ROLE OF TRICELLULIN DURING COLORECTAL CANCER PROGRESSION

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ABSTRACT

During CRC progression, epithelial cells undergo genetic and morphological alterations, which can lead to cell-cell adhesion disassembly. The tight junctions (TJs), components of the cell-cell adhesion system, play an important role in control of the cell homeostasis regulating events such as paracellular transport, cell polarity and transduction of signals. Tricellulin (TRIC) is a transmembrane protein that predominantly localizes at tricellular TJs, where the corners of three epithelial cells meet, however it can also be located at bicellular TJs, which are present between two adjacent cells. Few studies have shown the altered expression of TRIC in pathological conditions and cancer. For example, i) decreased expression of TRIC was observed in the squamous cells of tonsillar cancer compared to oral epithelium; ii) TRIC expression in pancreatic ductal adenocarcinoma showed a negative correlation with the level of differentiation; iii) loss of TRIC expression has been reported in human liver tumors. However, there are no studies that report the role of this protein during CRC progression. Furthermore, the molecular mechanisms that regulate the localization and function of TRIC are not yet fully elucidated. Thus, the goal of the present study is to evaluate the role of TRIC during the colorectal cancer progression. For this, CRC cell lines at different stages of differentiation were used as model to determine whether the expression and localization of TRIC are directly involved in the malignant behavior of these cells. To determine which signaling pathways could participate in the localization of TRIC in the cell contacts, HT-29 and HCT-116 cells were treated with LY29002, a specific PI3K inhibitor at different times. Furthermore, we evaluated the protein expression of TRIC in surgical specimens obtained from CRC patients (n=20) by western blot. This study has been approved by the INCA Research Ethics Committee (Protocol No: 84/04). Our results showed that there was significant difference in the protein levels of TRIC between cell lines analyzed, being higher in CaCo-2 (well differentiated) cell line in compare to the other cell lines studied. On the other hand, we observed a differential subcellular distribution of this protein. The presence of TRIC in the corners between three cells, which co-localized with occludin protein in this region, was found in Caco-2 and HT-29 cell lines (well differentiated and moderately differentiated, respectively). Nevertheless, HCT-116 cells (undifferentiated) presented poor staining of TRIC, which was distributed throughout the cytoplasm, with few staining on cell-cell contacts that did not co-localize with occludin. Preliminary results shown that treatment with the LY29002 did not affect the expression and localization of the TRIC in the HT-29 cells, but altered its localization in the regions of cell-cell contacts in HCT-116 cells. In patient samples, the TRIC protein levels was increased in tumor tissues as compared to adjacent normal, effect observed in samples from patients in advanced stages of the CRC. Together, our results suggest that the altered localization of TRIC, which is potentially regulated by PI3K/Akt pathway, may be a potential prognostic factor to understand the differentiation of CRC, contributing to new insights diagnostic for this type of tumor.

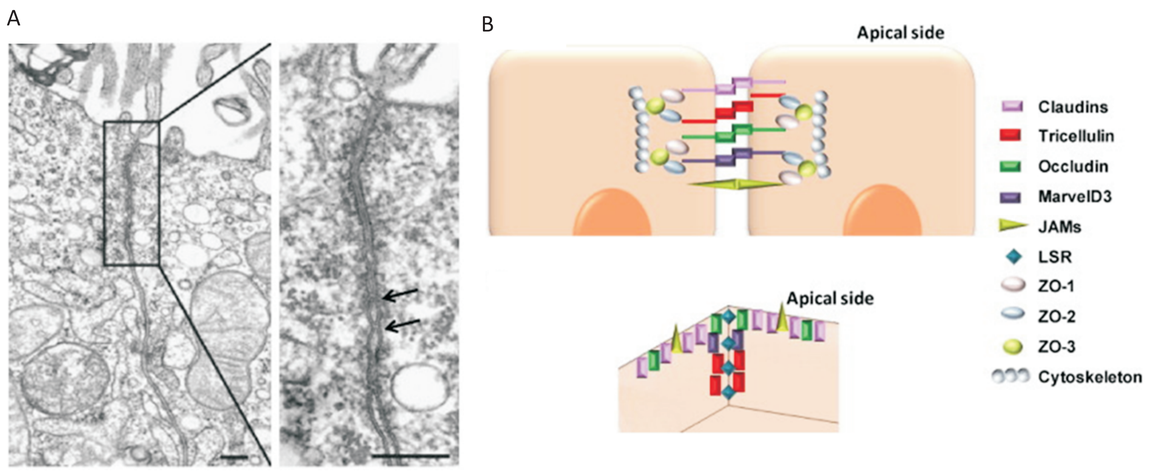


Figure 1 - Structure of tight junctions between two adjacent cells. **(A)** View of a tight junction by transmission electron microscopy reveals focal attachments of adjacent cells. **(B)** Schematic representation of proteins distribution at bicellular tight and tricellular tight junctions viewed from the cytoplasmic side of one of the cells. Scale bars, 200 nm. From: Eur. J. Cell Biol. 90 (2011) 787–796.

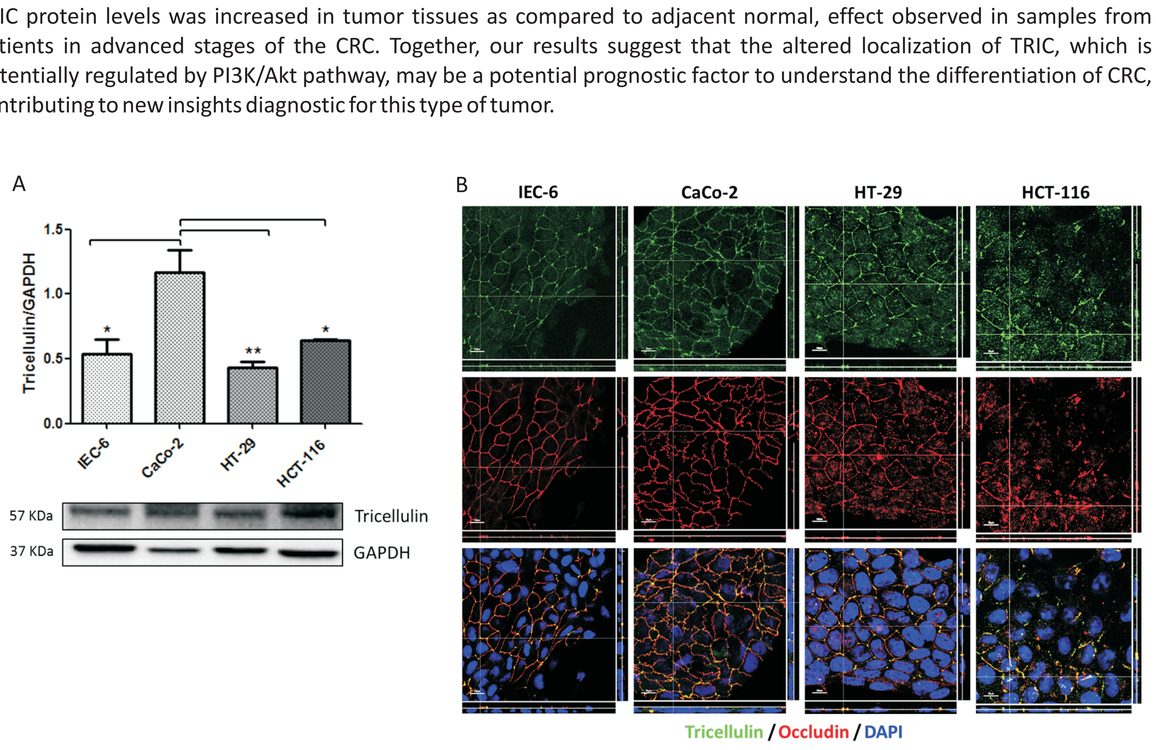


Figure 2 - Analysis of Tricellulin protein levels and localization in IEC-6, CaCo-2, HT-29 and HCT-116 cell lines. **(A)** Western blot and densitometry analysis showing that Tricellulin protein levels is different between the cell lines. **(B)** Immuno-fluorescence analysis showing the labeling profile of Tricellulin and Occludin. Occludin protein was used as control of the tight junction protein. Results are expressed as mean ± s.e.m. *p<0,05, **p<0,01. n=3

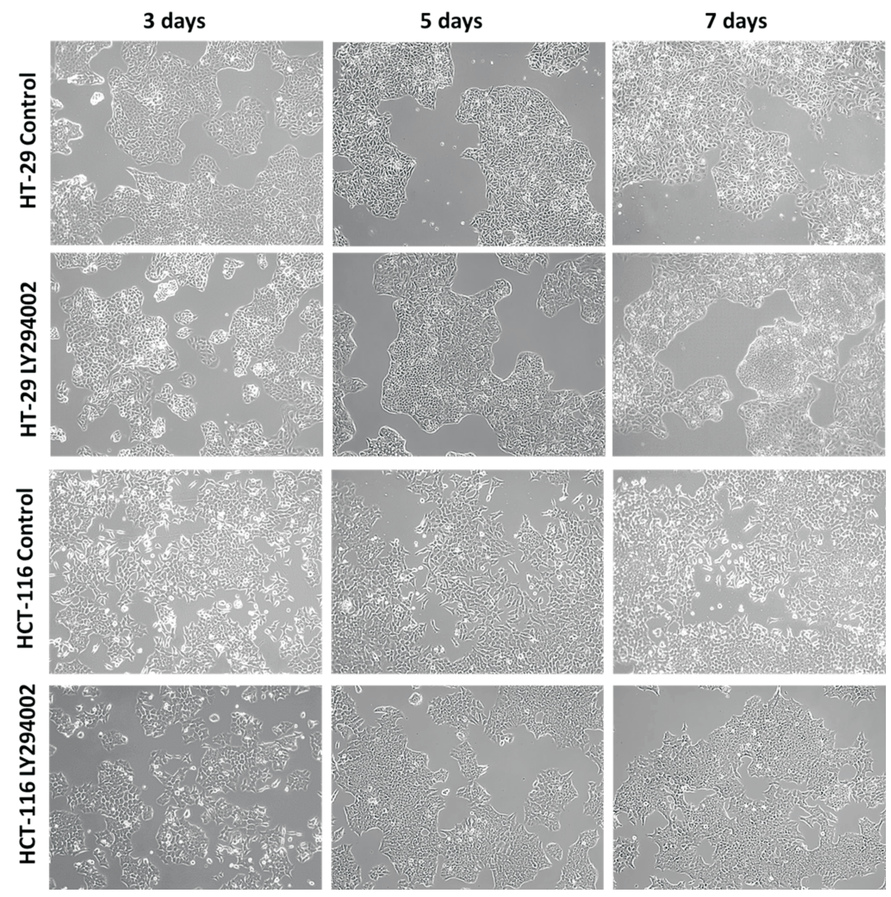


Figure 3 - Analysis of the cellular morphology of HT-29 and HCT-116 cells with or without treatment after 3, 5 and 7 days with LY294002. Representative images phase-contrast microscopy shows that PI3K inhibition induces cell differentiation.

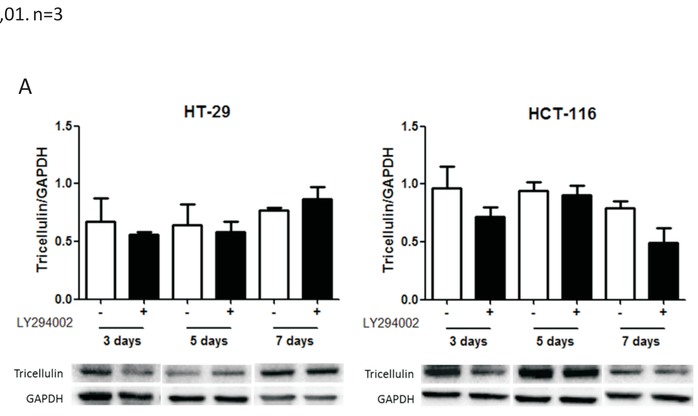
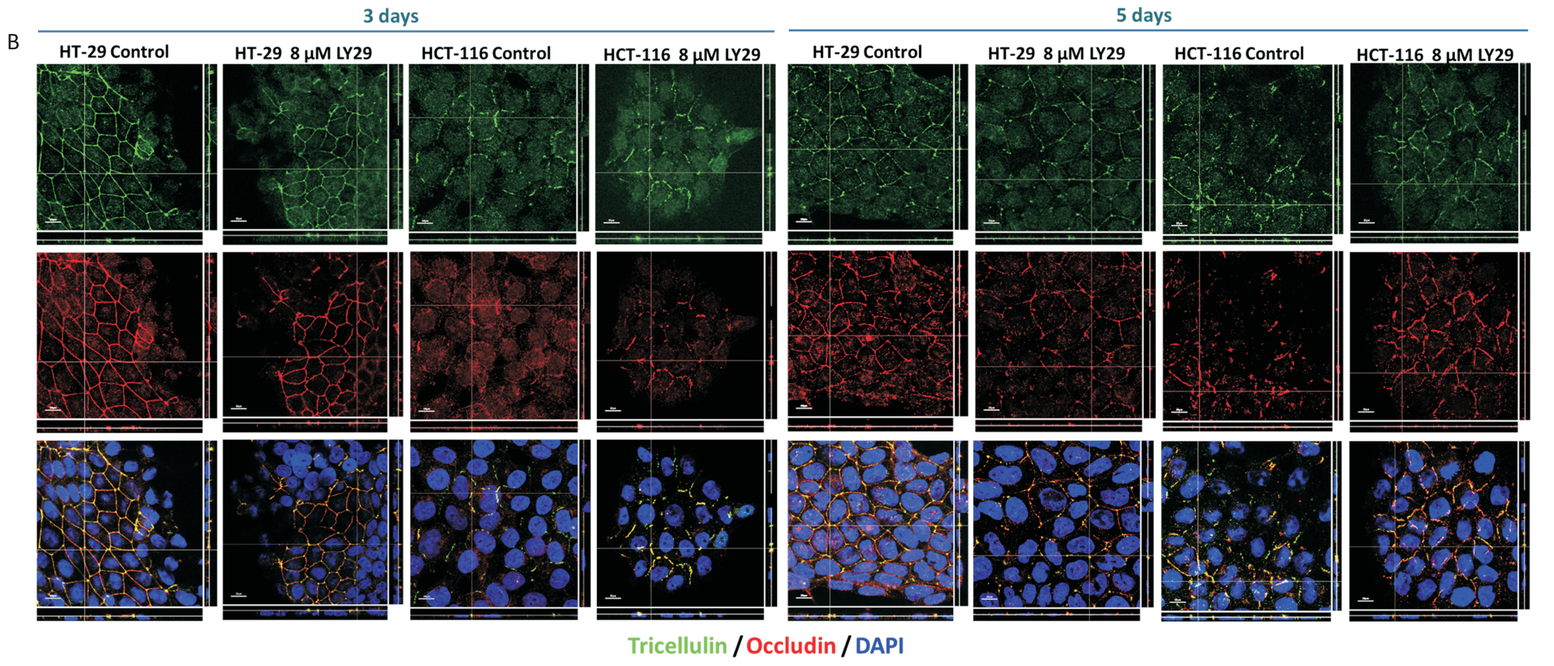


Figure 5- Analysis of protein expression levels and localization of Tricellulin with or without LY294002 treatment. **(A)** Western blot, densitometry analysis of the HT-29 and HCT-116 cell lines and **(B)** Immuno-flourescence analysis indicates that PI3K inhibition seems to affect the Tricellulin and Occludin levels in bicellular junctions of the HCT-116 cell. Results are expressed as mean ± s.e.m. n=2



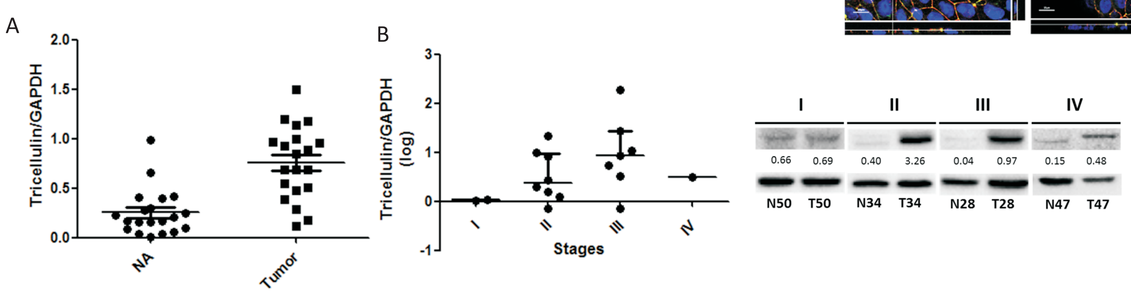


Figure 6-Expression of Tricellulin protein in tumor samples from CRC. The levels of Tricellulin in tumor tissues (T) were measured in relation to the levels of normal adjacent (AT) respective. **(A)** General information of the protein levels of Tricellulin in adjacent tissue and tumor samples. **(B)** Analysis of Tricellulin protein levels in tumor samples separated by staging. The panel presents immunoblot analysis of patients samples at different stages. n = 20.

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