REVERSING THE IMMUNE-EXCLUDED ("COLD") TUMOR IMMUNE MICROENVIRONMENT IN ORAL SQUAMOUS CELL CARCINOMA

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Background The tumor immune microenvironment (TIME), plays a major role in oral squamous cell carcinoma (OSCC) resistance to therapy, including immunotherapy. Many OSCC are considered poorly immunogenic tumors or "immune deserts" which lack immune infiltration, evade immune recognition and suppress immune system activation, all of which have been associated with early disease relapse and poor prognosis in OSCC patients. Somatic $TP53$ mutations, the most common genetic alterations in 75% to 85% of OSCCs. The loss or mutation of $TP53$ in a cancer can affect the recruitment and activity of myeloid and T cells, thereby enabling immune evasion and tumor progression. Here we generated a set of syngeneic mouse oral cancer cell lines (ROCs) from mice exposed to 4-nitroquinoline-1 oxide, a carcinogen that acts as a tobacco mimetic and causes DNA damage. The syngeneic murine ROC cell lines were tumorigenic, with different immune landscapes and response to immunotherapy.

Methods Orthotopic tongue injections were performed to (i) characterize tumor growth rate (ii) the TIME landscape by immunohistochemistry (IHC); and (iii) immunotherapy drug studies. We inactivated mutant $p53$ expression by shRNA, profile gene expression changes by RNAseq, and validated these findings by quantitative PCR, ELISA, co-culture and flow cytometry studies. Tumor response was achieved by combined therapy and TIME changes were evaluated by opal multiplex IHC.

Results We used the ROC1 cell line to investigate the effect of mutant $p53$ in the modulation of cell-intrinsic factors that shape the tumor immune landscape and affect sensitivity to immunotherapy. We observed that a carcinogen-induced $p53$ mutation promoted a cold TIME enriched with immunosuppressive M2 macrophages highly resistant to ICI therapy. $p53$-mutated cold tumors failed to respond to combination ICI treatment; however, the combination of a programmed cell death protein 1 (PD-1) inhibitor and stimulator of interferon genes (STING) agonist restored responsiveness.

Conclusions Our syngeneic OSCC models provide an experimental system, which can be used to understand the interplay between cell-intrinsic genetic changes and immunosuppressive mechanisms that promote tumor progression, and serve as a translationally-relevant platform for evaluating immunotherapy combinations to improve treatment strategies for OSCC.