Effects of 5-Fluorouracil in Combination with Novobiocin in Cancer Cells with Microsatellite Instability

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Therapeutic approaches for colorectal cancer (CRC) employ DNA-damaging agents, such as fluoropyrimidines, platinum compounds, and DNA topoisomerase inhibitors. To date, only the functional status of DNA mismatch repair (MMR) has been established as a molecular marker influencing treatment response in the oncology practice for CRC. MMR deficiency is clinically equivalent to a high microsatellite instability (MSI) phenotype, while tumors with functional MMR correspond to a microsatellite stability (MSS) phenotype. Induction of MSI has been observed in cells with non-functional MMR, as a result of DNA double-strand break repair involving DNA polymerase theta (POLQ). Thus, the aim of the present work was to study the effect of Novobiocin (POLQ inhibitor) on the cytotoxicity of 5-FU in CRC cell lines with different MMR status. For this purpose, human colon cancer cell lines, proficient (HT29) and deficient (HCT116) in MMR, were treated with 5-FU alone or in combination with Novobiocin. The evaluation of cell survival in our study confirmed the lower sensitivity of HCT116 cells with non-functional MMR to 5-FU, but no effect of the combined treatment with NVB was found by Sulforhodamine B (SRB) and clonogenic assays. In the HT29 cell line, after treatment (24 h) with 5-FU, cell cycle arrest in the G1/S phase was observed, in contrast to HCT116, where an accumulation of cells in G2 was observed. To investigate whether 5-FU and NVB induce DSBs, we assessed the formation of γH2AX foci, simultaneously labeling cells with EdU to distinguish dividing cells. While DNA double-strand breaks increased after treatment with 5-FU and 5-FU+NVB at 24 h in HT29, in HCT116 such an increase was observed after 48 h for the combined treatment, and after 72 h for 5-FU alone. The replication fork velocity, measured in Fiber assay, decreased after 24 hours of treatment with 5-FU and combination with NVB in both cell lines, while NVB alone had a stronger effect in HCT116. The inhibition of POLtheta with NVB, as monotherapy or in combination with 5-FU, showed different effects on replication and DSB formation in HCT116 (MSI) and HT29 (MSS) cell lines, which may be due to activation of different DNA repair pathways. However, the combined treatment with NVB did not enhance 5-FU toxicity in the studied cell lines.

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