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Topic: DRUG TOLERANCE

Development of predictive and therapeutic tools to overcome chemotherapy resistance in head and neck squamous cell carcinomas.

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Abstract

Introduction: Head and Neck Squamous Cell Carcinomas (HNSCC) are the 8th most frequent cancers worldwide, with a poor prognosis and a 5-year mortality rate of 55% that has not improved in the last decade. HNSCC is a heterogeneous disease that arises from the mucosal epithelium of the oral and nasal cavity, pharynx, larynx, and salivary glands, with the former three being the most frequent. They can be broadly classified into two groups: those associated with human papillomavirus (HPV) infection and those related to tobacco and alcohol consumption. The latter have worse prognosis as they have a greater mutational burden compared to HPV-positive tumors. First line treatment is surgery, combined with radiotherapy and/or chemotherapy. Advanced stage tumors which are unresectable or unsuitable for radiotherapy, are treated with standard chemotherapy, being cisplatin the gold standard. However, most of the patients develop resistance to cisplatin so they suffer relapses, which is associated with a very poor outcome. Although being used for decades, there are still no molecular biomarkers that predict tumor response to cisplatin and treatment is still chosen based on patient characteristics such as ECOG.

Objectives: The main objective of the study is the identification of genes that confer cisplatin resistance by performing whole-genome CRISPR/Cas9 KO screen and the functional validation of the genes identified in this CRISPR screen.

Methods: The screen was performed using a lentiviral library targeting all coding region of the genome. This library was transduced into an oral squamous cell carcinoma (OSC) cell line at a MOI <0.3. After the selection of successfully infected cells, these were split into three conditions: one that was collected after seeding, one that was left untreated for 7 days and one with a 7-day cisplatin treatment at the IC90 of the selected cell line. After this period, the cells were collected and the sgRNAs were amplified and sequenced. The sequencing data were analyzed and a list of several candidate genes was obtained. For the validation phase of the screen, we generated KOs using independent sgRNAs for each of the candidate genes and dose-response curves to cisplatin were performed. For the most promising candidate gene (*AMBRA1*), resistance to other chemotherapies was also analyzed.

Results: Of all the candidate genes studied in the validation phase of the screen, AMBRA1 KO cells showed the highest sensitivity to cisplatin treatment. Further studies showed that the KO cells were also more sensitive to carboplatin in three different OSC cell lines. Further mechanistic analyses are underway to elucidate which pathway is involved in conferring this sensitivity, such as cell cycle and apoptosis assays and mitophagy studies.

Conclusions: A successful whole-genome CRISPR/Cas9 KO screen has been performed and as a result AMBRA1 has been identified as a potential biomarker of response to platinum-based therapies.