Pharmacological strategies to reduce the tumor stem cell population.

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Glioblastoma multiforme is one of the most aggressive CNS tumors. It is characterized by its high invasiveness, proliferation, high levels of recurrence and death, as well as its chemo and radioresistance. Solid cancers are characterized by a hierarchical organization, where there is a small population of cancer stem cells (CSCs) or cancer initiator cells (CICs). These cells are capable of repopulating a tumor, which leads to recurrence. CSCs are characterized by performing asymmetric mitosis, where parts of the cells remain as CSCs and the other part differentiates. These differentiated cells are unable to repopulate a tumor because they have lost their stem cell capacity. CSCs have shown to be relatively resistant to conventional anticancer therapies such as chemo and/or radiotherapy. Doxorubicin (doxo) is an anticancer agent used in numerous types of tumors. Its main action is to inhibit topoisomerase II. Depending on the concentration of doxo employed, diverse behaviors are observed. For example, low doses (10-7 M) lead to cell senescence while high doses (10-5 M) lead to cell apoptosis. The aim of this study was to investigate the effect of doxo in CSCs derived from human glioblastoma. We performed the sphere forming assay, as an indicator of the presence of CSCs. Our assay utilized the cell line U87-MG and two types of culture medium. The first contained DMEM Low Glucose with FBS 10% and the second being a neuro stem cell medium (NSCM) which contained DMEM F12 with CSCs growth factors and being treated with two doses of doxo, 1 nM and 10 nM. We saw a decrease in the sphere formation in both types of medium and concentrations. Medium containing showed around 50.7±7.2 spheres in control against 2.5±2.2 and 1.8±0.8 in doxo, 1 nM and 10 nM respectively; NSCM showed around 312.1±17.6 spheres in control against 125±32.5 and 41±9.1 in doxo, 1 nM and 10 nM respectively. After we performed flow cytometry for two specific markers of CSCs, Oct4 and Nanog, an increased number of positive cells for Oct4 and Nanog was mainly seen using the differentiated medium treated with doxo 1 nM. We also measured the presence of senescence and results showed increased β-galactosidase positive cells after the treatment with doxo 1nM (12 %) and 10 nM (9 %) compared to control (1 %). These results indicated that doxorubicin, mainly in the 1 nM, may be selecting these CSCs by selecting spheres and lead to senescence within the differentiated cells.

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