ALTERED *N*-GLYCOSYLATION IN PROGENY FROM IRRADIATED COLORECTAL CANCER CELLS CONTRIBUTE TO ACQUISITION OF EMT-LIKE PHENOTYPE

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INTRODUCTION: 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. Recently, we demonstrated that transgenerational effects induced by radiation increase the malignant features on the progeny derived from irradiated parental HT-29 colorectal cancer cells.

OBJECTIVES: The aim of the present study is to investigate the changes in N-glycans in these radioresistant progenies and its relationship with acquisition of EMT-Like Phenotype.

METHODS: Irradiated human colorectal adenocarcinoma cell line HT-29 (HTB-38TM) was used as model of EMT-acquisition. β1,6-branched and α2,6-sialylated complex-type *N*-glycans were detected using the lectins L-PHA (*Phaseolus vulgaris L*) and SNA (*Sambucus nigra*) respectively. Cell migration was examined by transwell assay followed by crystal violet staining.

RESULTS: Our preliminary results have shown that the progeny of HT-29 colorectal cancer cells displayed both mesenchymal-like features and increased expression of β1,6-branched *N*-glycans. In addition, pharmacological inhibition of α-mannosidase II enzyme by swainsonine, thereby blocking the formation of complex-type *N*-glycans, have inhibited cell migration in HT-29 progenies.

CONCLUSION: Additionally, our in vitro results have suggested that the biosynthesis of *N*-glycans appears to be a potential therapeutic target to inhibit malignant phenotype displayed by these radioresistant progenies.

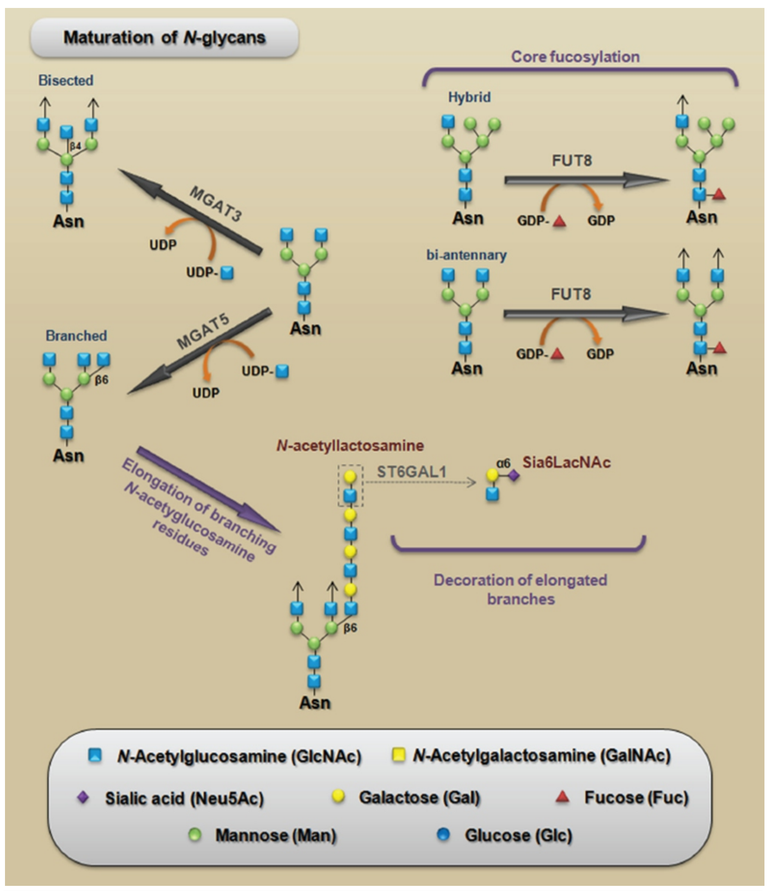

Figure 1: Schematic representation of the three components of maturation of N-glycans: (I) core fucosylation representing the addition of monosaccharides to the core; (II) N-acetyllactosamine structure, which represents the elongation of branching N-acetylglucosamine residues; and (III) the synthesis 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.

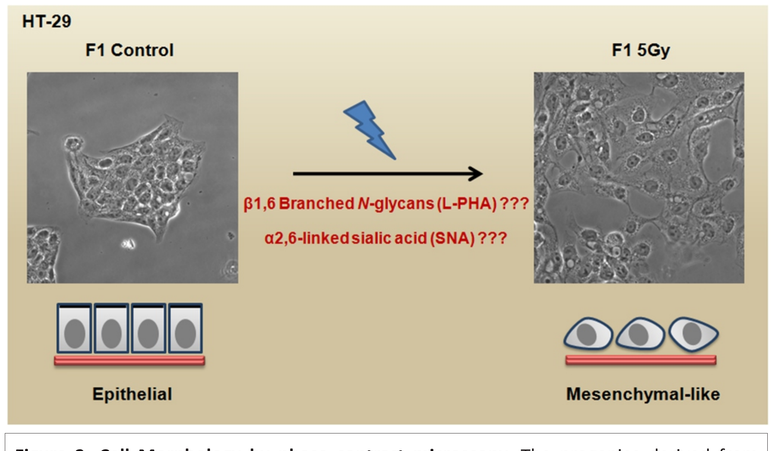

Figure 2: Cell Morphology by phase contrast microscopy. The progenies derived from irradiated cells showed an mesenchymal-like phenotype with abnormal colony formation and more dispersed cells when compared to control progenies.

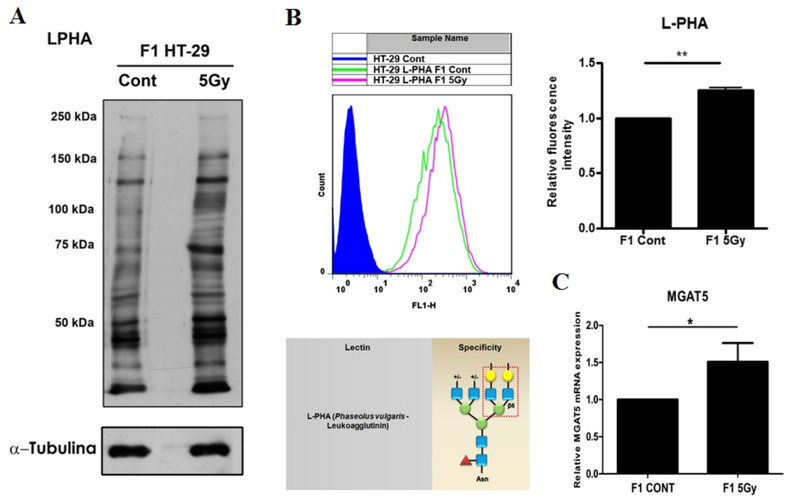

Figure 3: Radiation induces increase of β-1,6 branching structures. (A) Blotting using the L-PHA lectin indicated increased global expression of β-1,6 glycans branched irradiated progenies. (B) Analysis by flow cytometry using the same lectin and quantitation of fluorescence intensity showed a significant 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.

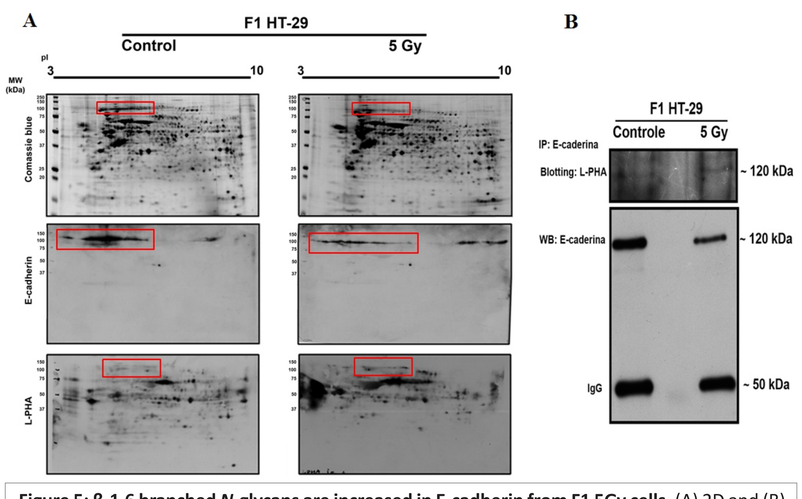

Figure 5: β-1,6 branched *N*-glycans are increased in E-cadherin from F1 5Gy cells. (A) 2D and (B) 1D electrophoresis were performed to evaluate E-cadherin expression and glycosylation. Reduced expression of E-cadherin in F1 5Gy progenies was accompanied by increased expression in β-1,6-branched *N*-glycans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

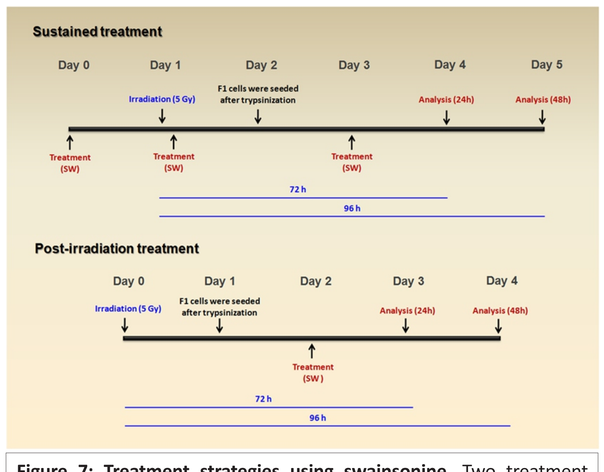

Figure 7: Treatment strategies using swainsonine. Two treatment protocols were developed, the sustained treatment and post-irradiation treatment. In the first, progeny is treated with swainsonine before and after exposure to radiation. In the second, progeny is treated with swainsonine only after exposure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

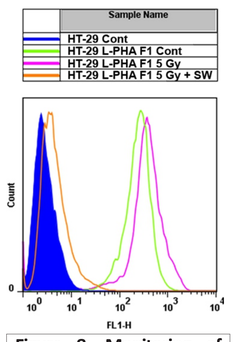

Figure 8: Monitoring of sustained treatment efficiency by flow cytometry using the lectin L-PHA. The sustained treatment with swainsonine prevents the increased expression of β-1,6-branched N-glycans observed in the F1 5Gy cells.

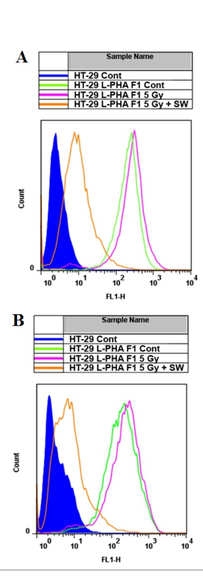

Figure 11: Monitoring the efficiency of post-treatment irradiated by flow cytometry using L-PHA lectin. (A) Analysis of post-treatment with swainsonine irradiated in a time of 24 hours and (B) 48 hours after irradiation is capable of inhibiting the synthesis of β-1,6 branched N-glycans structures.

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.

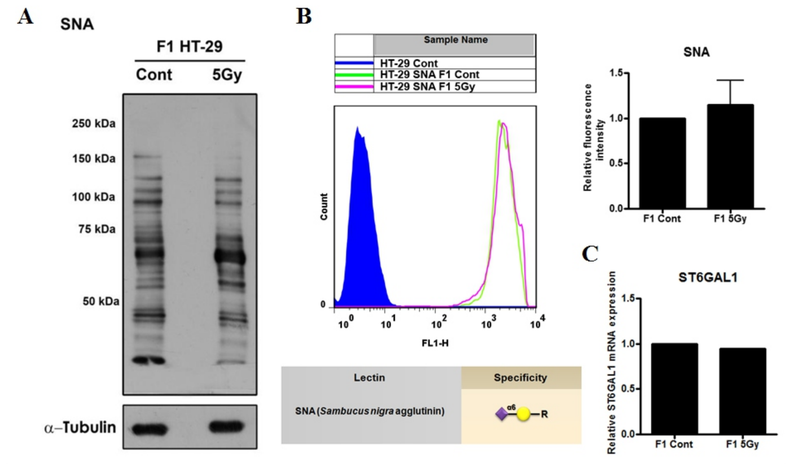

Figure 4: Radiation does not seem to interfere with the levels of expression of terminal sialylation. (A) Blotting using the SNA lectin indicated that the overall terminal glycans expression do not appear to change in 5Gy F1 progeny compared with those Control F1. Confirming that result in (B) Analysis 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) levels of expression of mRNA ST6Gal1 F1 5Gy. Mean ± S.E.M. (N = 2).

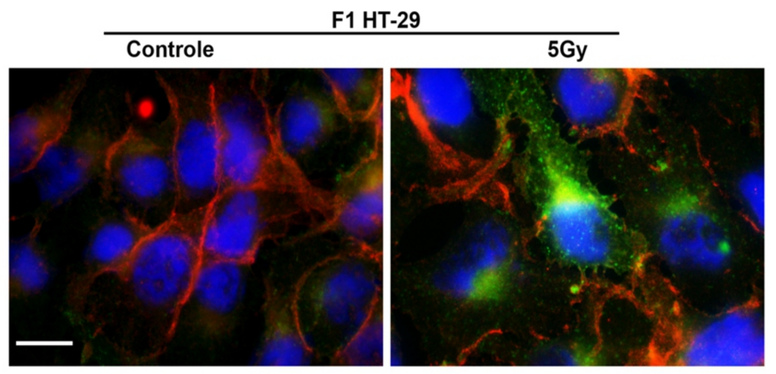

Figure 6: Subcellular localization of E-cadherin and N-glycan branching by immunofluorescence. In F1 5Gy cells there was an increase in labeling for L-PHA lectin (green) as well as reducing marking of E-cadherin (red). Representative data from a single experiment.

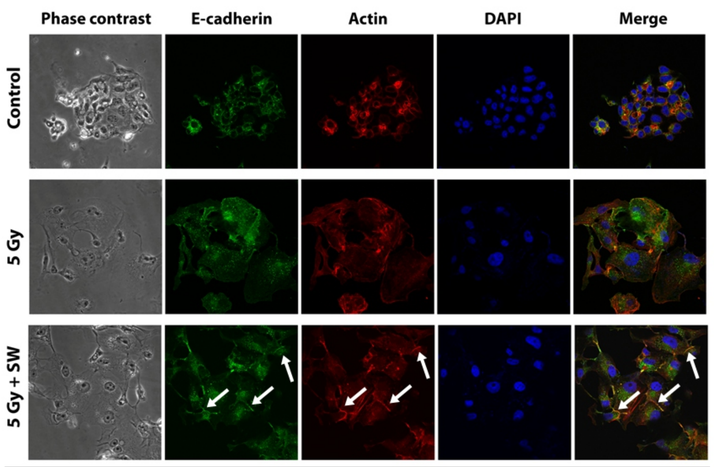

Figure 9 Analysis of the subcellular localization of E-cadherin and the cytoskeleton organization of progenies derived from radiation surviving cells after sustained treatment with swainsonine. Immunofluorescence of F1 5Gy+SW progeny have indicated that after 24 hours of sustained treatment with swainsonine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

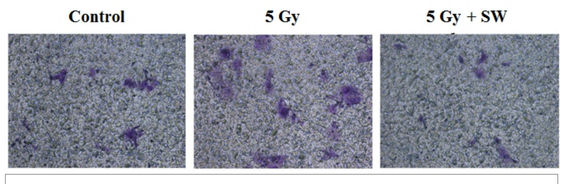

Figure 10: Analysis of invasive potential of progenies subjected to radiation by sustained treatment with swainsonine. The F1 5Gy+SW cells show a reduced invasiveness compared with F1 5Gy cells.

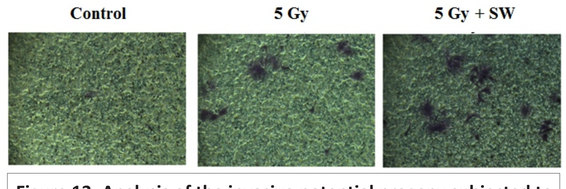

Figure 13: Analysis of the invasive potential progeny subjected to radiation after post-treatment with swainsonine. The post-irradiated treatment of surviving cells is not able to reverse the more invasive phenotype acquired by the cells after irradiation.

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