Ultra-high concentration nitric oxide (UNO) enhances anti-CTLA-4 treatment activity and induces a durable anti-tumor immune response

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Background

CTLA-4 blockade is a highly effective therapeutic modality but only in a subset of cancer patients. We previously demonstrated that intratumoral administration of ultra-high concentration nitric oxide (UNO) augmented response to the immune checkpoint inhibitor, anti-mPD-1. Specifically, more than half of the mice treated with 10-minutes UNO (50,000 ppm) and anti-mPD-1 were primary and secondary tumor-free at Day 100 post-UNO treatment (vs 25% of control mice treated with anti-mPD-1 alone). In addition, the combination of UNO and anti-mCTLA-4 assessed in an immunocompetent breast cancer model (4T1) demonstrated prolonged survival in mice compared to single-agent anti-mCTLA-4.

In CT26 and 4T1 mice, the immunodominant AH-1 antigen is associated with anti-tumor immune response. In this study, we assessed the systemic levels of CD8+ T-cells recognizing the AH-1 antigen following UNO treatment and the association with anti-tumor response.

Methods

Here we explored the effects of UNO treatment in combination with anti-mCTLA-4 therapy. Colon carcinoma (CT26) tumor-bearing mice were treated with intra-tumoral 100,000 ppm UNO for 5 minutes in combination with 5 mg/kg anti-mCTLA-4. Effects on tumor growth and anti-tumor immune response were assessed. AH-1 specific CD8+ T cells were quantified by flow cytometry.

Results

Combining 100,000 ppm UNO with anti-mCTLA4 in CT26 tumor-bearing mice resulted in considerable growth delay and regression of treated tumors. Furthermore, the combination induced a strong systemic response that led to the rejection of a distant metastasis-like tumor inoculated on the contralateral flank. Specifically, at Day 7 post-treatment, all UNO treated mice exhibited tumor-specific CD8+ T-cells in the blood and to a higher degree than in untreated mice. Moreover, systemic levels of tumor-specific CD8+ T-cells were higher in mice treated with the combination of UNO and anti-mCTLA4 compared to mice treated with monotherapy anti-mCTLA-4, indicating an increase in immune surveillance following UNO treatment. Finally, cured mice treated with combination therapy displayed higher systemic levels of antigen-specific CD8+ T-cells compared to untreated or naïve mice.

Conclusion

UNO stimulates a higher tumor-specific T-cell response than anti-mCTLA-4 and synergizes with this drug to generate an even more significant targeted immune response.