Targeting immunosuppressive classical monocytes prevents anti-PD-1/CTLA-4 treatment resistance

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Introduction

Immune checkpoint inhibitors have revolutionized the treatment of long cancer. However, most patients fail to respond or acquire resistance over time. Understanding resistance mechanisms to immune checkpoint blockade in NSCLC is critical to developing new therapeutic strategies and improving patient outcomes. Here we report our discovery that targeting classical monocytes with anti-Ly6C blockade can effectively prevent anti-PD-1/CTLA-4 treatment resistance.

Background

Fig 3. 129/Sv mice were treated weekly with IgG control, anti-PD-1_CTLA-4, or anti-PD-1_CTLA-4_Ly6C (A) mice were subcutaneously injected with B16-O VAmelanoma cells for 4 weeks. (B) B16-O VAtumors were processed for FACS analysis. Percentage of CD209a+CD11c+CD11b- mature dendritic cells from IgG control or combination therapy treated tumors. (C) B16-O VAtumors were processed for FACS analysis. Percentage of CD115+CD14+Ly6C+ monocytes was found to be much higher in combination therapy resistant tumors. (D) Expression of Ly6C was evaluated over time. Combination therapy resistant tumors treated with IgG control or combination therapy resistant tumors. (E) Monocytes sorted from 344SQ tumors were treated with IFNg for 12 hours. Expression of Ly6C was evaluated over time.

Results

Dual PD-1/CTLA4 blockade leads to long term acquired resistance due to infiltrating immunosuppressive cells

Fig 4. (A) 344SQ tumors were treated with IgG control or anti-PD-1_CTLA-4 after week 8. Tumors were processing for single cells FACS analysis. Percentage of CD209a+CD11c+CD11b- mature dendritic cells and subcutaneous B16-O VAmelanoma cells stratified based on expression of Ly6C. (B) Correlation between CD14/CD16a/CD16b and CCL2 or CCR2 expression in TCGA lung adenocarcinoma. (C) Correlation between CD14/CD16a/CD16b and CCL2 or CCR2 expression in TCGA lung adenocarcinoma.

Conclusions

Tumor-derived dendritic cells play a vital role in maximizing the immunosuppressive effect of anti-PD-1/CTLA-4 treatment

Fig 5. (A) 344SQ tumor bearing 129/Sv mice were treated weekly with anti-PD-1_CTLA-4, anti-Ly6C, or anti-PD-1_CTLA-4_Ly6C beginning on week2 after subcutaneous cell injection for 4 weeks. At week 8 mice were euthanized and to measure tumor weight (middle) and # of long metastatic lesions (right). Tumors from (A) were harvested for protein and cells for FACS analysis. (B) Anti-PD-1_CTLA-4_Ly6C was capable of tumoral control. (C) Percentage of CD14+CD16+CD16b+ monocytes was found to be much higher in combination therapy resistant tumors. (D) Percentage of CD14+CD16+CD16b+ monocytes was found to be much higher in combination therapy resistant tumors.

References


Nivolumab plus ipilimumab show improvement in NSCLC patients

Fig 7. (A) 344SQ tumor-bearing mice were injected with TCGA blood samples and PD-1 staining of CD14+CD16+Ly6C+ monocytes. (B) RT-QCR of CD14+Ly6C+expression of Ly6C- monocytes from IgG CTL or combo treated tumors. (C) RT-QCR of CD14+Ly6C- expression of Ly6C+ monocytes from IgG CTL or combo treated tumors. (D) Percentage of CD14+Ly6C+ or CD14+Ly6C- monocytes (from IgG CTL or combo treated tumors). (E) Percentage of CD14+Ly6C+ or CD14+Ly6C- monocytes (from IgG CTL or combo treated tumors). (F) Percentage of CD14+Ly6C+ or CD14+Ly6C- monocytes (from IgG CTL or combo treated tumors). (G) Percentage of CD14+Ly6C+ or CD14+Ly6C- monocytes (from IgG CTL or combo treated tumors). (H) Percentage of CD14+Ly6C+ or CD14+Ly6C- monocytes (from IgG CTL or combo treated tumors). (I) Percentage of CD14+Ly6C+ or CD14+Ly6C- monocytes (from IgG CTL or combo treated tumors). (J) Percentage of CD14+Ly6C+ or CD14+Ly6C- monocytes (from IgG CTL or combo treated tumors).

Loss of PD-1/PDL-1 in classical Ly6C+monocytes in the anti-PD-1/CTLA-4 resistant tumors prevents differentiation into immunosuppressive cells

Tumor-infiltrating monocytes trans-differentiation into dendritic cells by Ly6C blockade

Fig 8. (A) 344SQ tumor bearing 129/Sv mice were treated weekly with anti-PD-1_CTLA-4, anti-Ly6C, or anti-PD-1_CTLA-4_Ly6C beginning on week2 after subcutaneous cell injection for 4 weeks. At week 8 mice were euthanized and to measure tumor weight (middle) and # of long metastatic lesions (right). Tumors from (A) were harvested for protein and cells for FACS analysis. (B) Anti-PD-1_CTLA-4_Ly6C was capable of tumoral control. (C) Percentage of CD14+CD16+CD16b+ monocytes was found to be much higher in combination therapy resistant tumors. (D) Percentage of CD14+CD16+CD16b+ monocytes was found to be much higher in combination therapy resistant tumors. (E) Percentage of CD14+CD16+CD16b+ monocytes was found to be much higher in combination therapy resistant tumors. (F) Percentage of CD14+CD16+CD16b+ monocytes was found to be much higher in combination therapy resistant tumors. (G) Percentage of CD14+CD16+CD16b+ monocytes was found to be much higher in combination therapy resistant tumors. (H) Percentage of CD14+CD16+CD16b+ monocytes was found to be much higher in combination therapy resistant tumors. (I) Percentage of CD14+CD16+CD16b+ monocytes was found to be much higher in combination therapy resistant tumors. (J) Percentage of CD14+CD16+CD16b+ monocytes was found to be much higher in combination therapy resistant tumors.