Immunohistochemical staining. - Biopsy specimens from 98 patients with HCC were statistically evaluated using in vivo analyses, RNA-sequencing, cytokine array, and endothelial trans-endothelial migration assay. - Functional effects of LSECs-iGap on cancer cells were analyzed using MMPs inducer, hepatocellular carcinoma (HCC) cell line, Hepa1-6 cells, to assess the LSECs-iGap formation. - Mouse models using acetaminophen or thioacetamide followed by intrasplenic injection of monocrotaline (MCT), and inhibitor, doxycycline (DOX). - Mouse models of cancer cell broke the wall of the LSEC. - MMP9 is involved LSECs-iGap under the interaction between LSECs and cancer cells. - Intracellular gap formation of LSECs results from the destruction of endothelial cells (LSECs). - Engraftment of cancer cells to liver pre-metastatic niches and their MMP9 and ICAM1 expression in LSECs, which are involved in LSECs-iGap formation.

Materials and Methods - Mouse models using aceterminophen or thioacetamide followed by intrapericolic injection of hepatocellular carcinoma (HCC) cell line, Hepa1-6 cells, to assess the LSECs-iGap formation. - Functional effects of LSECs-iGap on cancer cells were analyzed using MMP inducers, monocrystalline (MCT), and inhibitor, doxycycline (DOX). - Morphological and molecular features of LSECs-iGap were examined by electron microscopic analyses, RNA-sequencing, cytokine array, and endothelial trans-endothelial migration assay in vivo mouse models and in vitro co-culture system. - Biopsy specimens from 98 patients with HCC were statistically evaluated using immunohistochemical staining.

Results

MMP9 is involved LSECs-iGap under the interaction between LSECs and cancer cells

Figure 3: (a) SEM images of mono- and co-cultured LSECs with Hepa1-6 cells. (b) Heat map using Z-score for normalization value (log2 based) (553 genes). (c) qRT-PCR analysis of TNF-α, CXCL1, CXCL2, CXCL5, ICAM1 expression in LSECs co-cultured with Hepa1-6 cells. (d) qRT-PCR analysis of IL-23 in LSECs, Hepa1-6 under co-cultured condition. (e) Immunoblot for TNF-α and IL-23 in media from mono-cultured LSECs and co-cultured LSECs-Hepa1-6 cells. Ponceau S, loading control. (g) Immunoblot of MMP9 in LSECs treated with rmIL23 in a dose-dependent manner. (h) IF staining with ICA1 in LSECs. Arrowhead indicated LSECs-iGap.

Cancer cells invade the liver directly through iGap in LSECs

Figure 4: IF staining with phalloidin and SEM images in LSECs. Arrowhead indicated LSECs-iGap. Scale bar in IF staining and SEM Images are 10 µm and 800 nm, respectively.

Interaction between Hepa1-6 and LSECs induces the TNF-α signaling pathway, causing induction of MMP9 and ICAM1 expression in LSECs, which are involved in LSECs-iGap formation.

Figure 5: Kaplan-Meier curves show the association between low and high levels of ICAM1, MMP9 staining and disease-free survival, overall survival time.

Treatment with MMP inhibitors reduces LSECs-iGap formation and attenuates liver metastasis

Figure 6: Representative SEM images of LSECs-iGap in liver sinusoid (yellow arrowheads) on day 2 after saline (control), DOX, MCT, or DOX/MCT treatment. Scale bar, 400 nm. Representative H&E staining of tumor foci in the liver (black circles) 3 days after intrasplenic injection of Hepa1-6 cells and quantitation of the average number of tumor foci per unit area. Scale bar, 20 µm

Conclusion

This study revealed that cancer cells induced LSEC-iGap formation via pro-inflammatory paracrine mechanisms and proposed MMP9 as a novel target for blocking cancer cell metastasis to the liver.

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