1105 TUMOR CELL AUTONOMOUS INNATE IMMUNE SIGNALING DRIVES THERAPEUTIC RESISTANCE IN HEAD AND NECK TUMORS

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Background Although immune checkpoint inhibition has moved into the frontline setting for advanced and metastatic head and neck cancer (HNC), only a minority of patients experience durable treatment responses.[1] Therapeutic resistance is associated with elevated hypoxia and STAT3 signaling and infiltration by myeloid derived suppressor cells (MDSC).[2 3] In this study, we tested the hypothesis that resistant phenotypes are driven by tumor cell autonomous innate immune signaling.

Methods Gene Set Enrichment Analysis was used to assess transcriptional signatures of immunity in the Cancer Cell Line Encyclopedia (CCLE) and The Cancer Genome Atlas (TCGA). Western blotting was used to assess HIF1α, HIF2α and pSTAT3 expression. ELISA was used to test the expression of cytokines including IL-6, IL-8 and VEGF-A. Inhibitors and blocking antibodies were used to disrupt TLR2, TLR4, RAGE, IL-1R1 and HIF1α signaling in human cell lines. Transplantable murine models of immunotherapy-sensitive (MOC1) and -resistant (MOC2) oral squamous cell carcinoma were used to test the immune effects of inhibiting HIF1α and IL-1α signaling. Flow cytometry was used to assess infiltration of immune populations in murine tumors.

Results We identified patterns of constitutive STAT3 and hypoxia signaling in HNC cell lines from CCLE and confirmed that these signatures were enriched in human HNC tumors (figure 1A-C). We confirmed enrichment of cell autonomous pSTAT3 activation and HIF1a accumulation in HNC cell lines compared with non-HNC controls (figure 2A,B). Cell autonomous inflammatory signaling was associated with secretion of IL-6 and IL-8 (figure 2C). We dissected the mechanism of spontaneous inflammatory signaling and demonstrated that it depends entirely on IL-1α signaling and partly on HIF1α and TLR signaling, which are associated with increased expression of innate pattern recognition receptors (figure 2D,E).

We next confirmed the presence of cell autonomous inflammatory signaling in the murine MOC2 model and its absence in MOC1 (figure 3A,B). Echinomycin, a HIF1α inhibitor, significantly impaired the growth of MOC2 tumors (figure 3C, D). Echinomycin-treated MOC2 tumors demonstrated enhanced immune infiltration, T and NK cell infiltration and decreased MDSC compared with placebo controls (figure 3E-G).

Conclusions This study establishes a basis for self-propagating inflammatory signaling in HNC tumors driven by HIF1α, IL-1α and STAT3. Disruption of this loop by targeting the IL-1α or HIF1A signaling pathways represent promising strategies to overcome therapeutic resistance and induce anti-tumor immunity.

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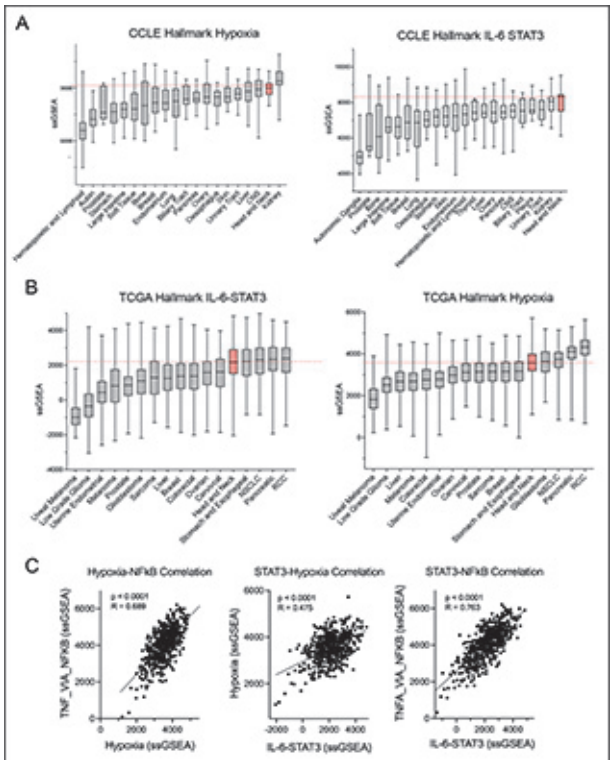
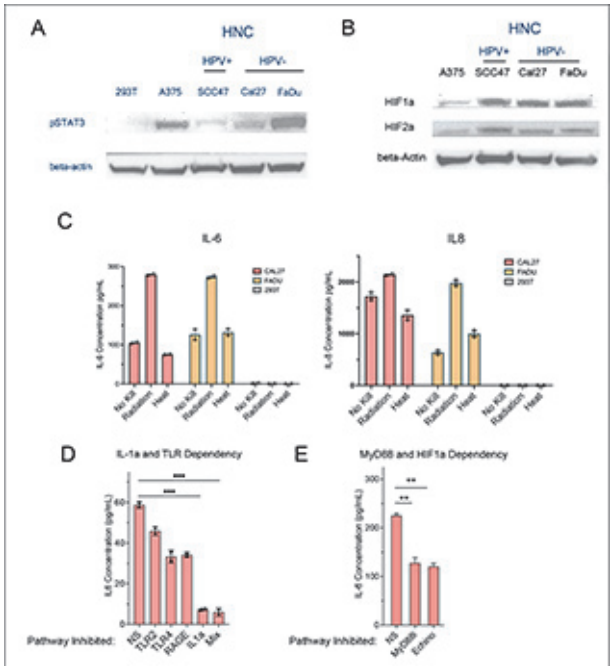
Ethics Approval

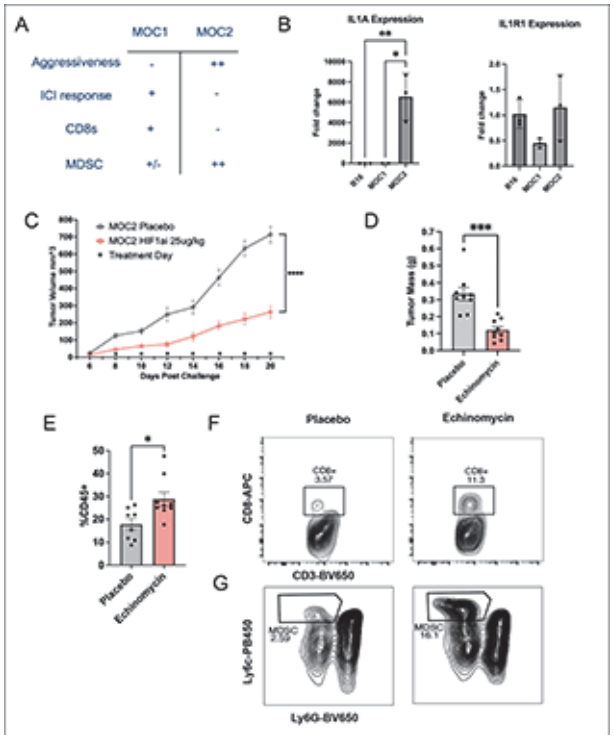
This study involved animals (mice) These studies were performed under IACUC approved protocol number 2023–20307.



Abstract 1105 Figure 1 Constitutive inflammatory signaling is common in head and neck cancers. (A) Head and neck tumor cell lines in CCLE are enriched for STAT 3 and hypoxia transcriptional signatures by ssGSEA compared wtih other tumor types. (B) STAT 3 and hypoxia signaling are also enirched in HNC biopsies in TCGA compared with non-HNC controls. (C) Hypoxia, NFkB and STAT 3 signaling are all highly corrleated in HNC tumor biopsies from TCGA.



Abstract 1105 Figure 2 Phenotypes of constitutive inflammatory signaling in HNC cell lines. HPV-HNC cell lines demonstrate constitutive signaling via pSTAT3 (A) and hypoxia (B). (C) Constitutive innate signaling in HNC cell lines is associated with the release of IL-6 and IL-8, both of which are enhanced upon radiation treatment. Spontaneous release of IL-6 can be completely abrogated by blockade of IL1a and partly abrogated by blockade of TLR2, TLR4, and RAGE (D, 96-well plate format). IL-6 release can also be partly abrogated by inhibition of MyD88 or inhibition of HIF1A via Echinomycin (E, 24-well plate format).



Abstract 1105 Figure 3 Targeting HIV1A enhances immunity in therapy-resistant murine models. (A) Compared with MOC1 oral squamous cell carcinoma, MOC2 is more aggressive, has decreased response to immunotherapy, fewer CD8+ T cells and more MDSC. (B) MOC2 demonstrates cell autonomous IL1a production in vitro, whereas MOC1 does not. (C) Treating MOC2 tumors with 25 ug/kg of the HIF1A inhibitor every 2 days beginning at d6 as shown significantly impaired the growth of MOC2 tumors compared with placebo controls. (D) On d25 of treatment, Echinomycin-treated tumors have significantly greater infiltration of CD45+ immune cells. Within the CD45+ immune fraction, Echinomycin-treated tumors have increased CD8+ T cell infiltration and decreased MDSC infiltration compared with placebo-treated controls.

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