IL-10 is a Dominant and Reversible Mechanism of Immune Evasion in Human Colorectal Cancer Liver Metastasis

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BACKGROUND

- Colorectal cancer liver metastasis (CRLM) is a leading cause of cancer death and morbidity.
- Long-term survival is poor.
- Microsatellite stable CRLM does not response to currently approved immunotherapies.
- Tumor slice culture (Fig. 1) accurately recapitulates human cancer microenvironment and immune response.
- Interleukin-10 (IL-10) is a major inhibitory cytokine whose role remains controversial in antitumor immune responses.

HYPOTHESIS

- IL-10 is a dominant but reversible mechanisms of immune suppression in human CRLM.

METHODS

- Tumor specimens were obtained from consenting CRLM patients at the time of surgery and cut into 250 μm-thick slices (Fig. 1).
- Tumor slices were treated for 4-6 days with control or neutralizing antibodies against PD-1 (EH12.1), IL-10 (JES3-9D7), IL-10 receptor alpha (IL-10RA, 3F9), major histocompatibility complex (MHC) class I (G46-2.6) or class II (Tu39).
- Tumor apoptosis was measured by cleaved caspase-3 (CC3) immunohistochemistry (IHC).
- Immune markers were measured by IHC and in situ hybridization (ISH).
- Student's t-test, paired t-test, or 1-way ANOVA with multiple test correction was used as indicated. Statistical significance was defined as $p < 0.05$.

RESULTS

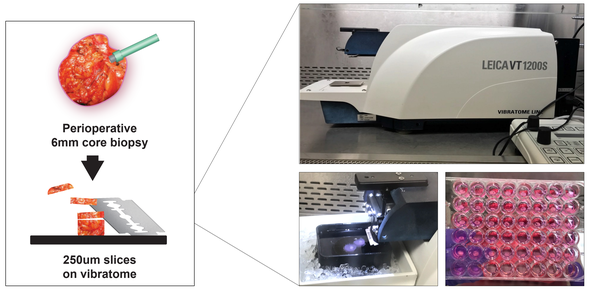
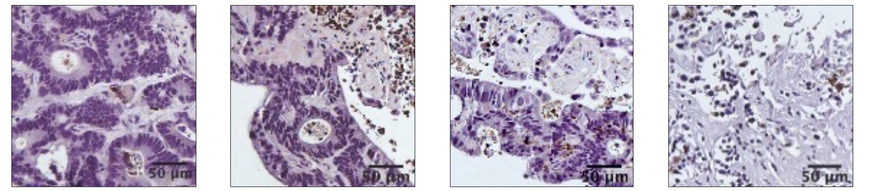


Figure 1. Tumor slice culture enables direct interrogation of human tumors with preserved immune infiltrate. 250 μm slices of freshly resected tumor are cultured on a PTFE membrane. Slices can then be treated and analyzed.



αIL-10 doubles tumor apoptosis, whereas αPD-1 does not.

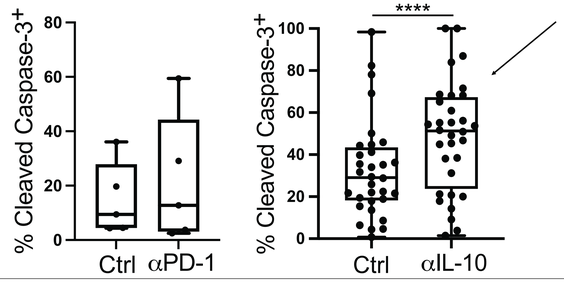


Figure 2. IL-10 blockade leads to increased tumor apoptosis in human CRLM slice culture. CRLM slices were treated with IgG control or blocking antibodies against PD-1 or IL-10 for 6 days, after which apoptosis was measured by CC3 IHC, with representative images (top) and quantification (bottom). Each dot represents a unique patient's tumor, n = 4 (αPD-1), n = 32 (αIL-10). *p<0.05.

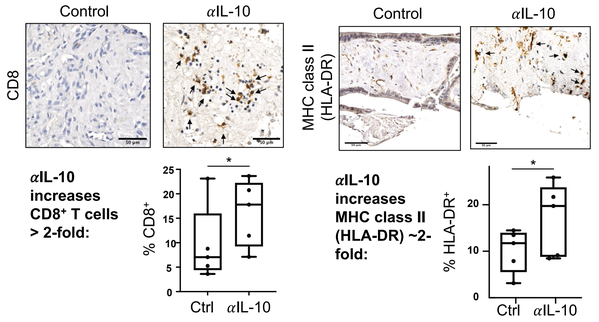


Figure 3. IL-10 blockade leads to increased immune activation in human CRLM slice culture. CRLM slices were treated as above for 4 days, after CD8 (left) and MHC class II (right) were assessed by IHC, with representative images (top) and quantification (bottom). Each dot represents a unique patient's tumor, n = 5. *p<0.05.

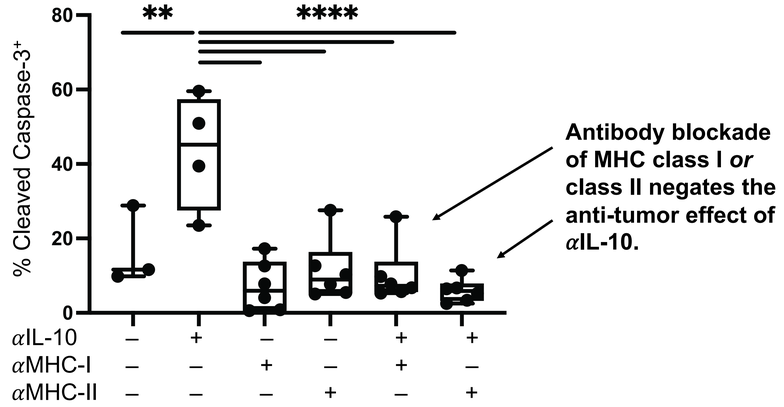


Figure 4. Mechanism of IL-10 blockade requires intact MHC class I and class II antigen presentation. CRLM slices were treated with blocking antibodies against IL-10, MHC class I or II as indicated. Tumor apoptosis was measured by CC3 IHC. Each dot represents a unique tumor slice from one patient's tumor, representative of 3 patients' tumors, n = 3-6 slices/grp. *p<0.05.

CONCLUSIONS

- IL-10 inhibits an effective immune response against human CRLM.
- Antibody-mediated blockade of IL-10 leads to significantly increased tumor death, through a mechanisms that requires intact $CD8^+$ T cell and antigen-presenting cell function.
- IL-10 is a **compelling new immunotherapeutic target** for patients with advanced CRLM.

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