Preliminary Evidence for a Resistance of HNSCC cells to Oxidative Stress and to Hindering of Apoptosis Signaling by Protein Kinase B (AKT) Phosphorylation

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Apoptosis is an essential mechanism for the control and selection of viable/functional cells. However, cancer cells acquire several mutations/alterations in order to escape or hinder the signaling to death, so they can proliferate under nutrient-poor or hypoxic environments, acquiring, in addition, a protective response against antineoplasic therapies. Although altered expression or phosphorylation of several proteins has been identified in cancer, its essence is largely unknown. Head and neck squamous cell carcinoma (HNSCC) have in common the high resistence to cisplatin and other drugs. In this connection, this study addressed the mechanisms that hinder apoptosis in HNSCC cell lines upon *tert*-butylhydroperoxide (*t*-BOOH)-induced oxidative stress. HEK293T (human embryonic kidney) and HN13 (oral carcinoma) cells were cultured in Dulbecco's Modified Eagle's Medium supplemented with 10% FBS, and kept in a humidified 5% CO_2 incubator at 37°C. Different *t*-BOOH concentrations (5-100 µM) and incubation times were tested. Apoptosis was followed by flow cytometry analysis of anexin/PI stained cells. Caspase-9 and caspase-3 activities were determined fluorimetrically and AKT phosphorylation by western blotting analysis. After 24 hs, *t*-BOOH (50 µM) induced apoptosis in 83% and 20% of HEK293T and HN13 cell lines, respectively. Caspase 9 and caspase 3 activities increased in HEK293T, but not in HN13 cells. AKT phosphorylation was assayed 0.5, 1, 4 and 6 hours after cells exposition to *t*-BOOH: HEK293T and HN13 cells exhibited pAKT decrease and increase, respectively. Collectively, these results point to a resistance of HNSCC cell lines to oxidative stress due to deregulation of apoptosis signaling via AKT phosphorylation.

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