ALTERED N-GLYCOSYLATION IN PROGENY FROM IRRADIATED COLORECTAL CANCER CELLS CONTRIBUTES TO ACQUISITION OF MESENCHYMAL-LIKE PHENOTYPE

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Colorectal cancer (CRC) represents the third most commonly diagnosed cancer in males and the second most commonly diagnosed cancer in females. CRC is a leading cause of cancer-related mortality, and it accounts for 8.5% of all cancer-related deaths. In rectal cancer, radiotherapy has been explored for improving the local control and survival of locally advanced disease promoting reduction of local recurrence risk. However, the dose fractionated protocols, which are important to preserve the normal tissue, in some cases may also allow the tumor repopulation by radioresistant cancer cells. Recently, we demonstrated that transgenerational effects induced by radiation increase the malignant features in progeny derived from irradiated parental HT-29 colorectal cancer cells. The aim of the present study is to investigate the changes in N-glycans expression profile in these radioresistant progenies and its relationship with acquisition of mesenchymal-like phenotype. First of all, irradiated HT-29 cells were used as a model of EMT-induction. Then, β1,6-GlcNAc branched and α2,6-sialylated complex-type N-glycans were detected through lectin blotting and flow cytometry using the specific lectins L-PHA (Phaseolus vulgaris L) and SNA (Sambucus nigra) respectively. Our results have shown that the progeny of HT-29 colorectal cancer cells displayed mesenchymal-like features and increased expression of β1,6-branched N-glycans concomitantly. In addition, an increase in the levels of the enzyme N-acetylglicosamyltransferase 5 (MGAT5), which is involved in the synthesis of this branched structures, was verified by qRT-PCR. Subsequently, using a 2D electrophoresis followed by immunoblotting it was observed that the progenies derived from the irradiation of HT-29 cells present an increase in E-cadherin N-glycosylation levels. Corroborating these results, the immunoprecipitation assay indicated a hyperglycosylation of that same protein. Increased labeling of branched complex N-glycans in cells that had a loss of E-cadherin localization at cell-cell contacts was also observed by immunofluorescence. Finally, the pharmacological inhibition of the α-mannosidase II enzyme by swainsonine, thereby blocking the formation of complex-type N-glycans, prevented the increased invasiveness observed only in the irradiated progenies, and also induced co-localization of E-cadherin and actin at cell-cell contacts. Together, our in vitro results suggest that the biosynthesis of N-glycans appears to be a potential therapeutic target to inhibit malignant phenotype displayed by these radioresistant progenies.

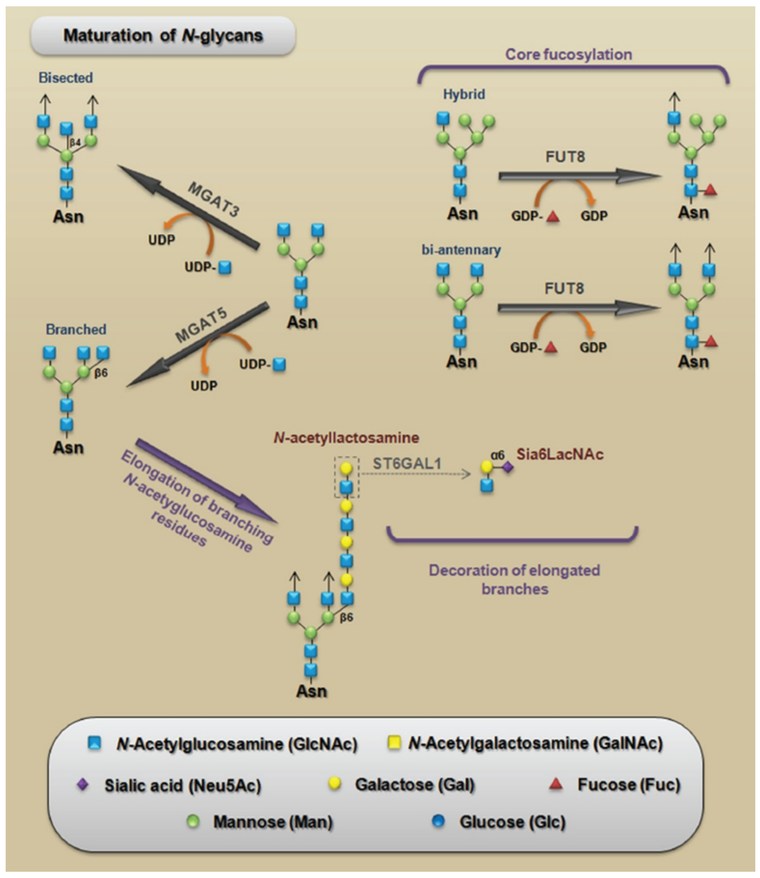

Figure 1: Schematic representation of the three components of N-glycans maturation: (I) core fucosylation representing the addition of monosaccharides to the core; (II) N-acetyllactosamine structure, which represents the elongation of branching N-acetylglucosamine residues; and (III) the synthesis of Sia6LacNAc, which represents the decoration of elongated branches. β1,6-branched, N-acetyllactosamine and Sia6LacNAc are examples of cancer-associated carbohydrate antigens in CRC. MGAT3 catalyzes the transfer of GlcNAc from UDP-GlcNAc to the core mannose in a β1,4 linkage, thus generating bisected N-glycans and MGAT5 catalyzes the transfer of GlcNAc in a β1,6 linkage, generating branched N-glycans. MGAT5, N-acetylglucosaminyltransferase V; MGAT3, N-acetylglucosaminyltransferase III; FUT8, α1,6-fucosyltransferase; ST6GAL1, α2,6- sialyltransferase.

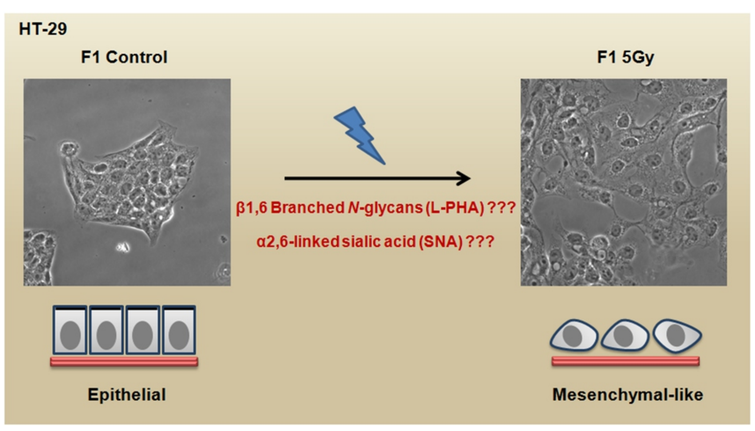

Figure 2: Cell morphology by phase contrast microscopy. The progenies derived from irradiated cells showed an mesenchymal-like phenotype with abnormal colony formation and more dispersed cells when compared to control progenies.

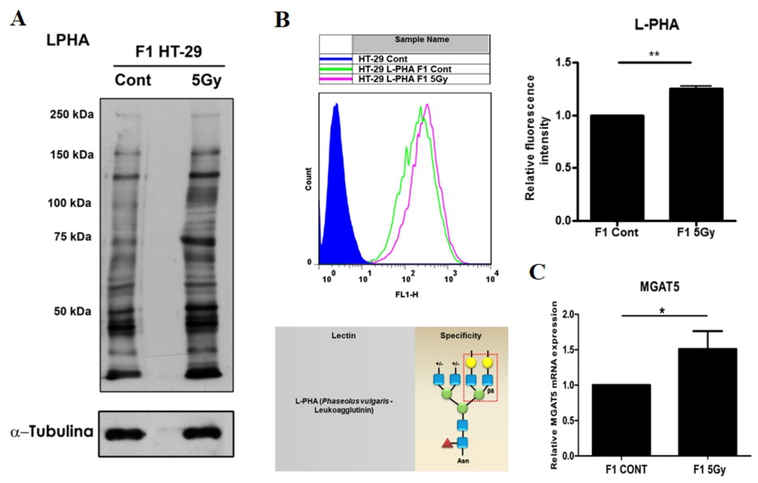

Figure 3: Radiation induces increase of β-1,6 branching structures. (A) Blotting using the L-PHA lectin indicated increased global expression of β-1,6-branched glycans in irradiated progenies. (B) Analysis by flow cytometry using the same lectin and quantification of fluorescence intensity showed a significant increase in the expression of the same arrangement in the irradiated progenies. (C) MGAT5 mRNA expression levels also increased in F1 5Gy. Mean + S.E.M. (N = 3), * P <0.05; ** P <0.01.

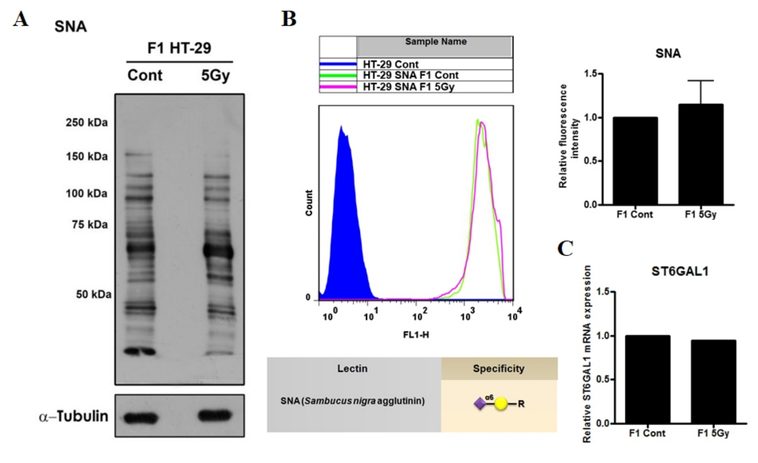

Figure 4: Radiation does not seem to interfere with the expression levels of terminal sialylation. (A) Blotting using the SNA lectin indicated that the overall terminal glycans expression do not appear to change in F1 5Gy progeny compared to those Control F1. Confirming that result, in (B) Analysis by flow cytometry using the same lectin and quantification also indicated no increase in fluorescence intensity of such structures. Mean ± S.E.M. (N = 3). As well as there was no change in (C) mRNA ST6Gal1 expression levels of F1 5Gy. Mean ± S.E.M. (N = 3).

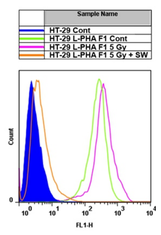

Figure 8: Monitoring of sustained treatment efficiency by flow cytometry using the lectin L-PHA. 24 hours after the sustained treatment with swainsonine the increased expression of β-1,6-branched N-glycans observed in the F1 5Gy cells was prevented.

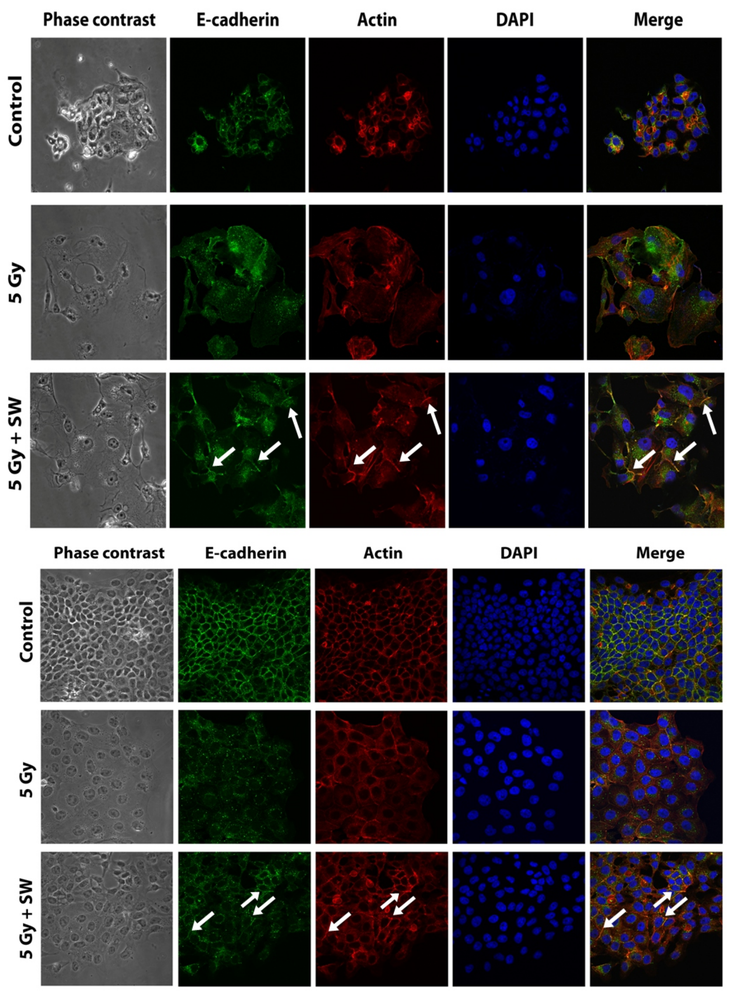

Figure 9: Analysis of the subcellular localization of E-cadherin and the cytoskeleton organization of progenies derived from radiation surviving cells after sustained treatment with swainsonine. Immunofluorescence of F1 5Gy+SW progeny have indicated that after (A) 24 hours or (B) 48 hours of sustained treatment with swainsonine the drug induced the co-localization of both E-cadherin and actin on cell contacts regions. Arrows: cell-cell contacts regions.

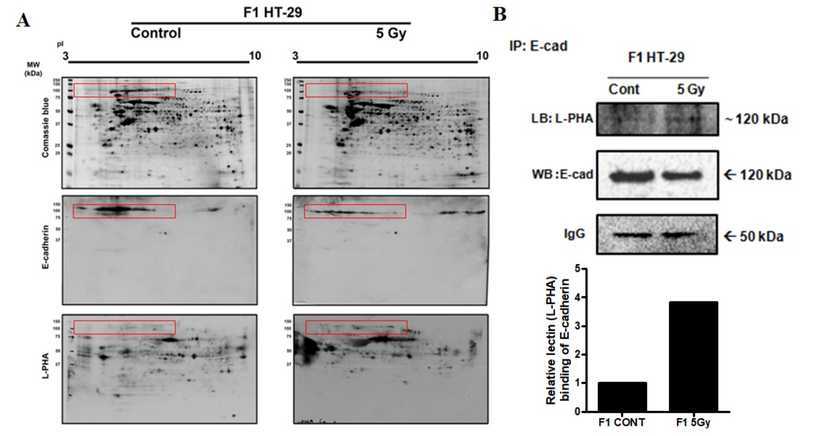

Figure 5: β-1,6 branched *N*-glycans are increased in E-cadherin from F1 5Gy cells. (A) 2D, (B) 1D electrophoresis and quantification of relative L-PHA binding to E-cadherin were performed to evaluate E-cadherin expression and glycosylation. Reduced expression of E-cadherin in F1 5Gy progenies was accompanied by increased expression in β-1,6-branched N-glycans (L-PHA positive N-glycans), suggesting that E-cadherin is hyperglycosylated in these progenies. Representative data from two independent experiments. Mean (N = 2).

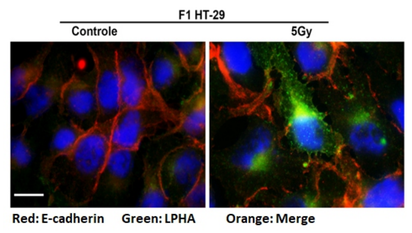

Figure 6: Subcellular localization of E-cadherin and *N*-glycan branching by immunofluorescence. In F1 5Gy cells there were an increase in labeling for L-PHA lectin (green) as well as a decrease in E-cadherin (red) staining. Representative data from a single experiment.

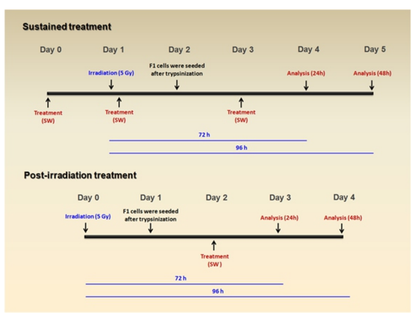

Figure 7: Treatment strategies using swainsonine. Two treatment protocols were developed, the sustained treatment and post-irradiation treatment. In the first, progeny is treated with swainsonine before and after exposure to radiation. In the second, progeny is treated with swainsonine only after exposure to radiation.

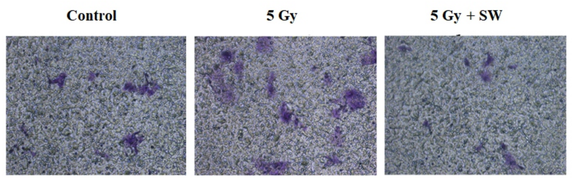

Figure 10: Analysis of the invasive potential in progeny subjected to radiation after sustained treatment with swainsonine. The F1 5Gy+SW cells show a reduced invasiveness compared with F1 5Gy cells.

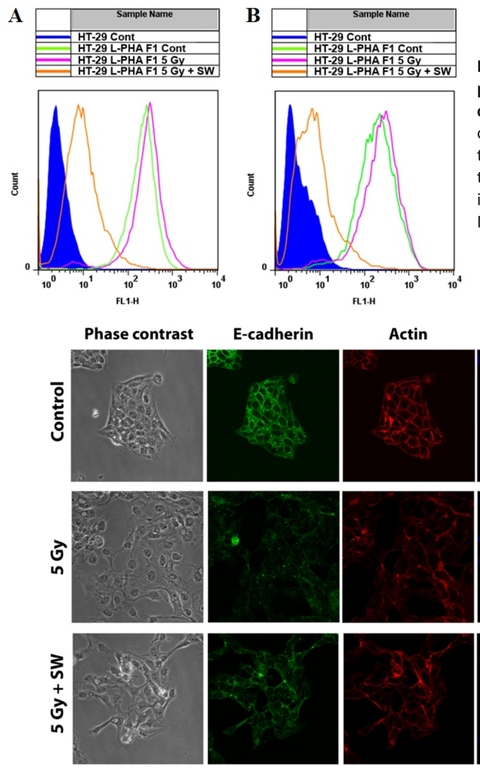

Figure 11: Monitoring the efficiency of post-treatment irradiated by flow cytometry using L-PHA lectin. (A) Analysis of post-treatment with swainsonine in a time of 24 hours and (B) 48 hours after treatment. Swainsonine is capable of inhibiting the synthesis of β-1,6 branched N-glycans structures.

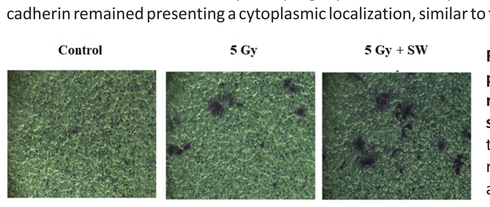

Figure 12: Analysis of the subcellular localization of E-cadherin and the cytoskeleton organization of progenies derived from radiation surviving cells after post-treatment with swainsonine. Immunofluorescence of F1 5Gy+SW progeny after 48h of post-irradiated treatment indicated that the E-cadherin remained presenting a cytoplasmic localization, similar to those observed in cells F1 5Gy.

Figure 13: Analysis of the invasive potential in progeny subjected to radiation after post-treatment with swainsonine. The post-irradiated treatment of surviving cells is not able to reverse the more invasive phenotype acquired by the cells after irradiation.

Projeto Gráfico: Área de Edição e Produção de Materiais Técnico-Científicos / INCA