Differential expression of claudin-3 during colorectal tumorigenesis and its role in modulation and interaction with other tight junction proteins

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ABSTRACT

Colorectal cancer (CRC) is the fourth leading cause of cancer-related deaths in the world. According to the International Agency for Research on Cancer data, Brazil belongs to the group of countries that presented an increase in both incidence and mortality average of the CRC in the last decade. The loss of the apical-basal polarity can lead to cancer progression, since neoplastic cells shows deficiency in the Apical Junctional Complexes (AJC), exhibiting a malign potential. Tight Junction (TJ) is one of the junctions found in the AJC. It is a structure that helps to maintain the polarity and also presents a barrier function, which regulates the transit of important molecules through the paracellular flow. Claudin protein family, are the main components of the TJ and are composed of transmembrane proteins. These proteins can interact with other TJ proteins such as occludins or scaffold proteins, essentially ZO-1/2. Our group have already showed that patients with CRC overexpress claudin-3 in the tumor tissue. Our goal was to evaluate the importance of the expression and interaction among claudin-3, ZO-1 and occludin proteins during the progression of the CRC. For the molecular analysis, samples were obtained from biopsy after patients were submitted to surgery at the Brazilian National Cancer Institute. Adenocarcinoma tissue and normal adjacent region were obtained through colectomy and classified by the TNM staging of tumor. The Immunoblotting essay was performed to quantify the expression of claudin-3 and occludin proteins in those patients in different stages of the disease. Moreover, the interaction between these proteins was identified through immunoprecipitation essay. *In vitro* essay was performed using transfected HT-29 human adenocarcinoma cells overexpressing claudin-3. In these cells we evaluated the expression levels of claudin-3 and ZO-1 and we also analyzed the subcellular distribution of claudin-3, ZO-1 and occludin by immunofluorescence. This study is being carried out with approval of the INCA Research Ethics Committee (Protocol 84/04). The analysis by immunoblotting of claudin-3 expression showed that the patients can be segregated in two groups: one where the level of this protein in the tumor is low and other group where its levels are high. It was not observed alteration in the levels of occludin when compared the tumor and the normal adjacent tissue. However, the patients where the tumor presented high levels of claudin-3 showed less interaction with occludin. *In vitro* essay using transfected HT-29 cells that overexpress claudin-3, allowed us to observe that there was a reduction in the protein levels of ZO-1 as well as a reduced co-localization between these in the regions of cell-cell contact. Associated with these results, we also observed claudin-3 and occludin colocalize in an intermittent fashion in the regions of cell-cell contact, being more evident in the region of claudin-3 accumulation in HT-29 cells. Altogether, our data suggest that in CRC there are groups of patients that show elevated level of claudin-3 in the tumor and other group that show low level. Further, it was observed that the increase of claudin-3 expression can be related to the decrease of the ZO-1 expression and a decrease in the interaction between claudin-3 and both, occludin and ZO-1. Our results can help to improve the understanding of the molecular mechanisms involved in the colorectal tumorigenesis, and point out potential prognostic markers aiming a therapeutic design to specific targets.

RESULTS

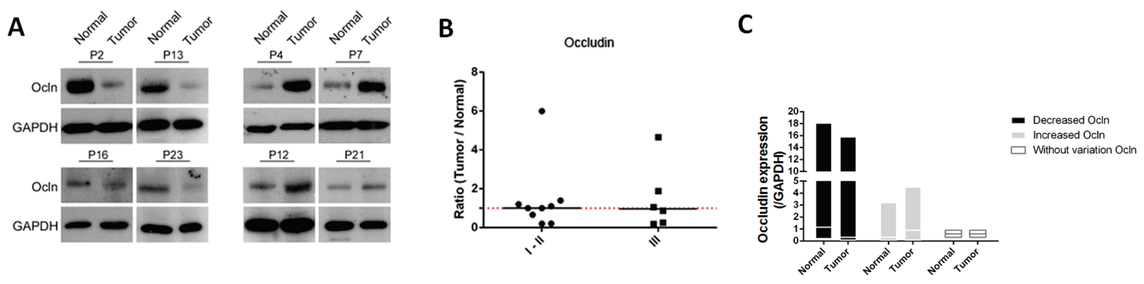

Fig 1. occludin protein expression in patients. Total proteic lysate were obtained through cirurgic specimens from patients with colorectal cancer. (A) Representative images of the immunoblotting for occludin. GAPDH protein was used as loading control. **(B)** Graphical representation of the ratio of occludin expression in the tumor compared to the normal adjacent tissue correlated with TNM staging progression. Samples used: staging I-II (n= 9), staging III (n= 6) **(C)** Segregation of patient groups according with alterations in occludin protein expression in tumor compared to the normal adjacent tissue. Samples used: decreased occludin (n= 8), increased occludin (n= 4), without variation occludin (n= 1).

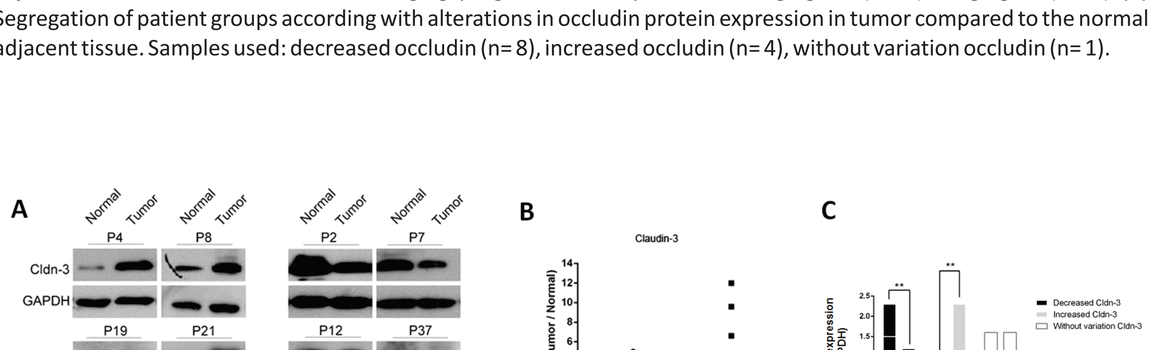

Fig 2. Expression of Claudin-3 protein in patients. Total proteic lysate were obtained through cirurgic specimens from patients with colorectal cancer. (A) Representative image of the immunoblotting for claudin-3. **(B)** Graphic represents the ratio of claudin-3 in the stages I and II (n = 12) and patients in stage III (n = 12) of CRC. **(C)** Specimens were segregated between decreased and increased expression of claudin-3. Samples used: decreased claudin-3 (n= 9), increased claudin-3 (n= 12), without variation claudin-3 (n= 2) (**p<0,01). Statistical analysis: ANOVA with Bonferroni's post test.

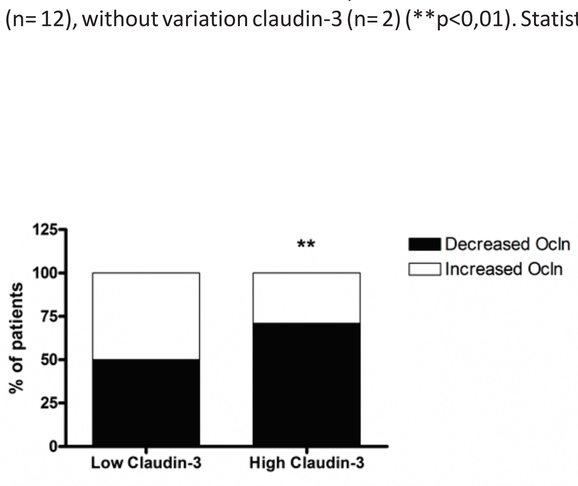

Fig 3. Correlation of the protein levels of claudin-3 and occludin in tumor samples. Total proteic lysate were obtained through cirurgic specimens from patients with colorectal cancer. Samples of patients that express high and low expression of claudin-3 in tumor were separated in two groups and correlated with the high and low expression of occludin. Samples used: decreased claudin-3 (n= 8), increased claudin-3 (n= 12) (**p <0.01, Fisher's test)

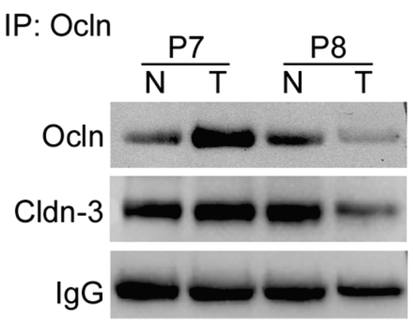

Fig 4. Interaction between occludin and claudin-3 in patients. Total proteic lysates of tumor tissue (T) and adjacent normal (N) of patients (P7 e P8) with colorectal cancer were immunoprecipitated with anti-occludin antibody. Analysis of the interaction between Claudin-3 and Occludin was performed by immunoblotting.

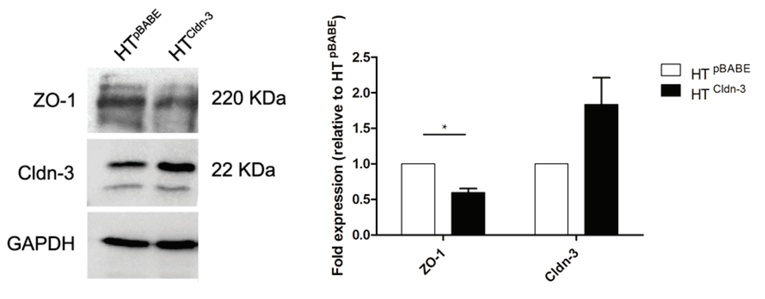

Fig 5. Tight junction proteins expression in HT-29 cells overexpressing claudin-3. Analysis of claudin-3 and ZO-1 protein expression in HT-29 cells transduced with retroviral-vectors containing claudin-3 c-DNA (HT^{Cld3}). HT^{pBABE} = cells transduced with empty vector. Results are representative of three independent experiments. (*p<0,05). Statistical analysis: ANOVA with Bonferroni's post test.

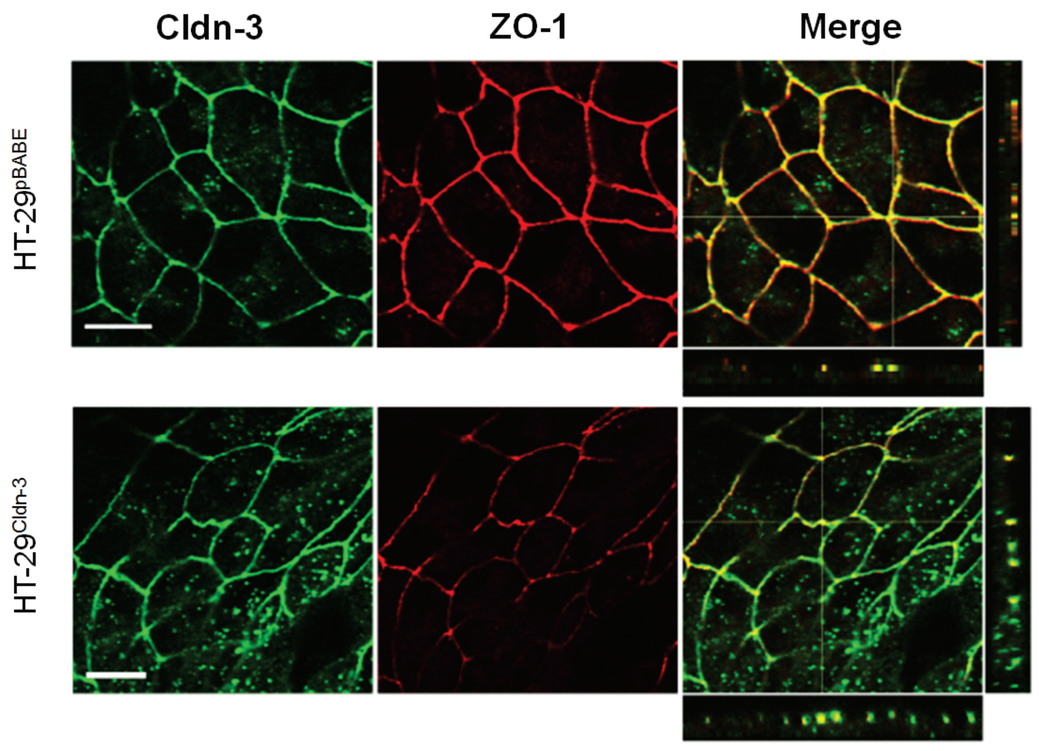

Fig 6. Subcellular localization of claudin-3 and ZO-1 proteins in HT-29 cells overexpressing claudin-3. Analysis of the cellular distribution of claudin-3 (green) and ZO-1 (red) protein in HT-29 cells. Scale bar 10µm.

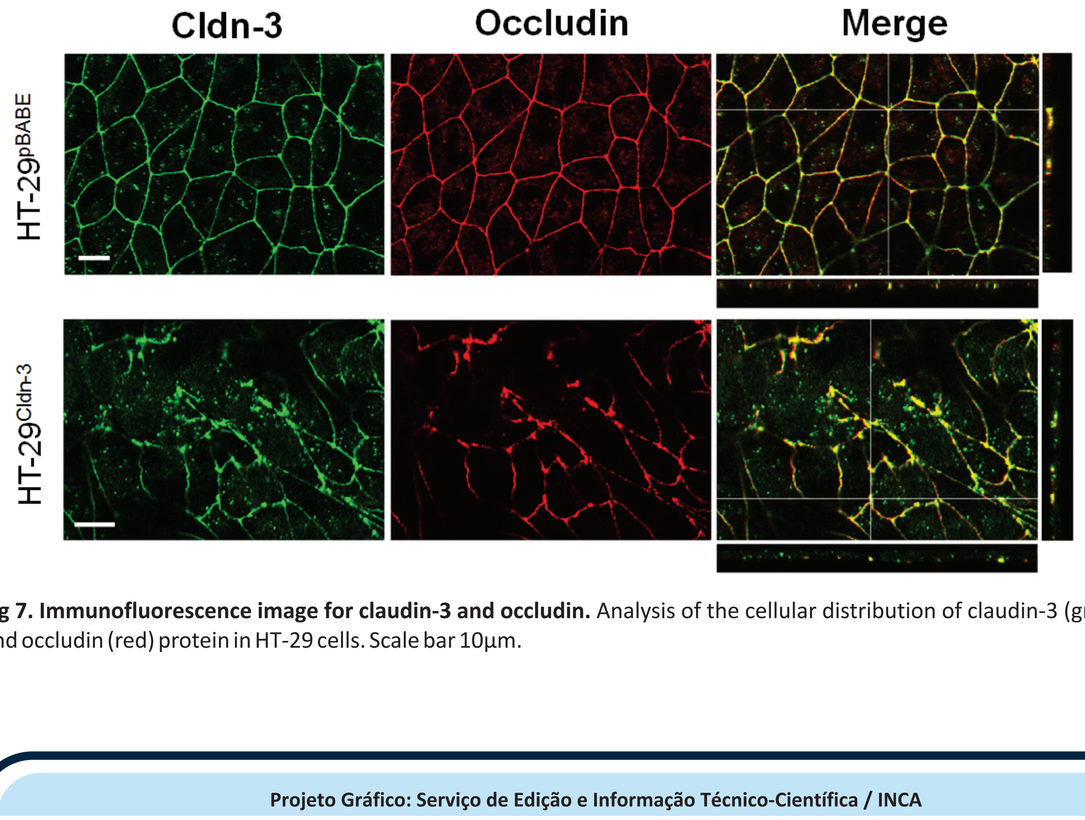

Fig 7. Immunofluorescence image for claudin-3 and occludin. Analysis of the cellular distribution of claudin-3 (green) and occludin (red) protein in HT-29 cells. Scale bar 10µm.

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