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Enhanced Susceptibility to TRAIL-mediated Apoptosis in Oral Squamous Cell Carcinoma Cells through Down-regulation of Cellular FLIP

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In general, oral squamous cell carcinoma (OSCC) cells are relatively resistant to tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-mediated apoptosis during culture in vitro. In this study, we investigated the roles of phosphatidylinositol 3-kinase (PI 3-K), epidermal growth factor receptor (EGFR), proteasome, and histone deacetylase (HDAC) in TRAIL-mediated apoptosis of OSCC cells. The PI 3-K inhibitors wortmannin and LY294002, EGFR inhibitors AG1478 and C225, proteasome-inhibitor MG132, and HDAC inhibitors suberoylanilide hydroxamic acid (SAHA) and trichostatin A (TSA) markedly accelerated TRAIL-mediated apoptosis in OSCC cells. The addition of TRAIL to these inhibitor-treated cells resulted in caspase-8 activation and the loss of mitochondrial membrane potential. Furthermore, the inhibitors of caspase-3, -8, and -9 reduced the acceleration effect of these inhibitors on TRAIL-mediated apoptosis. These results suggest that the pro-apoptotic effect of these inhibitors on TRAIL-mediated apoptosis may contribute to both the extrinsic and intrinsic pathways of apoptosis. Although the PI 3-K and EGFR inhibitors did not affect expressions of the TRAIL receptors DR4 and DR5, the proteasome and HDAC inhibitors enhanced the expressions of these receptors. Furthermore, we observed a marked reduction in the expression of cellular FLICE inhibitory protein (c-FLIP). Fig. 1 Down-regulation of c-FLIP expression by transfected siRNA in HSC-2 cells. c-FLIP protein expression was analyzed by Western blot analysis as described in the Materials and Methods. All experiments were performed four times independently. A, c-FLIP protein levels, Lane 1, control siRNA; Lane 2, c-FLIP siRNA. c-FLIP protein levels were analyzed by densitometry, and the c-FLIP to β-actin ratio was calculated the arbitrary units. Control showed as ratio of 1.0. B, treated HSC-2 cells were incubated with TRAIL (200 ng/ml) for 24 h, and cells were assayed for TRAIL-mediated apoptosis as described in the Materials and Methods. Results represent the mean ± SD of four experiments. *p<0.05, compared with control cells.
(c-FLIP) with these inhibitors. The knockdown of c-FLIP in OSCC cells with a small interfering RNA (siRNA) strongly enhanced TRAIL-mediated apoptosis. Although these inhibitors also modulated the expressions of members of the Bcl-2 and inhibitor of apoptosis protein (IAP) family, common mechanisms of modulation were not observed. These results suggest that the down-regulation of c-FLIP may represent a novel strategy for overcoming resistance to TRAIL-mediated apoptosis in OSCC cells.