HIF1-α KNOCKDOWN BY RNA INTERFERENCE POTENTIATES THE CYTOTOXIC EFFECT OF DOXORUBICIN IN HUMAN COLON CARCINOMA CELLS

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Colon adenocarcinomas are refractory to a number of widely used anticancer agents. Multifactorial mechanisms have been implicated in the poor response of these tumors to radio- or chemotherapy, including the presence of hypoxic regions inside the tumor mass; thus, factors regulating hypoxic adaptation may be good targets for anticancer therapy. In mammalian cells, the master regulator of hypoxic response pathways is the hypoxia-inducible transcription factor (HIF)-1. More than 60 putative HIF-1 direct target genes have been identified; particularly relevant to cancer progression are genes encoding proangiogenic factors (notably, the vascular endothelial growth factor, VEGF), glucose transporters and glycolytic enzymes, as well as factors involved in cell proliferation and survival and in tumor invasion.

In the present study we investigate the role of HIF-1 in the response of two colon adenocarcinoma cell lines, HCT116 and HT29 cells, to doxorubicin (DOX) under actual (pO_2 1 or 0.1%) or chemically-simulated (by the use of $CoCl_2$) hypoxic conditions; in addition, we evaluate the possibility to improve cellular response to the antracycline by inhibiting HIF-1 expression/activity .

As expected, incubation of HCT116 and HT29 cells under hypoxic conditions induces an increase in HIF-1α levels, evaluated by western blot analysis of cells lysates, and in HIF-1 transcriptional activity, determined by a luciferase assay on HCT116 and HT29 cells transfected with a plasmid containing the luciferase gene under control of three copies of a HIF-1-responsive element. Increase in HIF-1 was associated to decrease in the apoptotic response of the two cell lines to DOX, as assessed by flow cytometric analysis. To confirm the hypothesis of a causal role of HIF-1 in poor cellular response to DOX, we evaluated the possibility to restore DOX sensitivity under hypoxic conditions by inhibiting HIF-1α expression. To this aim, we transduced colon carcinoma cells with a lentiviral vector encoding a shRNA that induces HIF-1α mRNA degradation by RNA interference. As a result, HIF-1α levels were down-regulated in transduced cells and a significant increase in DOX-induced apoptosis was observed.

Taken together, our observations indicate HIF, or its HIF-1α subunit, as potential targets for polychemotherapeutic strategies in the management of colon adenocarcinomas.