LANDSCAPE OF HELPER AND REGULATORY CD4+ T CELLS IN MELANOMA

Background Within the tumor microenvironment, distinct CD4+ T cell subsets can play different and even opposite roles either promoting or suppressing anti-tumor responses through the recognition of antigens presented by human leukocyte antigen (HLA) class II molecules. However, how cancers co-opt these processes to shape the intratumoral CD4+ landscape and achieve immune evasion remains incompletely understood.

Methods We performed single-cell characterization of CD4+ tumor infiltrating lymphocytes (TILs) collected from four human melanoma with low or high HLA-class II expression and we utilized TCR reconstruction and antigen specificity screening to unambiguously discover the tumor reactivity of CD4+ TILs. By testing TCR-transduced T cells against autologous patient-derived melanoma cell lines or against autologous antigen presenting cells (APCs) loaded with tumor lysates, we assessed the capacity of CD4+ TCRs to directly or indirectly recognize tumor cells. We defined the antigen-specificity of antitumor CD4+ TCRs by assessing their reactivity towards personal neoantigens (NeoAg) or public melanoma associated antigens (MAAs). Finally, we correlated NeoAg burden and HLA-class II expression in a series of 116 melanoma specimens from 4 independent cohorts of patients.

Results Analysis of single-cell data showed that the cluster distribution of cells within each CD4+ TCR clonotype family was highly homogeneous and appeared to follow 3 distinct major phenotypes, corresponding to non-exhausted memory cells, exhausted cells and regulatory cells (T Regs). Strikingly, clonally expanded CD4+ T Reg-TILs were highly abundant within the tumor microenvironment of HLA class II pos melanomas. We found that TCRs from exhausted cytotoxic CD4+ T cells could be directly triggered by melanoma cells not only through recognition of HLA class II restricted antigens, but also through presentation of HLA class I restricted MAAs. T Reg-TCRs could be indirectly elicited through presentation of tumor antigens via APCs. Notably, numerous tumor-reactive CD4+ T Reg-TCRs were directly stimulated by HLA class II pos melanoma and demonstrated specificity for melanoma NeoAgs. In HLA class II pos melanomas, the clonal expansion of numerous tumor-reactive and NeoAg-specific T Reg-cIones appeared to be favored by a dramatically high tumor NeoAg load. Analysis of 116 melanoma specimens confirmed the association of elevated HLA-class II expression with extremely high NeoAg burden.

Conclusions Our data elucidate the landscape of infiltrating CD4+ T cells in melanoma and point to presentation of HLA-class II restricted NeoAgs and direct engagement of immunosuppressive CD4+ T Reg as a novel mechanism of immune evasion favored in HLA class II pos melanoma.

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