Silencing Of Discoidin Domain Receptor 1 (DDR1) Expression In Murine C26 Colon Carcinoma Cells Reduces Experimental Liver Metastasis. Blockage Of DDR1 Phosphorylation In Sinusoidal Cells Diminishes Prometastatic Features Of C26 Cells

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INTRODUCTION

Colorectal cancer (CRC) colonization of the liver and its further metastatic growth are the result of complex interaction between tumor cells, the collagenous extracellular matrix (ECM), and the hepatic sinusoidal cells (SCs), to conform a prometastatic liver microenvironment. Discoidin domain receptor 1 (DDR1) is an atypical tyrosine kinase receptor for collagens. DDR1 signaling regulates key cell functions for cancer development such as metalloproteinases (MMPs) synthesis, and cell proliferation and migration, suggesting that this receptor could play an important role in tumor progression. High DDR1 expression is a bad prognosis factor in several cancers, including CRC. During liver metastasis, CRC cells first interact with HSCs, KCs and LSECs, leading to tumor implantation and colonization of the hepatic stroma. However, whether liver SCs regulate cancer communication with the collagenous ECM and the underlying mechanisms remain poorly known.

AIM

To analyze the role of tumor DDR1 in the crosstalk between CRC cells and the hepatic SCs.

METHODS

1. DDR1 expression in hepatic metastatic tissue (Immunohistochemistry).

2. *In vitro* model of C26 CRC cells activated by SCs secretomes and/or collagen type I.
   - Prometastatic genes expression (qPCR).
   - DDR1 (WB) and MMPs secretion (Zymography).
   - Proliferation and invasion assays.

3. *In vivo* murine model of C26 CRC cells metastasis to the liver.
   - Silencing of tumor DDR1 (Transfection).
   - Metastatic development (H/E Staining).

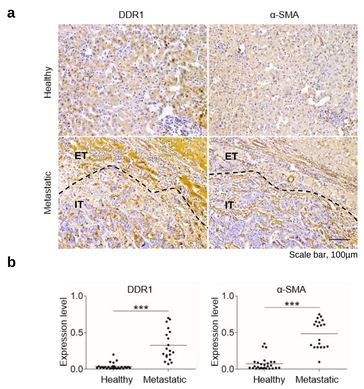

FIGURE 1. Immunohistochemical DDR1 and α-SMA expression in human hepatic tissue. (a, b) Hepatic tissue with metastasis from CRC expresses around 30% and 50% higher protein levels of DDR1 and α-SMA (marker of cancer associated fibroblasts recruitment) respectively, compared to healthy adjacent tissue. IT: intra-tumor, ET: extra-tumor.

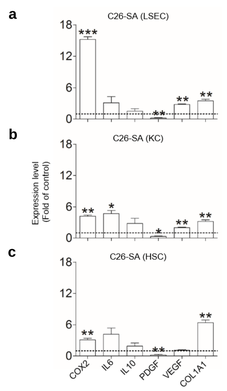

FIGURE 2. *In vitro* model of tumor-liver interaction. (a-c) Pro-inflammatory, pro-angiogenic and collagen genes expression in C26 CRC cells activated by SCs secretomes (C26-SA). qPCR results show that soluble factors from liver sinusoidal endothelial cells (LSECs), Kupffer cells (KCs) and stellate cells (HSCs), enhance C26 CRC cells prometastatic characteristics.

RESULTS

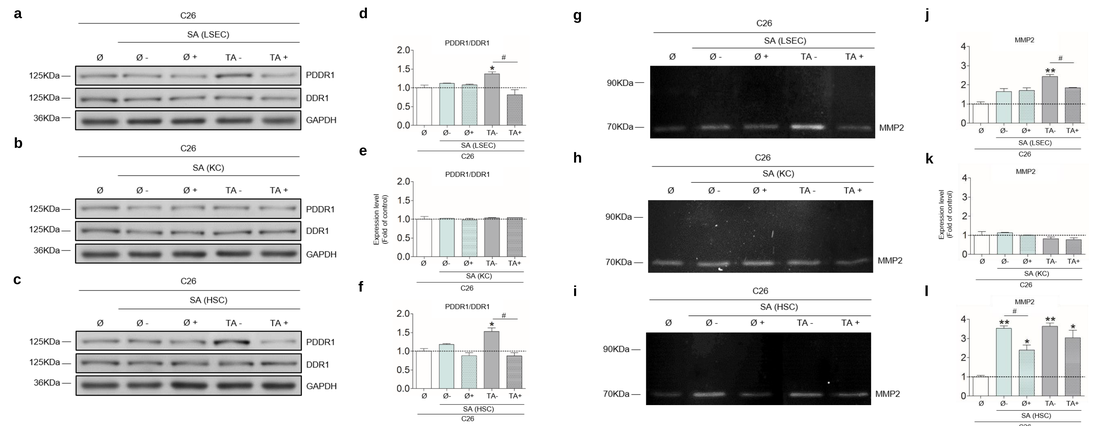

FIGURE 3. Effect of chemical blockage of sinusoidal DDR1 in basal (Ø) and stromal-activated (SA) C26 CRC cells DDR1 activation and MMPs secretion. (a-f) WB analysis show that DDR1 inhibition in SCs reduces DDR1 phosphorylation in C26 CRC cells incubated with secretomes from tumor activated (TA) LSECs and HSCs. (g-l) Zymography analysis show that blocking of sinusoidal DDR1 decreases MMP2 production in C26 CRC cells. Sinusoidal DDR1 regulates tumor DDR1 activation and MMP2 secretion.

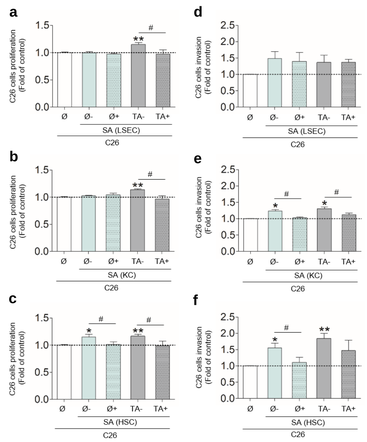

FIGURE 4. Effect of chemical blockage of sinusoidal DDR1 in basal (Ø) and stromal-activated (SA) C26 CRC cells proliferation and invasion. (a-f) Sinusoidal DDR1 inhibition reduces tumor growth and invasion throw collagen type I.

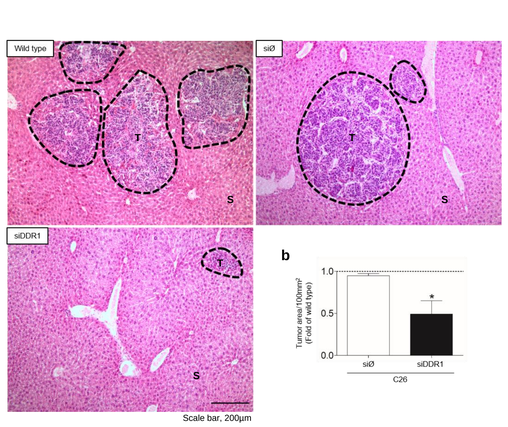

FIGURE 5. Effect of silencing of tumor DDR1 expression in C26 CRC cells metastasis development. (a, b) Lack of tumor DDR1 diminishes by half the *in vivo* metastatic activity of C26 CRC cells, by reducing liver volume occupied by metastasis. T: tumor, S: stroma.

CONCLUSIONS

Taken together, these findings indicates that colon carcinoma cells DDR1 participates in the tumor responses to hepatic sinusoidal cells.

REFERENCES

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