

melanoma cells. Potentially, research into substances like RA could help to clarify mechanisms that may provide protection to healthy normal cells while exclusively damaging neoplastic cell, thus presenting a new strategy for cancer patients undergoing radiotherapy.

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Radiosensitivity enhancement and MMP modulation: A dual role for epigenetic drugs in breast radiotherapy

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Introduction. Breast cancer is a leading death cause among 30 and 50 years old women from industrialized countries and distal metastases after treatment are the main responsible of this high death rate. Ionizing radiation (IR) along with chemotherapy has traditionally been used as first-line therapy against breast cancer. Protocol optimization led us to improve clinical breast cancer outcome. However, during the last decade, some epidemiological and translational studies have been pointing out some tumour-promoting properties in those cells that survive after radiation exposure. Matrix metalloproteinases (MMPs) have been proposed as one of the main candidates to enrol this effect since MMPs have been described as chief proteases involved in stromal fibre turnover.

Hypothesis. The purpose of this study was to evaluate in vitro the MMP contribution to second malignancies arising after breast radiotherapy (RT) and to demonstrate whether this effect might be epigenetically softened.

Materials and methods. Two breast cancer cell lines MDA-MB-231 and MCF-7 were cultured in DMEM supplemented with 10% FBS. Radiation-derived MMP levels were measured by both real-time PCR and WesternBlot. Gelatinolytic activity was detected by confocal microscopy and an ELISA-like assay using DQ gelatin. Invasion capability after radiation was also quantified.

Results. Our results revealed that MDA-MB-231 significantly enhanced invasion capability after IR exposure. Gene and protein expression analyses revealed increased amounts of MMP-1 (collagenase), MMP-3 (stromelysin) and both TIMP-1 and TIMP-2 (tissue inhibitors/activators of MMPs) after irradiation. MMP activity (measured by the amount of cleavage gelatine) was also increased in this breast cancer cell line. In order to soften this radiation-induced MMP activity, the histone deacetylase inhibitor valproic acid (VA) was tested. Our findings reflect that VA reduces both IR-enhanced MMP gene expression and activity. Moreover, our data confirm a dose-dependent radiosensitizing effect of VA.

Conclusions. Taken together, our results suggest that VA might be considered a potential adjuvant enhancer of breast radiotherapy effectiveness. Our data reveal that VA not only is able to radiosensitize breast tumour cells but also to reduce RT-derived tumour-promoting effects.

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Stem-like cells from breast cancer: Crucial players in cellular response to radiotherapy

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Introduction. Although radiotherapy approach has improved local control and survival rates with acceptable toxicity, recurrence is essentially seen in most patients and some of them develop radioresistance that might limit any further treatment. Stem-like cells (SLCs) isolation from breast tumors has allowed the analysis of the molecular mechanism involved in their origin, self-renewal, differentiation into tumor cells, resistance to radio and chemotherapy, and invasiveness and metastatic ability.

Hypothesis. The purpose of this work was to evaluate the role of SLCs in breast cancer response to radiotherapy. We have studied the influence of ionizing radiation (IR) exposure in tumor SLCs enrichment and whether this subpopulation contributes to tumor radioresistance. Evaluation of IR-induced tumor-promoting properties in those SLCs has also been carried out.

Materials and methods. Two breast cancer cell lines (MCF-7 and MDA-MB-231) and SLCs, sorted by flow cytometry from those cell cultures, were grown at 37 °C and 5% CO₂ in DMEM supplemented with 10% FBS and stem cell culture medium, respectively. Cells were irradiated 24 h after seeding and harvested 24 h later. Radiation-induced invasiveness-related matrix metalloproteinases

levels were measured by real-time PCR. Radiosensitivity parameters were determined by clonogenic assays. α and β coefficients were calculated using the linear-quadratic model $[-\ln(S) = \alpha D + \beta D^2]$.

Results. Flow cytometry sorting have revealed that surviving cells to IR exposure increased SLC markers (CD44, CD24 and aldehyde dehydrogenase – ALDH1) in a dose-dependent manner after seven days of incubation. In MCF7 cell line 4 Gy increased the ALDH activity to 17.8-folds, while CD24 was not detected. However, in the MDA MB 231 cell line the largest increase in cancer stem cell markers was induced with 6 Gy, increasing 8.6-fold ALDH activity, 5.2 the label for CD44 with a negative expression for CD24 suggesting that IR exposure might promote SLC phenotype. Clonogenic assays revealed significant enhanced radioresistance capability for SLCs. On the other hand MMP gene expression profiles after radiation exposure from both subpopulations are radically different.

Conclusions. Our results show that enrichment in SLC subpopulations after IR is highly dependent of the radiation dose and the cell line used. Taken together our data suggest a crucial role of SLCs in cellular response to radiation therapy.

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