SMAD4-Mediated Interaction of Epithelial and Dendritic Cells

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BACKGROUND

- Patients with inflammatory bowel disease are at increased risk of Colitis-associated carcinoma (CAC), a subtype of colorectal cancer.
- Although the prevalence of CAC is low (accounting for between 1-2% of all colorectal cancer), the prognosis of CAC is considered to be unfavorable[1].
- Importantly, CAC has been associated with the loss of a tumor suppressor gene, SMAD4.
- Previous studies in mice with induced colitis have shown that loss of SMAD4 is associated with upregulation of a notable chemokine, CCL20, which binds to a sole receptor, CCR6, on immune cells[2].
- The binding of CCL20 to CCR6 is believed to influence dendritic cell recruitment to the epithelium, which may impact gastrointestinal homeostasis.

AIMS

- Determine whether SMAD4 loss impacts dendritic cell recruitment to the epithelium.
  - Hypothesis 1: SMAD4 loss will increase dendritic cell recruitment to the gut epithelium.
- Determine how the CCL20/CCR6 axis influences dendritic cell recruitment to the epithelium in the setting of SMAD4 loss.
  - Hypothesis 2: The CCR6 receptor is necessary for dendritic cell recruitment to the gut epithelium in the loss of SMAD4. Upregulated CCL20 will increase dendritic cell recruitment.

RESULTS

Figure 1: An experimental outline demonstrating the culturing of dendritic cells is displayed.

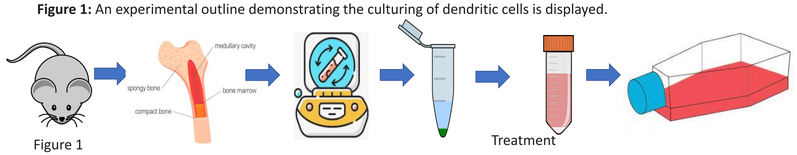

Figure 1

Figures 2,3,4: Bone marrow-derived cells treated with GM-CSF and either IL-4 or ß-Mercaptoethanol were analyzed for dendritic cell markers CD11c, CD103, and CD11b via RT-qPCR analysis.

Figures 5,6: SMAD4-/SMAD4+ epithelia were analyzed via RT-qPCR for the presence of dendritic cell markers from CCR6-/CCR6+ mice. Figure 6 tests for the presence of a marker of dendritic cell activation, CD86.

Figure 7: Immunohistochemistry was performed assessing recruitment of dendritic cells from CCR6+/CCR6- mice to SMAD4- epithelia.

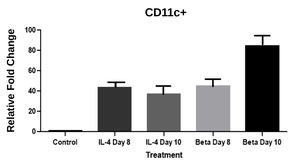

Figure 2

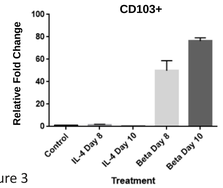

Figure 3

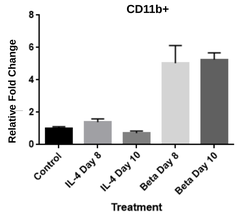

Figure 4

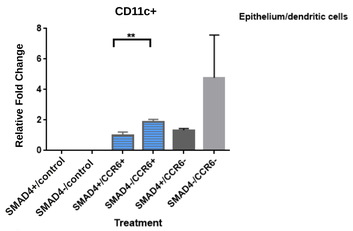

Figure 5

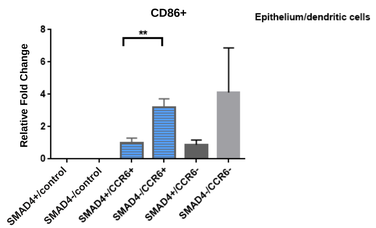

Figure 6

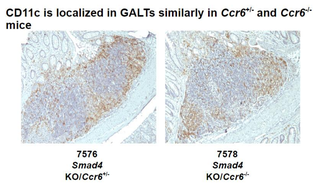

Figure 7

METHODS

- First, cells were extracted from the bone marrow of mice and were differentiated into dendritic cells using GM-CSF and either IL-4 or ß-Mercaptoethanol.
- RT-qPCR was performed to confirm the presence of dendritic cells using cell specific markers.
- A co-culture experiment was performed to assess the migration of CCR6+ and CCR6- dendritic cells from mice to epithelia that were SMAD4+ and SMAD4- .
- RT-qPCR was performed to characterize the dendritic cells that migrated during the co-culture.
- We also performed immunohistochemical (IHC) staining of dendritic cells in colon tissue from CCR6+ and CCR6- mice that were also SMAD4-.

CONCLUSIONS

- The most poignant result of the RT-qPCR performed on dendritic cells from the co-culture show that dendritic cells migrating to SMAD4- epithelia had higher levels of a marker of activation when compared to those migrating to SMAD4+ epithelia.
- This indicates that although the relative levels of dendritic cells may appear comparable, there may be a functional difference in the dendritic cells that are recruited.
- Understanding how SMAD4-mediated signaling within epithelial cells can alter the inflammatory response may lead to the identification of actionable targets.

References:
1. Maryńczak K, Włodarczyk J, Sabatowska Z, Dziki A, Dziki Ł, Włodarczyk M. Colitis-Associated Colorectal Cancer in Patients with Inflammatory Bowel Diseases in a Tertiary Referral Center: A Propensity Score Matching Analysis. J Clin Med. 2022 Feb 7;11(3):866.
2. Means AL, Freeman TJ, Zhu J, Woodbury LG, Marincola-Smith P, Wu C, Meyer AR, Weaver CJ, Padmanabhan C, An H, Zi J, Wessinger BC, Chaturvedi R, Brown TD, Deane NG, Coffey RJ, Wilson KT, Smith JJ, Sawyers CL, Goldenring JR, Novitskiy SV, Washington MK, Shi C, Beauchamp RD. Epithelial Smad4 Deletion Up-Regulates Inflammation and Promotes Inflammation-Associated Cancer. Cell Mol Gastroenterol Hepatol. 2018 May 24;6(3):257-276.