EPIGENETIC MECHANISMS OF SENSITIVITY TO IRINOTECAN IN COLORECTAL CANCER CELL LINES

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Promoter hypermethylation is an epigenetic phenomenon known to silence numerous genes involved in apoptosis, cell-cycle control, and mechanisms deregulated during colorectal cancer development. Other genes may be indirectly modulated through transcription factors or corepressors silencing. Demethylating agents may revert these modifications, change gene expression profile and favor arrest of proliferation and apoptosis. Irinotecan, a Topoisomerase I (Top I) inhibitor, is one of the most frequently used drugs in the treatment of advanced colorectal cancer. Although clinical studies reported an evident interindividual variability in the response to this drug, there are no data correlating Top I mutations with drug response. Moreover, it has been shown that Top I expression levels in colorectal cancer specimens are variables and may predict individual sensitivity to the irinotecan. Thus, pre-treatment of colorectal cancer cells with a demetilating agent (5-Azacytidine) may enhance their chemosensitivity to irinotecan by at least one of these three mechanisms: (1) direct demethylation of Top I promoter; (2) indirect activation of Top I expression; (3) induction of cell-cycle arrest and/or apoptosis in cells whose DNA has been damaged by irinotecan. Three p53-mutated (HT29, SW620 and WiDr) and one p53-wt (LS174T) colorectal cell lines were treated with the active metabolite of irinotecan SN-38, 5-Azacytidine, or a combination of the drugs. Cytotoxicity and cytofluorimetric assays were performed to study drug activity and ell-cycle modulation. Top I mRNA expression in cells treated with the 5-Azacytidine was measured by quantitative PCR and the methylation status of Top I promoter by Methylight PCR 5-Azacytidine at non-cytotoxic concentration enhanced the antitumor activity of irinotecan in HT29, SW620 and WiDr (IC_{50} reduction of 2.9-22.3-fold); this effect was coupled to an increased expression of Top I in two cell lines. In LS174T cells, opposite effects in terms of cytotoxicity and Top I expression were observed. Cytofluorimetric assays demonstrated that the synergistic interaction of 5-Azacytidine ad irinotecan may be due to activation of G1 checkpoint after DNA damage. Methylight PCR showed that Top I promoter wass normally hypomethylated, thus the increased Top I expression after 5-Azacytidine treatment was caused by activation of trancription factors regulating Top I promoter. In conclusion, pre-treatment of p53-mutated colorectal cancer cells with a demethylating agent enhances the antitumor activity of irinotecan through two distinct mechanisms, Top I up-regulation and cell-cycle modulation, and suggests the clinical use of this combination.