Silencing Of Hepatic Discoidin Domain Receptor 1 Reduces Murine Liver Metastasis By Colon Carcinoma. Role Of Sinusoidal Discoidin Domain Receptor 1 In Liver Metastasis

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INTRODUCTION

Liver metastasis is the main cause of death for patients suffering from unresectable colorectal cancer (CRC). Colonization of the liver by tumor cells largely depends on the formation of a favorable collagenous microenvironment by the hepatic capilaries (sinusoids). Discoidin domain receptor 1 (DDR1) is an ubiquitous receptor tyrosine kinase for collagen that functions as a central sensor of the extracellular matrix (ECM) microenvironment, and regulates cell proliferation, migration, and ECM remodeling. DDR1 is an independent prognosis factor for several cancers. Intensive research has been carried out to generate new therapies using DDR1 as a target, some of them are FDA-approved. However, its cellular localization, and its functional implications in healthy and diseased tissues remains unknown.

AIM

To analyze whether sinusoidal cells (SCs) express functional DDR1 and its potential implication in hepatic metastasis development by CRC cells.

METHODS

1. *In vitro* model of freshly isolated SCs activated by C26 CRC cells secretomes (TA-SCs) and/or collagen type I.
   - DDR1 expression and activation (WB and IF).
   - Chemical blockage of DDR1 phosphorylation (WB).
   - Metalloproteinases (MMPs) secretion (Zymography).
   - Silencing of HSCs DDR1 (Transfection).
   - Migration assay.

2. *In vivo* murine model of C26 CRC cells metastasis to the liver.
   - Silencing of hepatic DDR1 (Transfection).
   - Metastatic development (H/E Staining).
   - SCs infiltration, MMPs and proliferation (IF).
   - Collagen deposition (Sirius Red Staining)

RESULTS

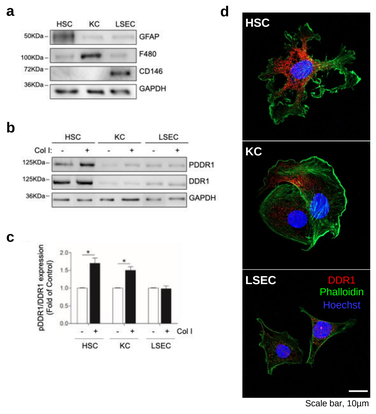

FIGURE 1. DDR1 expression and activation in SCs. (a, d) Monocultures of freshly isolated SCs (stellate cells, HSCs; Kupffer cells, KCs; and endothelial cells, LSECs) express DDR1 protein by WB and IF. HSCs express the largest amounts of DDR1. (b, c) KCs and HSCs express phosphorylated DDR1 (PDDR1) in response to exogenous collagen I (Col I) by WB. HSCs present the strongest DDR1 activation.

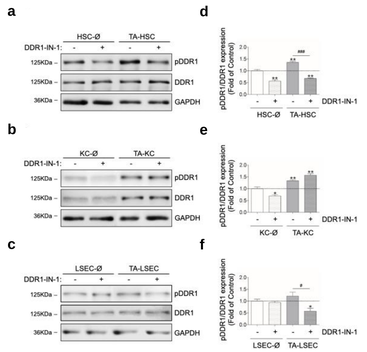

FIGURE 2. Chemical blockage of DDR1 activation in basal (Ø) and tumor-activated (TA) SCs. (a-f) WB analysis show that DDR1-IN-1 compound inhibits DDR1 phosphorylation in HSCs-Ø and KCs-Ø, and HSCs-TA and LSECs-TA.

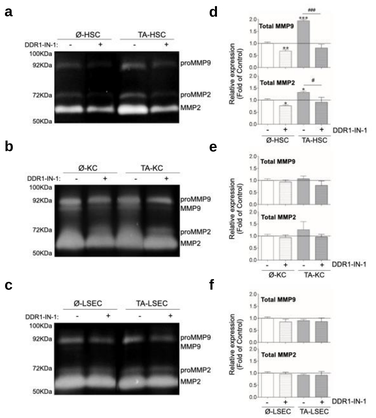

FIGURE 3. Effect of DDR1 inhibition in basal (Ø) and tumor-activated (TA) SCs MMPs secretion. (a-f) Zymography analysis show that DDR1 phosphorylation blockage reduces MMP2/9 production in HSCs. DDR1 regulates MMPs secretion in HSCs.

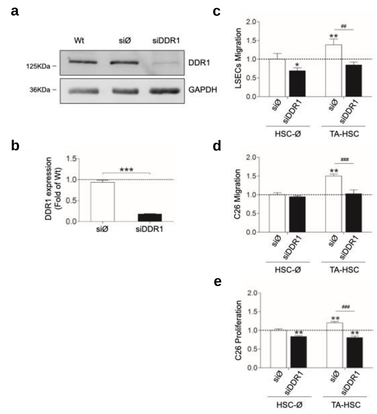

FIGURE 4. Effect of DDR1 silencing in basal (Ø) and tumor-activated (TA) HSCs in LSECs and C26 CRC cells migratory capacity and C26 cells proliferation rate. (a, b) DDR1 mRNA silencing validation by WB. (c-e) DDR1 silencing in HSCs decreases their secretion of migratory and proliferative factors for LSECs and tumor cells.

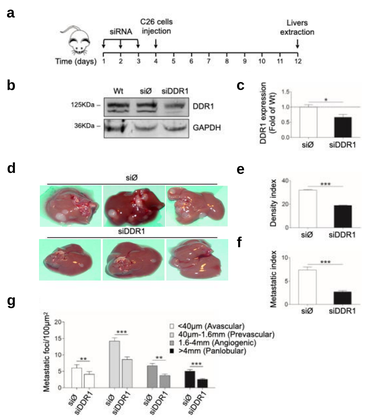

FIGURE 5. Effect of hepatic DDR1 silencing. (a) siRNA injection scheme. (b, c) DDR1 mRNA silencing validation by WB. (d-g) DDR1 silencing by siRNA injection to mice prior to tumor inoculation reduces experimental C26 CRC cells metastasis to the liver.

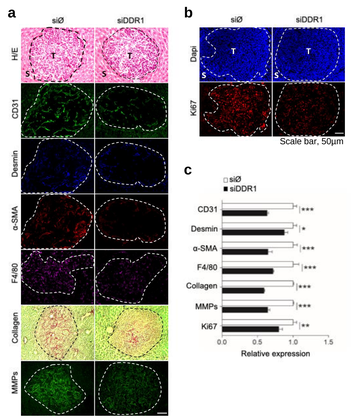

FIGURE 6. Effect of hepatic DDR1 silencing. (a-c) DDR1 silencing by siRNA injection to mice prior to tumor inoculation reduces the recruitment of activated SCs, MMPs activity, the number of proliferating cells and collagen deposition in the liver. T: tumor, S: sinusoids.

CONCLUSIONS

Taken together, these findings indicates that DDR1 signaling contributes to the prometastatic response of the hepatic sinusoidal cells in experimental colon carcinoma metastasis to the liver.

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