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Sur8, a determinant protein in colorectal cancer tumor progression

TANIA LÓPEZ BRIONES, *MARÍA PILAR DE LUCAS, ANA BELÉN CÁMARA, JUDITH ORTEGA RODRÍGUEZ, ASUNCIÓN FERNÁNDEZ BARRAL, PILAR BUSTAMANTE MADRID, ALBA COSTALES CARRERA, PABLO MATA MARTÍNEZ, NATASHA ZARICH, JOSÉ LUIS OLIVA, DANIEL PEÑA JIMÉNEZ, VICTORIA LÓPEZ ALONSO, MARÍA JESÚS FERNÁNDEZ ACEÑERO, ANTONIO BARBÁCHANO, ALBERTO MUÑOZ, BERTA ANTA, JOSÉ MARÍA ROJAS CABAÑEROS*

Instituto de Salud Carlos III Unidad de biología celular

Colorectal cancer (CRC) has the highest incidence rate in the Spanish population. The most important challenge consists on the discovery of efficient disease treatments, due to high mortality rates in highly developed stages. Sur8 is a scaffold protein that positively modulates ERK signaling pathway, which has a major role in the progression and metastasis in colorectal cancer. The main goals of our research are to determine the role that Sur8 plays in the development and progression of CRC and to analyze its possible therapeutic potential. For this purpose, our group has developed an inducible conditional mouse model msur8$^{f/f}$VillinCre^{ERT2}. In order to determine Sur8 action in the colonic tissue, we have developed organoids from the colon epithelium of healthy mice and have analyzed gene expression pattern by an RNAseq approach. Sur8 KO affects oncogenic CRC transcription factors expression, as well as the modulation of some Wnt pathway regulators. In regard to miRNA data, we have observed deregulation of miRNAs related to CRC in Sur8 KO organoids. To determine the role that Sur8 plays in the development and progression of CRC, we have subjected our inducible conditional mice to chemical carcinogenesis and we have observed that Sur8 KO males display less and smaller tumors and do not present any adenocarcinoma. In addition, we have carried out Sur8 silencing in human CRC cell lines by infection with constitutive shRNA lentiviruses. We have observed that Sur8 silencing produces decreases of cell tumor proliferation, and reduction of p-ERK levels. Finally, we are evaluating the effects of putative therapeutic agents against Sur8 in human CRC cell lines. Concretely, we are testing Celastrol, which has been described that binds and blocks the action of Sur8 *in vitro*. We have observed that Celastrol treatment diminishes the cell tumor proliferation in this model. Altogether, our results indicate that Sur8 may have a determinant role in CRC progression and that Sur8 could be a potential molecular target for the design of novel strategies against CRC.

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NADPH oxidase 1 as a new regulator of the WNT pathway and the protective effect of vitamin D in colorectal cancer.

ELIEZER NAVARRO-RAMIREZ, *JOSÉ LUIS ROMÁN-FERNÁNDEZ, MARÍA GUTIÉRREZ-SALMERÓN, ANA RAMÍREZ-SÁNCHEZ, ALBERTO SÁNCHEZ-DE LA CRUZ, ANA CHOCARRO-CALVO, ROSA MARÍA MARTÍN-OROZCO, CARMEN FIUZA, MARÍA JESÚS LARRIBA, ALBERTO MUÑOZ, JOSÉ MANUEL GARCÍA-MARTÍNEZ, CUSTODIA GARCÍA-JIMÉNEZ*

Universidad Rey Juan Carlos, Alcorcón, Madrid Departamento de Ciencias Básicas de la Salud

Worldwide, colorectal cancer (CRC) is the third most common malignant neoplasm and the second leading cause of cancer-associated mortality, with an estimated increase in global prevalence of 60% by 2030 (1,2). Mutational inactivation of adenomatous polyposis coli (APC) is the hallmark of CRC and leads to an overactivation of WNT signaling that favors the development and progression of CRC (3). Large epidemiological studies suggest that the diabetic population is at increased risk for site-specific cancers, including CRC (4).

Our laboratory has shown that hyperglycemia induces the accumulation of ROS in CRC but not healthy cells, driving the activation of a newly described ROS/AMPK/EP300 axis that enhances Wnt/b-catenin signaling. Increased EP300 leads to increased acetylation of β-catenin at K354, a requirement for nuclear accumulation and transcriptional activation of WNT target genes (5,6).

The critical role driven by ROS suggest a possible involvement of the NADPH oxidases (NOX family, as a source of ROS. Specifically, NOX 1 and NOX 4 are expressed in colon epithelial cells, and their overexpression in CRC cells promotes cell proliferation and invasiveness (7,8,9,10). Our results indicate that hyperglycemia significantly increases NOX1 levels, in correlation with increased ROS production in CRC cells, suggesting a possible regulation of the ROS/AMPK/EP300 axis by NOX1.

Antioxidant mechanisms dealing with NOX1-induced ROS should be effective against CRC. Vitamin D (1α, 25-dihydroxyvitamin D3) is a powerful antioxidant that inhibits proliferation and promotes differentiation of CRC cells at least partially through inhibition of Wnt/β-catenin signalling. Consequently, vitamin D deficiency is associated with poor survival to CRC (11,12).

Our results indicate that vitamin D causes a reduction in the levels and / or activity of some members of the NOX family by turning off the ROS/AMPK/EP300/β-catenin axis and its proliferative and tumorigenic effects. The data suggest a new antitumor mechanism of vitamin D linked to its anti-oxidant action. Our results integrate independent epidemiological links between vitamin D deficiency, diabetes and cancer in one overarching and unifying mechanism.

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Sur8 lipid rafts tethering impairs S338 C-Raf phosphorylation and consequent ERK activation.

MARÍA PILAR DE LUCAS, *NATASHA ZARICH, GONZALO LEÓN, LARA MARÍA DURÁ, TANIA LÓPEZ-BRIONES, JUDITH ORTEGA, ANA BELÉN CÁMARA, MANUEL PARRILLA, PABLO MATA-MARTÍNEZ, BERTA ANTA, JOSÉ LUIS OLIVA, JOSÉ MARÍA ROJAS-CABAÑEROS*

UFIEC - ISCIII Unidad de Biología Celular

Sur8 protein is a positive modulator of RAS signaling and Sur8 mutants have been found in a group of pathologies called RASopathies, because they share a set of specific phenotypes found in patients with mutations in RAS and RAS-related signaling proteins. Nevertheless, the way of action of the best-characterized Sur8 mutation in this pathology, S2G, is controversial. Although this mutation places Sur8 at the plasma membrane, more concretely at the lipid rafts domain, authors differ on the ability of this mutated Sur8 version to influence on ERK activation. Sur8 can be found in different locations inside cells, being its membrane association an important factor for the RAS to RAF signal transduction in the RAS/RAF/MEK /ERK pathway. In this work, we aim to clarify the specific function of Sur8 at the concrete lipid rafts membrane location. For this purpose, we have directed Sur8 by its fusion with peptides of different proteins that favor their anchoring to lipid-rafts. We have observed that Sur8 lipid-raft tethering blocks ERK phosphorylation in different human cell lines, such as HEK 293T and HeLa; when concomitantly expressed with M-, H- or K-Ras V12, as well as upon EGF stimulation. Furthermore, this impairment can be explained by the inability of C-RAF to be phosphorylated at S338, as we do not observe changes in C-RAF S259 phosphorylation status when compared to non-directed Sur8. Concerning other pathways, we have observed that Sur8 lipid-raft tethering also blocks the phosphorylation of AKT at S473. In addition, we have explored C-RAF phosphorylation status in C260Y and E457K Sur8 mutants, described in *C. elegans*. Our data indicate that overexpression of Sur8 E457K mutant inhibits C-RAF S259 dephosphorylation and promotes C-RAF S338 phosphorylation. Lipid raft location of Sur8 E457K mutant did not affect C-Raf S259 dephosphorylation but impairs C-Raf S338 phosphorylation, which confirms that Sur8 needs to be out of this membrane location to favor C-Raf S338 phosphorylation. Finally, Sur8 lipid-raft attachment is able to repress proliferation and differentiation in different cell lines, accordingly to a reduction of p-ERK levels.