A CXCR4 PARTIAL AGONIST TFF2-MSA SENSITIZED ADVANCED GASTRIC CANCER TO PD-1 BLOCKADE BY SYSTEMATICALLY REDUCING PMN-MDSC ACCUMULATION, IMMUNOSUPPRESSION, AND GENERATION IN BONE MARROW

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Background: Polymorphonuclear myeloid-derived suppressor cell (PMN-MDSC) is pathologically activated immature neutrophil that exerts immunosuppressive functions. PMN-MDSC is short-lived and constantly replenished through bone marrow (BM) myelopoiesis. Within the BM, histidine decarboxylase (HDC) expressing immature neutrophil constitute a histaminergic niche that enforces hematopoietic stem cell quiescence and inhibits pathological myelopoiesis. TFF2, a partial agonist for CXCR4, inhibits CXCL12-dependent chemotaxis. Here we engineered a stabilized TFF2 peptide linked to mouse serum albumin (TFF2-MSA), and investigated its effect on PMN-MDSC and combination with PD1 inhibitor in advanced gastric cancer mouse models.

Methods: A syngeneic gastric cancer cell line ACKP (Atp4b-Cre; Cdh1-/-; LSL-KrasG12D; Trp53-/-) was grafted subcutaneously into HDC-GFP transgenic mice to trace HDC-GFP+ MDSCs. Once tumors reach 200 mm³, mice received TFF2-MSA or/and anti-PD-1 antibody. The CXCR4 antagonist AMD3100 served as comparison to TFF2-MSA. We further established a spontaneous lung metastasis model in which mice bearing ACKP tumors received surgical tumor resection and subsequent TFF2-MSA/anti-PD-1 treatments. In another orthotopic model, ACKP-luciferase cells were implanted to stomach submucosa, and the treatments started after 1 week. At 1 month, flow cytometry and single cell RNA sequencing (scRNA-seq) were performed to study treatment induced immune profile changes.

Results: Either TFF2-MSA or anti-PD-1 monotherapy showed little inhibition of subcutaneous ACKP tumor (15% and 25%, N=10, p>0.05), but their combination dramatically suppressed ACKP growth by 78% in a synergistic manner (p<0.0001). In the lung metastasis model, the combination completely abrogated metastasis in 80% mice (N=10, p<0.0001) and prolonged mice survival (p<0.0001), in contrast to minimal effect of either monotherapy (p>0.05). In the orthotopic model, although either monotherapy only slightly delayed tumor growth, their combination successfully eradicated tumor in 80% mice (N=5, p<0.001). Mechanistically, TFF2-MSA systematically reduced HDC+ PMN-MDSCs in the TME, spleen and blood, and its combination with anti-PD-1 profoundly increased polyfunctional Tim-3-Lag-3-CD8⁺ T cells in the TME and TCF1⁺ T cells in the TDLN. Inside BM, TFF2-MSA decreased PMN-MDSC generation from its precursors (MPP3/CMP/GMP/GP) to a level similar to tumor-free mice, and restored hematopoietic stem cell quiescence via histamine feedback loop. Importantly, flow cytometry, scRNA-seq and functional analysis suggested TFF2-MSA treated PMN-MDSCs display a more mature profile with less immunosuppressive property. In contrast, AMD3100 plus anti-PD-1 failed to decrease tumor growth and TME MDSCs possibly due to destruction of BM hematopoietic homeostasis.

Conclusions: TFF2-MSA systematically reduced PMN-MDSC, and its immunosuppression and generation in BM, thereby sensitizing advanced gastric cancer to PD-1 inhibitors.