TRANSIENT RECEPTOR POTENTIAL VANNILOID TYPE 4 CHANNEL (TRPV4) IS A KEY REGULATOR OF COLORECTAL CANCER DEVELOPMENT

1)Romano BR. 2)Petti LP. 3)Piontini AP. 4)Correale CC. 5)Izzo AA. 6)Danese SD. 7)Vetrano SV.

Department of Pharmacy, University of Naples Federico II

Colorectal cancer (CRC) is the third most common carcinoma worldwide. To date, the molecular mechanisms involved in CRC are still poorly understood. TRPV4 is a polymodal ionic channel activated by various stimuli including chemical activators. It is recently emerging the role of TRPV4 in carcinogenic phenomena mostly by acting as a critical regulator of tumor angiogenesis. The role of TRPV4 in CRC is unknown to date. A preliminary analysis conducted on CRC patients revealed a down-regulation of TRPV4 gene expression in CRC tissues compared to healthy area, suggesting its potential role in intestinal carcinogenesis. Therefore, we aimed to uncover the knowledge on the role of TRPV4 in colon carcinogenesis. TRPV4 localization on healthy and tumoral human tissues was performed by immunohistochemical analysis. Primary human epithelial cells and human intestinal microvascular endothelial cells (HIMEC) were isolated from healthy and tumor-affected area of CRC patients and quantitatively analyzed for TRPV4 expression by using RT-PCR. Caco-2 cells were stimulated with selective TRPV4 agonists and/or a selective antagonist and then analyzed for the proliferation and migration capabilities by 3H-thymidine incorporation and scratch assay, respectively. The xenograft mouse model of CRC was performed in nude mice treated or not with the TRPV4 activator. Ki67 immunofluorescence staining was evaluated on xenograft tumor frozen sections by confocal microscopy. Immunostainings performed on healthy colonic mucosa revealed TRPV4 expression mainly on the epithelial and endothelial cells and on CD3+ T cells of lamina propria. By contrast, in tumor-involved area its expression was strongly down regulated on epithelial cells, but not on endothelial cells and T cells. Indeed, mRNA levels of TRPV4 markedly decreased in primary epithelial cells isolated from tumor-affected area of 10 CRC patients compared to healthy area, whereas its expression was not altered between healthy and tumoral HIMEC. In vitro studies revealed that TRPV4 was differently expressed in several colorectal cancer cell lines in relation to the metastatic capacity. Interestingly, the selective TRPV4 stimulation of Caco-2 cells, which faintly expressed TRPV4, not only greatly enhanced its expression, but also caused significant effects on cell growth rate inhibiting cell proliferation and migration. In confirmation of this, the use of TRPV4 antagonist did not affect neither proliferation nor Caco-2 cell migration. Similar results were observed in vivo after repeated intra-tumoral injections of the TRPV4 activator that resulted in a reduced tumor growth as demonstrated also by a reduced Ki67 positive cells than mice treated with only vehicle. On the whole, our data support that TRPV4 is a master regulator of CRC development controlling proliferation and migration of epithelial tumor cells. In consequence, these results highlighted the relevant role of TRPV4 as a new target for CRC and importantly as a novel therapeutic approach for the treatment of CRC.