Combined treatment with everolimus and 5-aza-2′-deoxycytidine: a new therapeutic strategy in medullary thyroid cancer.

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Medullary thyroid carcinoma (MTC) is a neuroendocrine malignancy highly resistant to chemo- and radiotherapy. Several reports demonstrated that epigenetic changes, such as aberrant DNA methylation, contribute to the induction of drug resistance in cancer cells. The aim of this study was to evaluate the potential antitumor effects of everolimus, an mTOR inhibitor, in combination with 5-aza-2′-deoxycytidine (AZA), a demethylating agent, in two MTC cell lines (TT and MZ-CRC-1). Both drugs significantly inhibited MTC cell proliferation in a dose-dependent manner after 6 days of treatment. We performed these experiments with MTT assay and the resulting data were elaborated by the dedicated software Calcusyn in order to identify potential synergistic doses between everolimus and AZA. Using this mathematical model, synergistic conditions occur when the combination index (CI) was below 1.0. Simultaneous exposure of everolimus and AZA resulted in synergistic antiproliferative effect in both cell lines. The optimal results (CI = 0.1) were obtained in MZ-CRC-1 cells with the following concentrations: IC_{25}everolimus (2.1 nM) plus IC_{75}AZA ($1.4x10^{-7}$M). Thereafter, we evaluated whether the mechanisms of the anti-tumor synergistic interaction involved cell cycle kinetics and apoptosis. After 6 days of incubation, MZ-CRC-1 cells were labelled with propidium iodide and evaluated by FACS. The synergistic combination of two drugs significantly induced a mild decrease of MZ-CRC-1 cells in S phase (-27% *vs* untreated control). This effect was comparable to that observed after everolimus alone (-21.5% *vs* untreated control). Interestingly, while either agent alone did not induce apoptosis (everolimus: + 0.9%, AZA: + 2.7% *vs* untreated control), everolimus plus AZA induced a potent stimulation of apoptosis in MZ-CRC-1 cells (+82% *vs* untreated control), as determined by Annexin-V/propidium iodide staining and flow-cytometric analysis. In conclusion, this is the first evidence that epigenetic therapy enhances the antitumor activity of everolimus through the induction of apoptosis, suggesting a new therapeutic strategy in MTC.