Inhibition of Novel Cetuximab Resistance Pathway Leads to Improved Outcomes in Head and Neck Cancer

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INTRODUCTION

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer worldwide, with more than 400,000 new cases and 300,000 deaths annually. In the US, despite advances in medical and surgical management, five-year survival rates have slightly improved over the last two decades to around 65% for all stages across all subtypes. This has prompted the development of novel molecular therapeutic approaches that target tumor cells while preserving normal tissue function.

The epidermal growth factor receptor (EGFR) remains the only non-chemotherapeutic molecular target that has been successfully translated into a therapeutic modality with clinical benefit for patients with HNSCC. Targeting this transmembrane tyrosine kinase has served as an attractive and rational strategy given that more than 90% of HNSCC tumors overexpress EGFR. Cetuximab, a chimeric monoclonal antibody that binds competitively to the EGFR, is used clinically for treatment of HNSCC, and has been shown to improve overall survival in both locally advanced and recurrent metastatic disease, especially when combined with radiation or chemotherapy.

Although cetuximab has shown great promise, it has been limited by intrinsic as well as acquired resistance. While several mechanisms have been reported, recent evidence has pointed to the shutoff of EGFR to the nucleus as an important mechanism used by cells to evade the action of the cell surface antibody. Furthermore, concurrent with clinical observations, this upregulation in nuclear EGFR (nEGFR) has been correlated with highly proliferative and therapy resistant tumors and linked with poor clinical outcomes in breast, ovarian, and HNSCC.

Several studies have demonstrated an important role for nEGFR in DNA repair, specifically, EGFR has been shown to translocate to the nucleus in response to double strand breaks, and inhibition of this pathway induces a DSBR repair deficiency. The Mre11, Rad50, and Nbs1 (MRN) complex plays a critical role in the recognition and repair of DNA double-strand breaks (DSBs), and has been shown to be upregulated in advanced HNSCC. Furthermore, Nbs1, the most upstream protein in this complex, has been linked with processes outside of DNA repair, including PI3K/Akt signaling through regulation of SFK protein expression.

This study, therefore, looked to evaluate if cetuximab resistance in HNSCC was regulated by MRN complex mediated upregulation of SFK expression and function, increasing nEGFR in HNSCC cells. Furthermore, our study investigated whether Mirin, a novel MRN inhibitor, could lead to downregulation of this pathway, and increase human HNSCC sensitivity to cetuximab treatment.

METHODOLOGY

Cell lines: Two human HNSCC cell lines, JHU006 and JHU020, derived from human tumor explants, were propagated and maintained in our laboratory for use in the present study.

Cell growth and survival analysis: Cells were seeded into 96-well plates at a density of 2.5 x 10^3 cells per well. Growth was evaluated for 5 or 6 consecutive days following treatment using a standard MTT assay.

Western Blot Analysis: Expression of proteins was confirmed using Western blot as described previously. Cells were plated in 6 well tissue culture plates at a density of 3 x 10^4 and allowed to adhere overnight. They were subsequently treated as indicated on the figures legend, and at the appropriate time points, growth media was aspirated and Trisyn was added to the culture flask. Media was used to neutralize the Trisyn. Cells were then pelleted by centrifugation at 3000 rpm for 5 minutes. Pellets were washed with phosphate-buffered saline (PBS) twice. PBS was washed away and the cells resuspended in 100 µL sodium dodecyl sulfate (SDS) gel loading buffer (50 mMol Tris- HCl at pH 6.8, 100 mMol L- Dithiothreitol, 2% SDS, and 0.1% bromophenol) warmed to 85°C. Supernatant equivalent to 1 x 10^5 cells was run on 8% SDS-PAGE gel then electrophoretically transferred onto Hybond ECL nitrocellulose membrane (Amersham Biosciences, Piscataway, NJ). Non-specific binding sites were blocked using 5% nonfat dried milk in Tris-buffered saline with 0.1% Tween20 (TBS-T). All antibodies used for this study were obtained from Cell Signaling Technologies, Danvers, MA.

Statistical Analysis: Statistical analysis was performed with SigmaStat 2.03 software (Systat, Point Richmond, CA). The two-tailed t-test for independent samples was used for the analysis of all the data. The level of significance was set at p<0.05.

RESULTS

Statistical significance was set at p<0.05. Analysis of variance was performed with Prism 4 software (GraphPad, San Diego, CA). The significance of differences was determined using a one-way ANOVA test with Tukey’s post-hoc test, and was considered significant at *p<0.05, **p<0.01, ***p<0.001. All experiments were independently conducted at least three times, with SEM shown.

SUMMARY

I. Cetuximab treatment exhibits a dose-dependent increase in MRN and SFK expression in resistant cells

II. Cetuximab treatment leads to increased SFK-mediated EGFR phosphorylation at Y845 and nuclear translocation of EGFR in resistant cells

III. Treatment with Mirin decreases MRN expression and SFK expression in HNSCC cells

IV. Treatment with Mirin leads to decreased SFK-mediated phosphorylation of EGFR at Y845, and nuclear translocation of EGFR in HNSCC cells

V. Mirin inhibits Cetuximab induced nuclear localization of EGFR in resistant cells

VI. Mirin sensitizes HNSCC cells to Cetuximab treatment

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