Dysfunction of HPV16-specific CD8+ T cells derived from oropharyngeal tumors is related to the expression of Tim-3 but not PD-1

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Introduction

Human papillomavirus (HPV) type 16 infection is currently considered to be one of the most significant risk factors for oropharyngeal squamous cell carcinoma (OPC) development in both men and women. Patients with HPV-positive OPC tumors, which express highly immunogenic HPV16 E6 and E7 oncoproteins, significantly better prognosis than patients with HPV-negative squamous cell carcinomas of the head and neck. However, tumor cell constitutive expression of HPV16 E6 and E7 oncoproteins efficiently inhibits HPV-specific T cell immunity, which may contribute to the better prognosis of patients with HPV-induced tumors.

Aim of study

The aim of this study was to analyze the frequency, phenotype and function of HPV16 E6/E7-specific T cells in oropharyngeal tumors and consider the effect of anti-PD-1 blockade, inhibition of PD-Tim3 pathway and immunotherapy with HPV16-directed therapeutic vaccines on HPV16 E6/E7-specific tumor infiltrating lymphocytes (TILs) in vitro.

Methods

In order to confirm the effect of PD-1/PD-L1 pathway blockade on IFN-γ production, OPC tumor cells were co-cultured with OT-I T cells, anti-PD-1 mAb nivolumab and soluble Tim-3 (n = 4). The change was counted from the baseline represented by IFN-γ production without any treatment.

To further characterize the phenotype and function of expanded TILs, we assessed the levels of PD-1, PD-L1, PD-L2, CD4, CD8, CD95, CD28, and Tim-3 in culture supernatants.

Correlation between mRNA levels of Tim-3 (x-axis) and PD-1, CTLA-4, LAG-3, and TGF-β (y-axis) was explored in expanded TILs using qPCR.

Conclusions

In this study, we characterized the phenotype and function of HPV16 E6/E7-specific tumor infiltrating lymphocytes (TILs) derived from oropharyngeal tumors. We showed the presence of HPV16-specific tumor infiltrating T cells in 70% of HPV-positive oropharyngeal tumors characterized by production of IFN-γ upon E6/E7 peptide pool stimulation. These HPV16-specific TILs predominantly expressed PD-1 but not Tim-3, identifying Tim-3 rather than PD-1 as a marker of T cell dysfunction. Specific IFN-γ production induced by HPV16 E6/E7 stimulated TILs was significantly inhibited by PD-1/PD-L1 pathway blockade. In contrast, the combination of PD-1/PD-L1 and PD-Tim3 pathway blockade resulted in a significant increase in IFN-γ production compared to PD-1 blockade alone. Our data suggest that the efficacy of complementary treatment combining anti-PD-1 blockade and inhibitors of other immune checkpoint pathways such as Tim-3 might be substantially supported by means of combining with HPV-directed therapeutic vaccines.