Differential expression of claudin-3 and its role in regulation of other tight junction proteins in colorectal cancer

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RESULTS

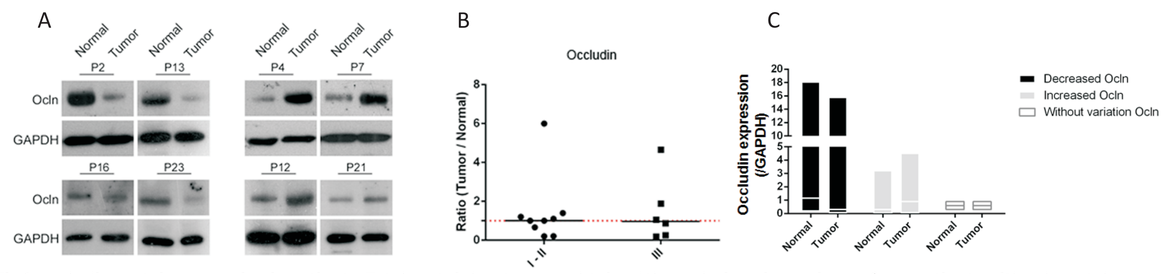

Fig 1. occludin protein expression in patients. Total proteic lysate were obtained through cirurgic specimens from patients with colorectal cancer. (A) Representative images of the immunoblotting for occludin. GAPDH protein was used as loading control. **(B)** Graphical representation of the ratio of 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).

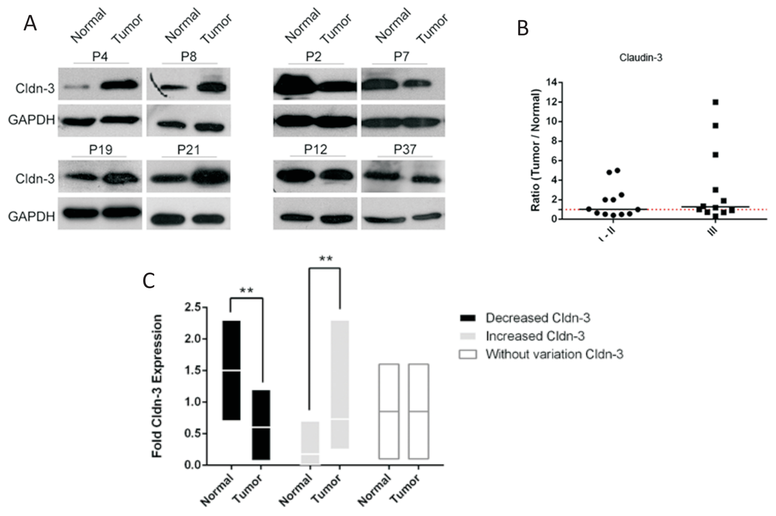

Fig 2. Expression of Claudin-3 protein in patients. Total proteic lysate were obtained through cirurgic specimens from patients with colorectal cancer. **(A)** Representative image of the immunoblotting for claudin-3. **(B)** Graphic represents the ratio of claudin-3 in the stages I and II (n = 12) and 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.

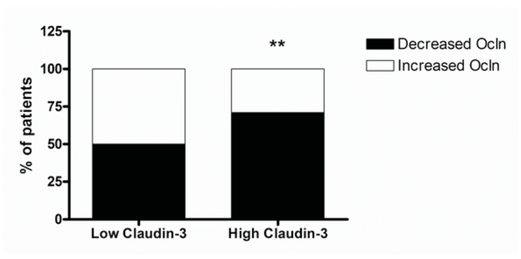

Fig 3. Correlation of the protein levels of claudin-3 and occludin in tumor samples. Total proteic lysate were obtained through cirurgic specimens from patients with colorectal cancer. Samples of patients that express high and low expression of claudin-3 in tumor were separated in two groups and correlated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

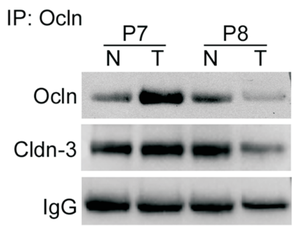

Fig 4. Interaction between occludin and claudin-3 in patients. Total proteic lysates of tumor tissue (T) and adjacent normal (N) of patients (P7 e P8) with colorectal cancer were immunoprecipitated with anti-occludin antibody. Analysis of the interaction between Claudin-3 and Occludin was performed by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

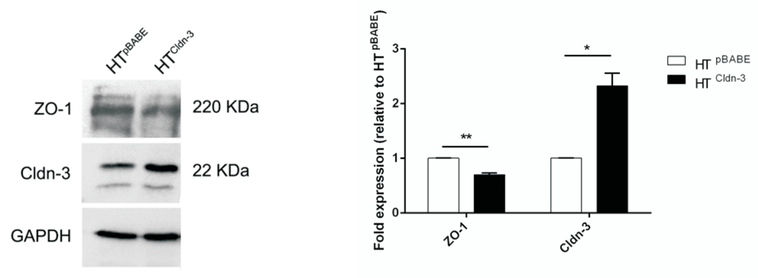

Fig 5. Tight junction proteins expression in HT-29 cells overexpressing claudin-3. Analysis of claudin-3 and ZO-1 protein expression in HT-29 cells transduced with retroviral-vectors containing claudin-3 c-DNA (HT^{Cld3}). HT^{pBABE} = cells transduced with empty vector. Results are representative of three independent experiments. (*p<0.05, **p<0.01). Statistical analysis: ANOVA with Bonferroni's post test.

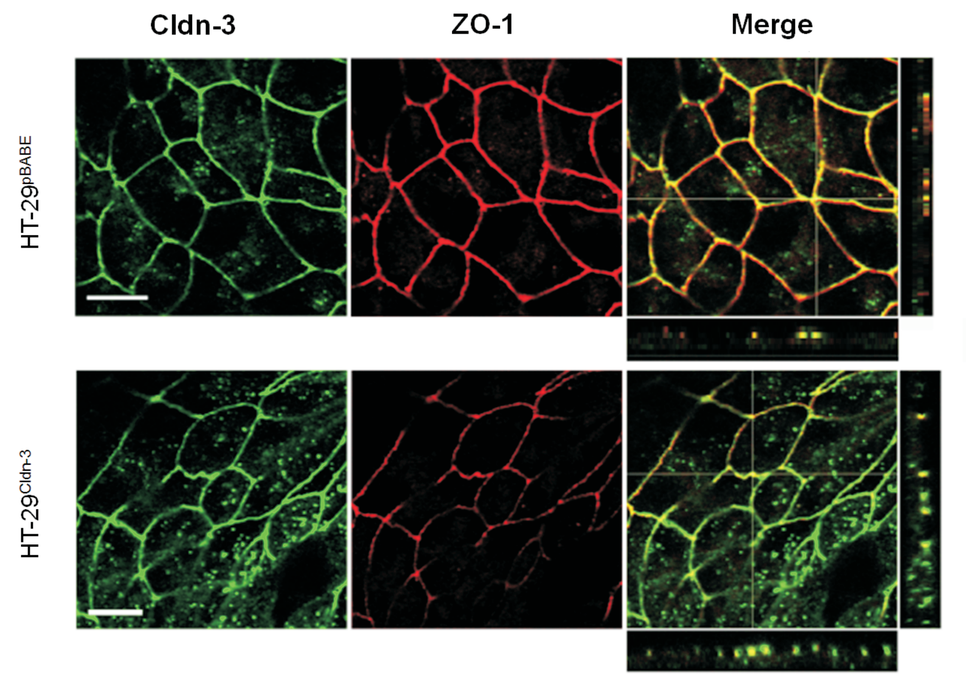

Fig 6. Subcellular localization of claudin-3 and ZO-1 proteins in HT-29 cells overexpressing claudin-3. Analysis of the cellular distribution of claudin-3 (green) and ZO-1 (red) protein in HT-29 cells. Scale bar 10µm.

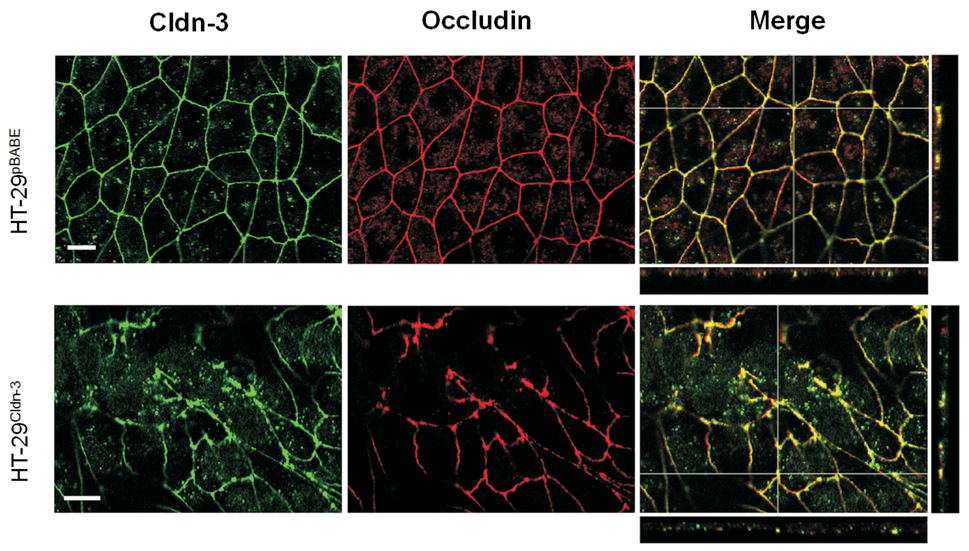

Fig 7. Subcellular localization of claudin-3 and occludin proteins in HT-29 cells overexpressing claudin-3. Analysis of the cellular distribution of claudin-3 (green) and ZO-1 (red) protein in HT-29 cells. Scale bar 10µm.

ABSTRACTS

BACKGROUND: During colorectal cancer (CCR) progression, epithelial cells undergo cell-cell adhesion disassembly increasing their malignant potential. In this context, the tight junction proteins, claudins and occludin, play important role regulating events related with carcinoma progression. Previous studies have showed altered expression of claudin proteins in human CCR samples. Nevertheless, the molecular interactions between Tight Junction (TJ) proteins and their role regulating the malignant potential remain to be defined. **AIMS**: Evaluate the importance of the expression and interaction among claudin-3, ZO-1 and occludin proteins during the progression of the CRC. **METHODS:** Human colorectal specimens were obtained from surgical resection of CCR patients treated in Brazilian National Cancer Institute. In all cases, we collected adenocarcinoma specimens and their paired normal mucosa, which were classified by TNM staging. The protein levels were analyzed by imunoblotting and the interaction between these proteins were evaluated by immunoprecipitation. Moreover, *in vitro* essays using transfected HT-29 human adenocarcinoma cells overexpressing claudin-3 were performed. 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 (Prot 84/04). **RESULTS:** 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. Furthermore, in patients where the tumor presented high levels of claudin-3 was observed 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 that 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. **CONCLUSIONS:** 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.

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