Summer 8-10-2022

Targeting Macrophages to Reduce Colorectal Cancer Metastasis: Diminished Effect in the Alcohol-injured Liver

Winnie Ladu
University of Nebraska Medical Center

Ashley M. Mohr
University of Nebraska Medical Center

Heather Richard
University of Nebraska Medical Center

Benita McVicker
University of Nebraska Medical Center

UNMC
UNIVERSITY OF NEBRASKA MEDICAL CENTER℠

Summer Undergraduate Alcohol Research Program

Targeting Macrophages to Reduce Colorectal Cancer Metastasis: Diminished Effect in the Alcohol-injured Liver

Winnie Ladu[1,2], Ashley Mohr[3], Heather Richard[4,5], Benita McVicker[4,5]

[1]University of Nebraska Medical Center SUARP; [2]University of Nebraska at Omaha; [3]Dept. of Biochemistry, University of Nebraska Medical Center; [4]Gastroenterology and Hepatology, University of Nebraska Medical Center; [5]VA Nebraska-Western Iowa Health Care System Research Service

Background and Aims

The dissemination of colon cancer to the liver is a major cause of morbidity and mortality in colorectal cancer patients. Alcohol use is a risk factor for colorectal liver metastasis (CRLM) yet contributing mechanisms are undefined.

Macrophages play a crucial role in alcohol-associated liver disease (AALD) as well as in CRLM. Studies indicate that treatment with gadolinium chloride (GdCl3) leads to the inactivation of Kupffer cells (KCs), the resident liver macrophage population. Also, it is known that carcinoembryonic antigen (CEA) from colorectal cancer cells is internalized by KCs leading to protumor cytokine and chemokine production and the promotion of liver metastasis.

However, it is not known whether targeting macrophages with $GdCl_3$ to reduce CRLM would be effective considering the flux of macrophage phenotypes in the liver. Furthermore, it is not known how preexisting alcohol-mediated liver injury will affect $GdCl_3$ inactivation of macrophages and hepatic colorectal cancer tumor burden.

The aim of this study was to determine the effect of $GdCl_3$ inactivation of KCs after CRLM establishment in healthy and alcohol-injured livers.

Methods

Preclinical model of AALD: C57BL/6 mice were fed control (C) or 5% ethanol (E) Lieber-DeCarli diets for 4 weeks to establish alcohol-associated liver injury.

Alcohol-CRLM model and treatments: The C/E-fed mice were intravenously injected with $GdCl_3$ (20mg/kg) or saline 9 and 12 days after the intrasplenic delivery of $1x10^6$ MC38+CEA colorectal cancer (CRC) cells.

Assessments: Animals were sacrificed 13 days following CRC cell injections and continued C/E diets. Tumor burden, AALD, macrophage phenotype, and cytokine expression were assessed in serum and liver tissue. Assays performed by W.L:

PCR:
- RNA was isolated from frozen liver tissue using the PureLink RNA Mini Kit (ThermoFisher).
- Quantification of isolated RNA was conducted using the Ribogreen Assay.
- cDNA was generated from RNA using the High-Capacity cDNA Reverse Transcription Kit
- TaqMan Real-Time PCR was conducted on cDNA to detect cytokine/chemokines: TNF-α, Nos2, TGF-β, IL-10, CD163, and HO-1.

Immunohistochemistry:
- Formalin-fixed, paraffin-embedded liver sections were dewaxed and subject to antigen unmasking using citric acid-based solution.
- The sections were incubated overnight at 4° with primary antibodies specific for smooth muscle α-actin (SMA, Abcam 5694), caspase-3 (R&D AF835), and heme oxygenase 1 (HO-1, Abcam 189491).
- Secondary antibody incubation was done using anti-rabbit HRP (Cell Signaling).
- Immunofluorescent detection was performed by tyramide signal amplification using AlexaFluor 647, 568, and 488 tyramide (ThermoFisher).
- Fluorescent micrographs were captured using a Nikon Eclipse 80i microscope.

Study design and validation of AALD

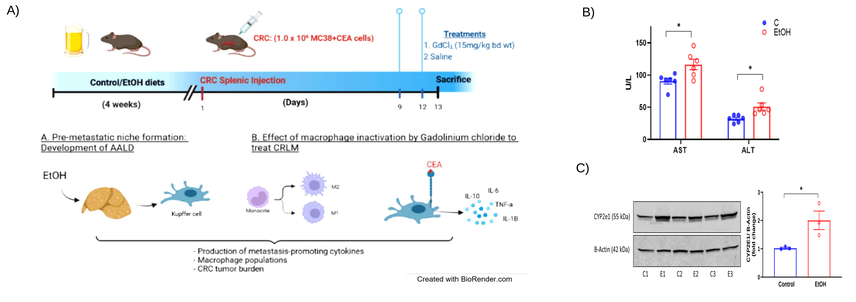

Fig.1. Alcohol-CRLM preclinical mouse model. A) Experimental design to evaluate colorectal liver metastasis (CRLM) in mice fed ethanol-containing or isocaloric control diets. B) Serum levels of aspartate and alanine aminotransaminases (AST and ALT). C) Hepatic protein expression of CYP2E1 normalized to β-actin.

CRLM is increased in AALD

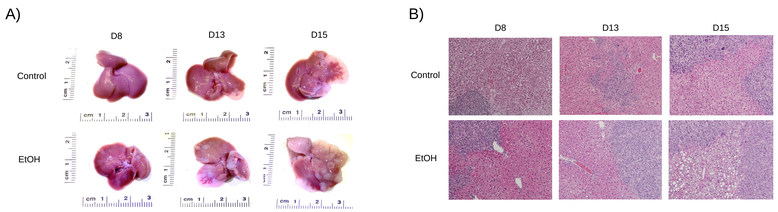

Fig. 2. Colorectal liver metastasis in the alcohol-injured liver. C57BL/6 female mice were fed C/EtOH diets for 4 weeks followed by intrasplenic injection of MC38+CEA colorectal cancer (CRC) cells. A) Visual assessment of CRLM at days 8, 13, and 15-days post CRC injections. B) H&E images, 10x magnification.

Effect of $GdCl_3$ treatment on CRLM

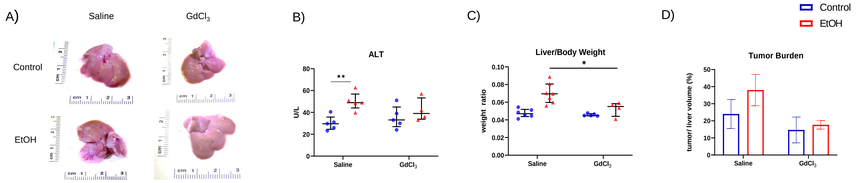

Fig. 3. The effect of Kupffer cell inhibition by $GdCl_3$ on CRLM. Control and ethanol-fed mice were injected with gadolinium ($GdCl_3$) after the delivery of MC38+CEA cells. A) Liver tumor burden by gross examination. B) Serum alanine aminotransaminase (ALT) levels. C) Liver to body weight ratios. D) Tumor burden as a percentage of total liver section volume. n=4-6 mice per group. *P <0.05, **P <0.01

$GdCl_3$ treatment reduces tumor-promoting factors

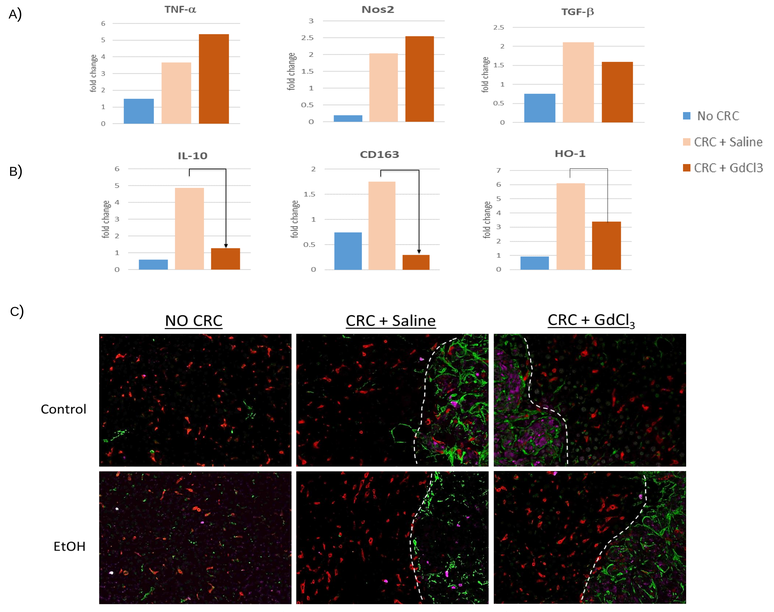

Fig. 4. The effect of gadolinium treatment on CRLM. Control and ethanol-fed mice were treated with gadolinium chloride ($GdCl_3$) after establishment of liver metastasis of colorectal cancer cells (CRC). Gene expression is shown for mice fed C/E alone (No CRC), or C/E-fed CRLM mice after treatments (CRC+saline or CRC+$GdCl_3$). Data is presented as the fold-change of genes in E-fed mice compared to control for proinflammatory (A) or protumor (B) factors. C) Immunofluorescent detection of smooth muscle actin (SMA), caspase-3, and heme oxygenase-1 (HO-1), 20x magnification. Macrophage HO-1 expression is known to dampen inflammatory responses, promote metastasis outgrowth and reduce apoptosis of CRC cells in CRLM (white dashed line).

Conclusions and Future Directions

- Liver metastasis of colorectal cancer is enhanced in the alcohol-injured liver.
- Targeting hepatic macrophages to treat CRLM shows potential benefit with a reduction of factors associated with angiogenesis and metastasis.
- Inactivation of macrophages with $GdCl_3$ is associated with an increased expression of caspase-3 and tumor cell death in CRLM. This effect is diminished in the alcohol-injured liver.
- This study highlights the importance of targeting hepatic macrophages against Kupffer cell-activating agents such as CEA.
- Future studies aim to define macrophage expression profiles and related processes including hepatic stellate cell activation and T cell responses during CRC metastatic growth in the liver.