Effective Reduction of PD-L1 Expression by Simultaneous Blockade of EGFR and HER3 (ErbB3) in Head and Neck Cancer

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Abstract

Background: We previously reported that simultaneous blockade of EGFR and HER3 by antibodies cetuximab and MM-121, respectively, could more potently reduce tumor growth in both squamous cell carcinoma of head and neck (SCCHN) cell lines and patient derived xenograft (PDX) models (Jiang et al, MCT, 13:1826-36, 2014; Wang et al., CCR, 23:677-86, 2016). Recently, we tested a new anti-HER3 antibody CDX-3379 (provided by Celldex Therapeutics, Inc) in combination with cetuximab for treatment of SCCHN. The effects of this combination on expression of the immune check point regulator PD-L1 and regulatory pathways underlying these effects were examined. A Phase II clinical trial combining CDX-3379 with cetuximab in checkpoint and cetuximab refractory patients with advanced SCCHN has already been initiated.

Method: Antitumor effects of cetuximab and CDX-3379 combination were investigated in SCCHN PDX animal models. Both HPV positive and negative SCCHN cell lines, 93-VU-147T (HPV+), FaDu (HPV-), and SqCCY1 (HPV-), were used for western blot analysis to examine EGFR and HER3 downstream pathways and PD-L1 expression in the presence and absence of interferon γ (IFNγ) after treatment with cetuximab, CDX-3379, or both agents in combination.

Results: The PDX study indicated that, similar to MM-121, combining CDX-3379 with cetuximab inhibited tumor growth more potently than cetuximab alone or the untreated control (control vs. combination: p < 0.0001; cetuximab vs. combination: p < 0.0001). Mechanistic in vitro studies demonstrated that in addition to co- blocking ERK and AKT pathways, and regardless of HPV status, the cetuximab and CDX-3379 combination reduced PD-L1 expression more effectively than any of the single agents in the presence or absence of IFNγ which has been previously reported to be secreted by natural killer (NK) cells through cetuximab-mediated antibody-dependent cell mediated cytotoxicity, leading to induction of PD-L1 expression in vivo (Concha-Benavente et al., CR, 76:1031-43, 2016). Using an AKT inhibitor, we confirmed that the AKT is one of the major pathways involved in regulation of PD-L1. Considering that HER3 exerts its inhibitory effect mainly through regulation of the AKT, reducing AKT activity by addition of CDX-3379 to cetuximab contributed significantly to the reduction of PD-L1 expression.

Conclusion: In addition to its anti-tumor effect, the combination of cetuximab and CDX-3379 significantly reduced PD-L1 expression. The role of this combination in the regulation of tumor immunity and the tumor microenvironment in SCCHN deserves further investigation.

Objective of the Study

➢ To determine the effect of co-targeting EGFR and HER3 on expression of PD-L1 and tumor growth of SCCHN.

Materials and Methods

- SCCHN cell lines: 93-VU147T(HPV+), FaDu (HPV-), SqCCY1, (HPV-) and UM-SCC1-C (a cetuximab resistant cell line).
- Western blot analysis with or without IFNγ.
- SRB cell growth assay.
- SCCHN cell line xenograft and PDX animal models.

Results

1. Combination of cetuximab and CDX-3379 inhibits mainly AKT signaling pathway and reduces PD-L1 in SCCHN.

Cells were treated with EGFR Antibody cetuximab (CTX, 0.5 µg/ml), HER3 antibody CDX-3379 (CDX, 1 µg/ml), and their combination (Combo) with untreated control (CNT). A: Percentage of survival after the treatment determined by SRB assay; B: Lysate of cells were collected 48 hours after the treatment and separated by 10% SDS-PAGE for western blot analysis. Proteins were probed with corresponding antibodies. The experiment were repeated at least 3 times.

2. Cetuximab, CDX-3379 or their combination inhibit IFNγ-induced pAKT, pERK, and PD-L1.

Conclusions

- Co-targeting EGFR and HER3 by cetuximab and CDX-3379, respectively, inhibits tumor growth and reduces PD-L1 expression in SCCHN.
- Reduction of PD-L1 in the presence of IFNγ varies among different cell lines and depends on multiple signaling pathways.

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