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Targeting Apoptosis to Overcome Cisplatin Resistance: A Translational Study in Head and Neck Cancer

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Introduction

Cisplatin is a primary agent for organ-sparing treatment of advanced head and neck squamous cell cancers (HNSCC). Cisplatin resistance remains a significant barrier to organ sparing therapy and to the survival of patients with advanced HNSCC. Our group has established that pretreatment tumor biopsies of treatment failures in several clinical trials using cisplatin-based induction chemotherapy are characterized by high expression of Bcl-xL and wild type p53.

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Materials and Methods

Pretreatment tissue biopsies were used to construct tissue microarrays. Immunohistochemical analyses were performed as described. The tissue microarrays were stained for p53 (Ab-6, clone DO-1, Lab Vision, Fremont, CA) and Bcl-xL (Ab-2, clone 7D9, Lab Vision, Fremont, CA).

Results

In the Department of Veterans Affairs Laryngeal Cancer Study, patients enrolled on the chemotherapy arm who had high expression of p53 protein in their pretreatment tumor biopsy were more likely to keep their larynx than patients whose tumors did not express the p53 protein (80% vs. 58.5%, $p = 0.03$).¹ In fact, the relative risk of laryngectomy was 2.1 fold in patients with p53 negative vs. p53 positive tumors. When p53 is wild-type and functional it has a short half-life so levels are low. Overexpression of p53 is usually on the basis of a mutation in p53 and/or other alterations in the p53 pathway. Bcl-xL is an anti-apoptotic protein that blocks programmed cell death. Bcl-xL is commonly overexpressed in head and neck tumors.² High Bcl-xL expression has been associated with resistance to cisplatin-based chemotherapy regimens in head and neck tumors.^{2,3}

Specifically, patients whose tumors expressed low Bcl-xL had higher rates of larynx preservation than patients who expressed high levels of Bcl-xL (90% vs. 60%, $p = 0.02$). Most importantly, combining these biomarker profiles has led to identification of a favorable biomarker profile (low tumor expression of p53 and Bcl-xL) in which patients enjoyed 100% larynx preservation contrasted with an unfavorable biomarker profile (low tumor expression of p53 and high tumor expression of Bcl-xL) in which patients had only a 50% rate of larynx preservation.

We have shown previously that a single cycle of induction chemotherapy can allow selection of patients with advanced larynx cancer for combined chemoradiation. Patients treated according to this new treatment paradigm (UMCC 9520) had improved survival relative to historical controls (Figure 1A).⁴ Furthermore, biomarker studies revealed that patients whose tumors expressed the lowest levels of Bcl-xL (assessed with immunohistochemistry of tissue microarrays) enjoyed 100% disease-free survival (Figure 1B).

We tested these same biomarkers in a similar clinical trial conducted in advanced oropharynx cancer patients. Patients whose tumors expressed low levels of p53 and high levels of Bcl-xL had the poorest overall survival ($p=0.01$) and the shortest time to recurrence ($p=0.04$) relative to tumors with elevated levels of p53 (intermediate risk) and tumors with low p53 and low Bcl-xL (lowest risk). These clinical data, taken together, suggest that HNSCC characterized by low (wild-type) p53 and high Bcl-xL are cisplatin resistant and patients with these cisplatin-resistant tumors have a poor outcome.

HNSCC cell lines and cisplatin-resistant progeny were studied to further investigate this phenomenon. The parental lines had mutant p53 and low or moderate levels of Bcl-xL. In contrast, the cisplatin-resistant cells contained wild type p53 and high expression of Bcl-xL, suggesting that wild type p53 and Bcl-xL are responsible for the resistant phenotype.⁵ We used lentiviral vectors to modify p53 status and Bcl-xL expression in these tumor cell lines. Expression of wild type p53 in tumor cell lines with low Bcl-xL resulted in enhanced sensitivity to cisplatin-induced apoptosis. Expression of Bcl-xL alone did not significantly increase cisplatin resistance in cells with mutant p53. However, expression of Bcl-xL and wild type p53 together caused tumor cells to become highly cisplatin resistant, via a mechanism involving cell cycle arrest, DNA repair and escape from apoptosis.

Based upon this data, we hypothesized that agents designed to inhibit Bcl-xL or activate p53 function could be highly effective for the treatment of cisplatin-resistant HNSCC. (-)-Gossypol, a small molecule inhibitor of Bcl-xL, binds to the BH3 pocket of Bcl-2 and Bcl-xL and displaces proapoptotic BH3 proteins. (-)-Gossypol induces high levels of apoptosis in cisplatin-resistant HNSCC.^{5,6} (-)-Gossypol also suppresses tumor growth in HNSCC cells with wild type p53 and Bcl-xL in a xenograft murine model.⁷ We conclude that targeting Bcl-xL is a rational concept for targeting cisplatin-resistant HNSCC. Recently, we have begun testing another novel therapeutic strategy to target resistant HNSCC cells with wild-type p53 status using an inhibitor of the MDM2-p53 interaction. In HNSCC cells with wild type p53, p53 is effectively inhibited by its endogenous cellular inhibitor, the MDM2 (or HDM2) protein. MDM2 is transcriptionally activated by p53 and in turn, inhibits p53 activity through multiple mechanisms mediated by direct binding to p53. MDM2 thus functions as a potent and highly effective endogenous inhibitor of p53. Blocking the interaction between MDM2 and p53 using a potent small-molecule inhibitor can effectively reactivate p53 function in HNSCC cells with wild type p53, which leads to cell cycle arrest and apoptosis. Design and development of non-peptide small molecule inhibitors to target the MDM2-p53 interaction is an exciting and new therapeutic strategy for the treatment of cisplatin-resistant HNSCC with wild type p53.

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Figure 1.

Treatment selection based upon response to induction chemotherapy improves survival rates in advanced larynx cancer (**A**). Improved survival of patients selected for concomitant chemoradiation based upon favorable response to induction chemotherapy (UMCC 9520) vs. historical control (VA268). (**B**). Patients whose tumors expressed the lowest proportion of Bcl-xL in UMCC 9520 had 100% disease free survival.