthotopic HCC mouse model with fibrosis background by administration of thioacetamide acid in the drinking water;
  - Spontaneous HCC model developed by Mdr2 knockout mice up to eleven months

Figure 1. The study design of three in vivo mouse models. Representative liver tumour photos were listed. White arrows point at tumour tissues. White dash line circles the tumour of TAA-induced liver fibrosis mouse model.

Results

- As shown in Figure 2A and 2B, CD36+ CAF subpopulation (quiescent marker) was not found in TACE-treated HCC patients, whereas CD36+ CAFs consisted 63.87% of all CAFs in untreated patients.
- The proportion of the activation markers, ACTA2 and COL1A1 (activation markers), were much higher in TACE treated patients.
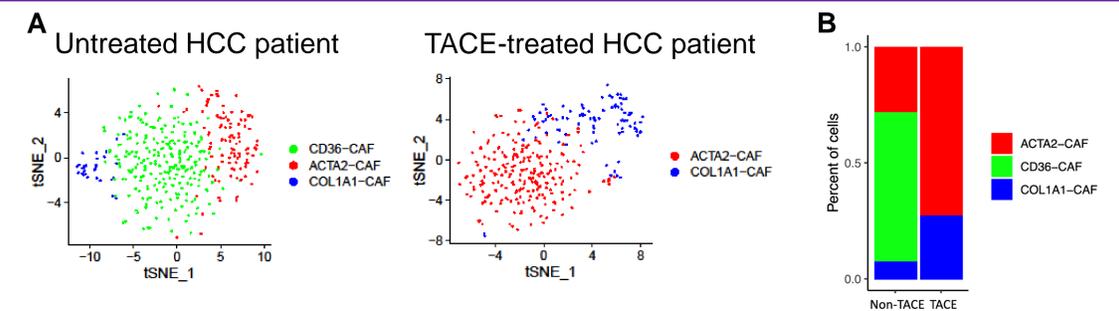- Hence, HCC patients with TACE treatment had a higher level of HSC activation.

Figure 2. Singe cell RNA sequencing analysis of CAF subpopulation in TACE-treated or untreated HCC patients.
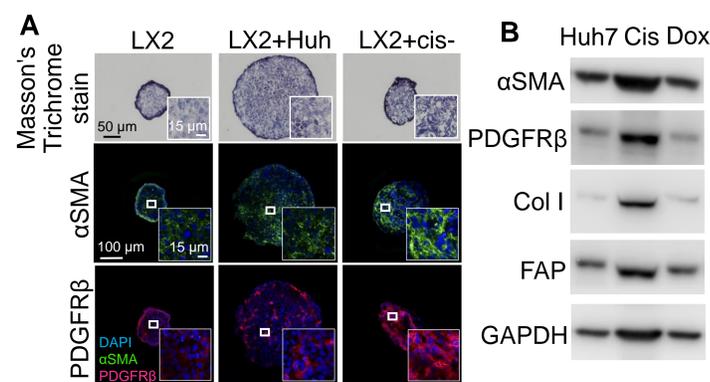

Figure 3. Activation of LX2 cells in cisplatin-pretreated mixed cell spheroids and cisplatin pretreated Huh7 CM. A. Representative images of Masson's trichrome stain for collagen and immunofluorescence staining of activation markers of mixed cell spheroids at day 5. **B.** Western blots of activation markers in primary HSCs cultured in Huh7 CM, cisplatin CM or doxorubicin CM.

Figure 4. Increased ROS level in LX2 cells in cisplatin CM. A. Real-time monitoring of intracellular ROS level in LX2 cells cultured in different CM groups. **B.** Quantification of ROS levels by CFP/YFP fluorescence intensity.

- Increased collagen deposition and activation markers expression in LX2 and cisplatin pretreated Huh7 mixed cell spheroids (Figure 3), which indicates HSCs were activated by paracrine effect of cisplatin pretreated Huh7 cells.
- In Figure 4, intracellular ROS level of LX2 cells elevated at 3-hour cultured in cisplatin CM and reach and maintain the maximum level up to 6 hours and this increased ROS in LX2 cells were only observed in cisplatin pretreated Huh7 CM.
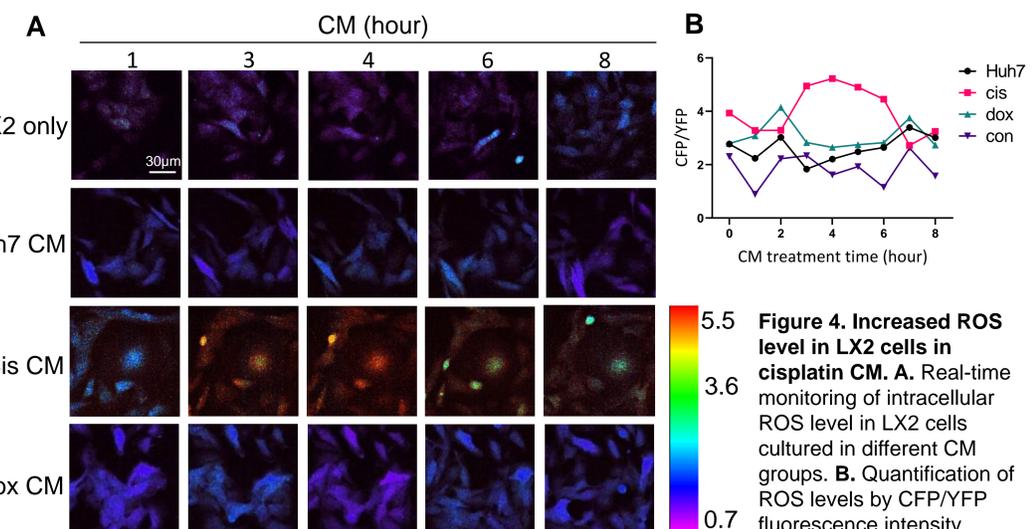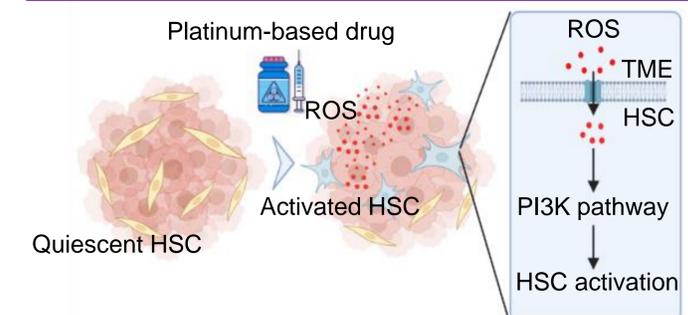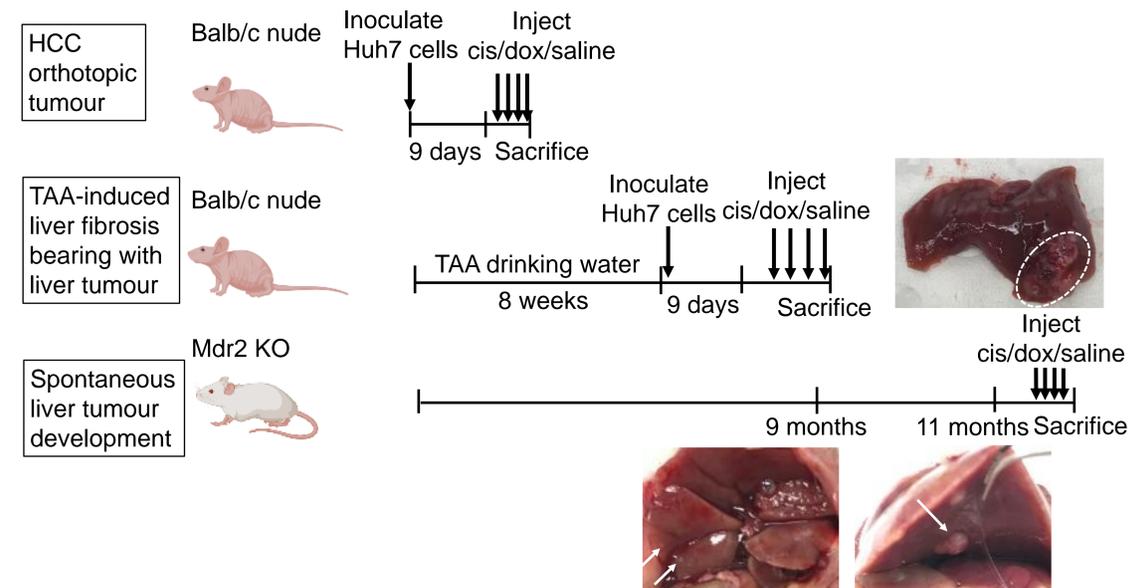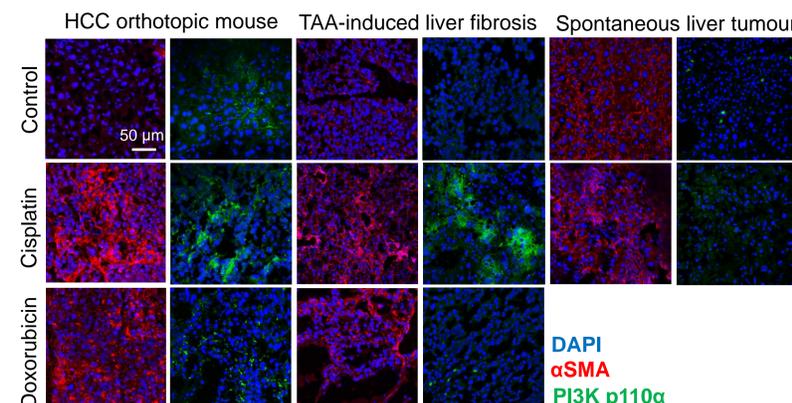

Figure 5. Representative immunofluorescence staining for αSMA and PI3K p110α accumulation in three in vivo mouse models co-stained with DAPI.

- In transcription profile, several genes related to PI3K pathway were upregulated. PIK3R3 was the top upregulated gene.
- Both αSMA and PI3K p110α were highly expressed in the cisplatin treated tumour tissues in orthotopic mouse model and mice with liver fibrosis. These increased expressions were not observed in cirrhotic liver (Figure 5).

Conclusion

- HSCs can be activated by cisplatin pretreated tumour cells via paracrine effects mediated by ROS release and PI3K-related pathway, especially PI3K p110α.
- The findings were validated in the tumour tissues obtained from HCC orthotopic mouse model and in fibrotic liver.
- Cisplatin cannot further activate HSCs in the tumours from cirrhotic livers.

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3. Liao, X., et al., Remodeling of hepatic stellate cells orchestrated the stroma-derived oxaliplatin-resistance through CCN3 paracrine in hepatocellular carcinoma. BMC Cancer, 2019. 19(1): p. 1192.