Influence of claudin-3 in the response to ionizing radiation in colorectal cancer cells

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Abstract

The occurrence of heterogeneous response to drug or ionizing radiation treatment may be associated with the high mortality rates related to colorectal cancer. During the cancer progression, epithelial cells undergo morphogenetic alterations. Tight Junction (TJ), one of the junctions found in the Apical Junctional Complex, play an important role by regulating the paracellular flow and signalling pathways. In this context, claudin proteins have been related with events of the colorectal cancer progression. Although the therapeutic failure and dysregulation of claudin-3 expression have been related to the progression of colorectal cancer, it still not well established the role of claudin-3 in the modulation of the response to radiotherapy treatment in this type of cancer. Our aim is to evaluate the molecular mechanisms that modulate the response to ionizing radiation in progenies derived from irradiated colorectal cancer cells. Parental HT-29 colon adenocarcinoma cells (HT-29) were irradiated with a 5Gy and the progenies (F1) of these cells were plated to extract RNA to analyze the gene expression by Microarray and the levels of claudin-3 by qRT-PCR. After irradiation, proteins were extracted to perform the Immunoblotting. Phase-contrast microscopy was used to analyse the cell morphology after irradiation. Immunofluorescence assay was also used to identify the subcellular localization of the claudin-3 after irradiation. The analysis by DNA Microarray from the progenies of the HT-29 cells indicated a higher upregulation of SNAI2 (also known as SLUG). Our results have shown that IR decreases the mRNA levels as well as protein levels of the claudin-3 in the progenies of irradiated cells when compared to control. Analysis by Phase-contrast microscopy shows a morphological change in the cells, when they turn into a mesenchymal-like cell. Furthermore, the irradiated cells showed a translocation of claudin-3 from the cell-cell contact to the cytoplasm, which was not seen in the cells that were not irradiated. Our data suggest that ionizing radiation increases SNAI2 expression, which may be related to both the downregulation of claudin-3, and indirectly modulates the subcellular redistribution of this protein to the cytoplasm, leading the cells into an Epithelial-Mesenchymal Transition. Alterations may be involved with an increased malignant potential of the HT-29 cells.

Support: FAPERJ, CNPq, CAPES, INCA-MS, INCT.

Introduction

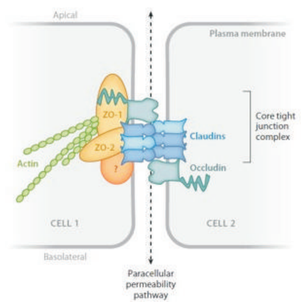
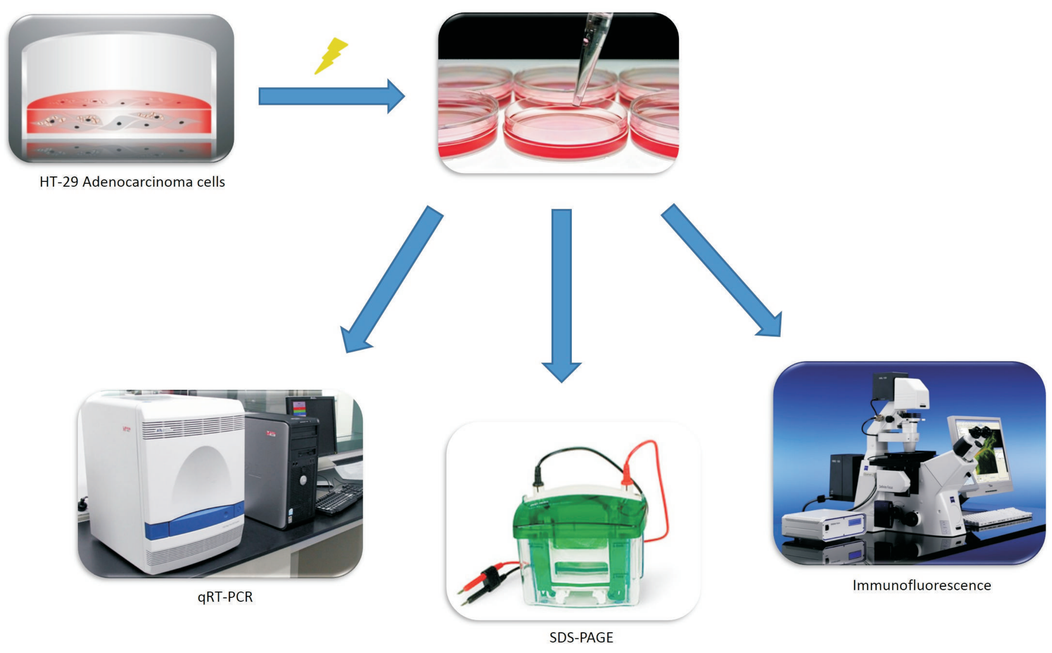


Fig 1. Schematic image of tight junction proteins. Core tight junction proteins forming the complex that helps to maintain the apical-basolateral permeability of the cell. In this image is possible to identify claudins, occludin and scaffold proteins such as ZO-1, -2 and actin filaments, the main component of the cytoskeleton. Adapted from Koval, 2013

Gene Symbol	Gene Assignment	Fold-Change (IR vs. Control)
SNAI2	Snail homolog 2 (Drosophila)	2,25
CLDN6	Claudin-6	1,28
VIM	Vimentin	1,22
CLDN15	Claudin-15	1,20
SNAI1	Snail Homolog 1 (Drosophila)	1,17
CDH1	E-cadherin (epithelial)	1,15
CDH2	N-cadherin (neuronal)	1,05
CLDN3	Claudin-3	1,01
CLDN11	Claudin-11	-1,22
CLDN1	Claudin-1	-1,22
ZEB1	Zinc Finger E-box Binding Homeobox 1	-1,07
ZEB2	Zinc Finger E-box Binding Homeobox 2	-1,08
TWIST1	Twist Homolog 1 (Drosophila)	-1,24

Fig 2. Dynamic gene alteration expression. After irradiation of HT-29 adenocarcinoma cells. Chip array was used to analyze the expression of genes related to the EMT process as well as claudin genes in F1 control and F1 5Gy HT-29 irradiated cells.

Methods



Results

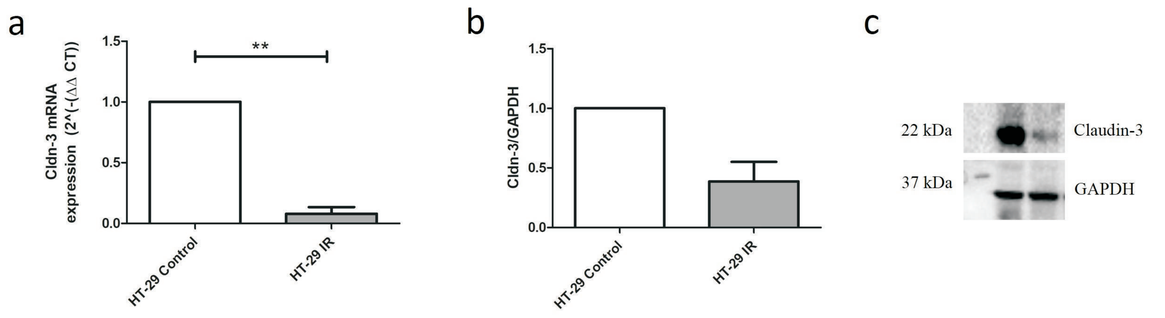


Fig 3. CLDN3 gene and protein levels are decreased after irradiation. a) RT-qPCR analysis of claudin-3 shows a decrease in its expression after F1 HT-29 adenocarcinoma cells radiation with 5Gy compared to the F1 HT-29 adenocarcinoma control cells. **b-c)** Total proteic lysate were obtained from F1 HT-29 adenocarcinoma cells. Data are presented as triplicate assays of three independent experiments

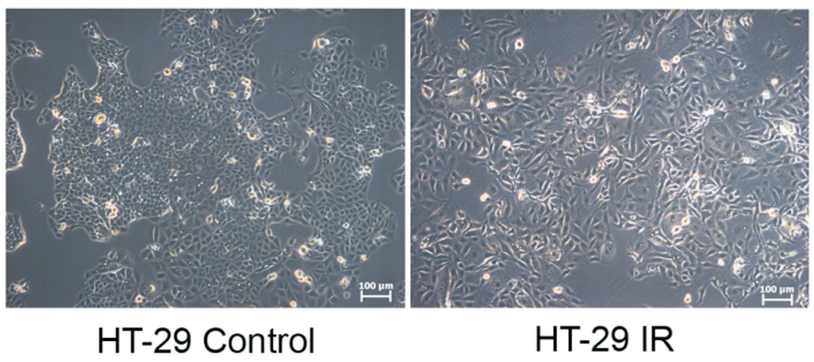


Fig 4. Effect on the morphology of the cells after irradiation. HT-29 cells were plated in bottles of 25cm3 until semiconfluence and its morphology were analyzed by phase contrast microscopy.

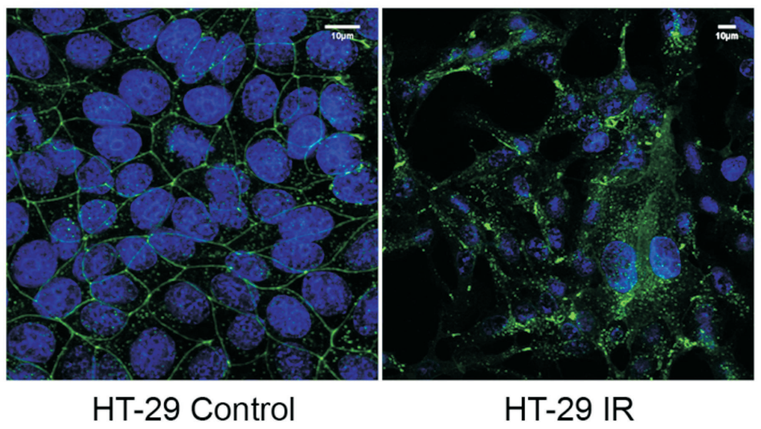


Fig 5. Effect on the subcellular localization of Claudin-3 after radiation. F1 Control or F1 5Gy of HT-29 cells were plated on coverslips until semiconfluence and subjected to immunofluorescence assay analysis.

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