IDENTIFICATION OF THERAPEUTIC TARGETS FOR CHEMOTHERAPY-RESISTANT COLON CANCER STEM CELLS

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Current first-line chemotherapy generally consists of cytotoxic agents. While these agents may initially control disease by effectively debulking tumors, the tumors invariably recur due to the ability of cancer stem cells (CSCs) to survive and repopulate the tumor mass. To identify potential molecular targets in 5-fluorouracil (5-FU) refractory colon CSCs, we developed 5-FU resistant cell lines over a period of a year. Here we show that 5-FU maintains colon CSCs in an undifferentiated state in vitro. While untreated control cells passaged in parallel progressively acquired a differentiated morphology comprising crypt-villus structures, cells treated with an IC50 dose or escalating doses of 5-FU organized in round colonies with defined edges. Cells from these chemoresistant colonies exhibited a high nucleus-to-cytoplasm ratio and prominent nucleoli, features of pluripotent stem cells. Chemoresistant cells showed a reduced proliferation rate with respect to their counterparts. While immunofluorescence analysis revealed chemoresistant cells expressed a panel of pluripotency markers, flow cytometric analysis indicated a change in CD antigens associated with tumorigenicity and pluripotency in chemoresistant cells as compared to untreated cells. These results suggest colon CSCs, while under the selective pressure of chemotherapy in vitro, are likely to display an adaptive plasticity resembling fate reprogramming. Three-dimensional growth in Matrigel revealed untreated cells were able to arrange themselves in a ring around a well-defined central lumen. Conversely, chemoresistant cell-derived organoids had poorly defined or no central lumens. While untreated cells generated fast-growing, well-differentiated tumors, resistant cells generated slow-growing, moderately differentiated tumors in vivo. Finally, a large number of genes were screened in cells from both groups using Real-Time PCR arrays. Through this analysis, we identified molecular targets that might assist in the development of therapeutic strategies which will counteract the mechanisms of chemoresistance.