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ABSTRACT TITLE: Steady IFNα therapy prevents the development of colorectal cancer liver metastases through the activation of liver sinusoidal endothelial cells.

Background: The liver is the most common site of colorectal cancer (CRC) metastases and liver CRC metastatization is a leading cause of cancer-related deaths in CRC. Thus, strategies aimed at reducing the risk of hepatic CRC colonization or at decreasing minimal residual disease after surgery are of strong interest.

Methods: Using mouse models of CRC metastatic spreading to the liver, either through intravascular seeding in the mesenteric vein of CT26 or MC38 CRC cell lines or by orthotopically implanting in the cecal wall $CT26^{LM3}$ cells that spontaneously metastasize to the liver, we tested the efficacy and mode of action of steady intraperitoneal IFNα therapy.

Results: We show that steady administration of IFNα in a neo-adjuvant setting, before CRC intravascular seeding, or in an adjuvant setting, after cecal wall orthotopic implantation, impairs hepatic tumor spreading of CRC cells. IFNα antitumor activity is mediated by its direct action on liver sinusoidal endothelial cells (LSECs), while it does not rely on IFNAR1 expression on tumor cells, hepatocytes, stellate cells or dendritic cells. Mechanistically, while IFNα does not alter the number of CRC cells that reach the liver and arrest intravascularly, it impairs transendothelial migration of invading CRC cells, due to the induction of a low grade endothelial inflammatory state, extracellular matrix remodelling, increased glycocalyx deposition and LSEC defenestration, reducing CRC cell colonization of the liver, and inducing a sustained overall survival of IFNα-treated mice. Furthermore, RNAseq analysis of isolated liver endothelial cells reveals that IFNα-treatment upregulates the expression of antigen processing and presentation genes, genes involved in cytokine/chemokines production as well as several pathways related to innate and adaptive immune responses that, together with the early intravascular reduction of tumor burden, impairs liver CRC tumor growth and progression. In fact, IFNα-mediated intravascular antigen reduction together with LSEC cross-presentation and cross-priming of Ag-specific CD8 T cells is necessary to eradicate CRC cells, promoting long-term immunological memory and tumor clearance upon secondary CRC challenge.

Conclusions: Overall, these findings, provide a rational for the use of steady adjuvant IFNα therapy to reduce CRC metastatic spreading to the liver and control minimal residual disease in early adjuvant settings.

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