**Abstract**

Despite remarkable responses to immune checkpoint blockade across multiple tumor types, the clinical benefit in colorectal cancer (CRC) is limited to metastatic colorectal tumors. PD-L1 expression is a negative prognostic marker in CRC but correlates with a better response to PD-1 blockade. Here, we investigated the role of PD-L1 in colorectal tumorigenesis and evaluated the utility of targeting myeloid-derived suppressor cells (MDSCs) in combination with PD-1 blockade in murine models of CRC. We generated knockin mice that conditionally express the murine PD1 gene (R26SL-PD1-EGFP) and crossed them with LysM-Cre mice to overexpress PD-L1 specifically in the myeloid lineage. AOM/DSS-treated mice formed tumors at 10 weeks and developed adenocarcinoma at 17 weeks post-AOM. AOM/DSS treatment led to a significant expansion of myeloid cells, particularly CD11b+Gr-1+ MDSCs, compared to untreated mice. Furthermore, there was a significant decrease in intratumoral CD8+ T cells, indicating attenuated anti-tumor immunity. AOM/DSS-treated PD-L1-overexpressing LysM-Cre; R26-PD-L1 mice showed markedly enhanced early colorectal tumorigenesis, with a significant increase in tumor number and size. Trefoil factor 2 (TFF2), a secreted anti-inflammatory peptide, inhibits colon tumor growth by suppressing the expression of CD11b+Gr-1+ MDSCs. TFF2 fuses with a retroviral-terminal peptide and three Flag motifs (TFF2-CTP-Flag) prolonged the circulation time in blood but retained bioactivity. We induced tumors in R26-PD-L1 and LysM-Cre; R26-PD-L1 mice with AOM/DSS, administered fusion recombinant TFF2-CTP-Flag and/or anti-PD-1 therapy. Anti-PD-1 in combination with TFF2-CTP showed a marked reduction in tumor growth while anti-PD-1 monotherapy failed to suppress growth. Interestingly, combined treatment showed greater anti-tumor activity in PD-L1-overexpressing mice than control animals. Treatment responders showed significantly increased tumor-infiltrating CD8+ T cells and concomitant MDSC subsets during CRC development.

**Conclusions**

These early findings suggest that TFF2 augments the response rate of CRC to PD-1 blockade, possibly through suppressing MDSC expansion, and further supports the potential of TFF2-CTP in combination I-O treatment for CRC.

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**CD11b+Gr-1+ MDSCs markedly increased as tumors progress**

**CD8+ T cells : Treg ratio is decreased as tumors develop**

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**TFF2F-CTP + anti-PD-1 showed greater anti-tumor activity in PD-L1-overexpressing mice**

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**TFF2F-CTP enhances anti-tumor activity of PD-1 blockade in colorectal cancer by suppressing MDSCs**

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**Figure 1.** Mice (C57BL/6 WT) received azoxymethane (AOM: 10 mg/kg i.p.) followed one week later with 2.5% dextran sodium sulfate (DSS) in the drinking water for 7 days. (A-B) AOM/DSS-treated mice formed tumors at 10 weeks and developed adenocarcinoma at 17 weeks post-AOM. (B) Gross images. Scale bars, 5mm. Tumors were more frequently observed in the distal colon. (C) Macroscopically visible tumors were counted and tumor area was measured using ImageJ. (D) H&E stains. Suppressed intratumoral immune cell infiltrates were detected at 10 weeks post-AOM.

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**Figure 2.** (A) Immunostaining for CD45, CD11b and PD-L1 on colon tissues from AOM/DSS-treated C57BL/6 WT mice. CD11b+ myeloid cells and PD-L1 expression were increased as tumors progressed. (B) Immunophenotyping of intratumoral myeloid cells by flow cytometry (% of CD45, CD11b+Gr-1+ MDSCs and both granulocytic (CD11b+Ly6G+) and mononuclear (CD11b-Ly6G-Ly6C+) MDSC subsets were markedly increased). (C) Micrographs (MG): CD11b+Ly6C+F4/80+ and dendritic cells (DC; CD11c+F4/80-).