
Proceedings Paper:
Caballero-Ruiz, B, Morales-Alcala, CC, Wood, HM orcid.org/0000-0003-3009-5904 et al. (2 more authors) (2021) PTCH1 C-terminal domain truncations in colorectal cancer increase mitogenic signalling and autophagy. In: Cancer Research. AACR Annual Meeting 2021, 10 Apr - 21 May 2021, Virtual. American Association for Cancer Research .

https://doi.org/10.1158/1538-7445.AM2021-2421

PTCH1 C-terminal domain truncations in colorectal cancer increase mitogenic signalling and autophagy

Begoña Caballero-Ruiz[1,2], Cintli C. Morales-Alcala[3], Henry M. Wood[3], Gianluca Canettieri[1] and Natalia A. Riobo-Del Galdo[2,3].

1. Department of Molecular Medicine, Sapienza University of Rome, Rome, Italy
2. School of Molecular and Cellular Biology, University of Leeds, Leeds, United Kingdom
3. Leeds Institute of Medical Research, University of Leeds, Leeds, United Kingdom

The canonical Hedgehog (Hh) signalling pathway, driving activation of SMO and GLI1, has been implicated in colorectal cancer (CRC) stemness and resistance to chemotherapy. We recently reported that the C-terminal domain (CTD) of the Hh receptor PTCH1 limits autophagic flux and ERK signalling by non-canonical mechanisms (independently of SMO/GLI1). Given that those novel functions are implicated in tumorigenesis, we explored the existence of PTCH1 CTD mutations in cancer specimens. Analysis of the TCGA Cancer Atlas database and the Genomics England 100,000 Genomes Project revealed relatively frequent somatic mutations in the CTD of PTCH1 in CRC: S1203(fs) and R1308(fs), which result in premature truncations of the CTD in about 4% of cases. PTCH1 CTD mutations were strongly associated with BRAF mutations (61% samples) and with right-sided disease (10/10 of samples with sidedness information). Co-immunoprecipitation analysis of truncated PTCH1(S1203*) and PTCH1(R1308*) showed loss of interaction with ATG101 and GRB2, required for regulation of autophagic flux and ERK signalling, respectively. To determine the pathogenic role of those mutations, we engineered SW620 metastatic CRC cell lines using CRISPR/Cas9 to create two clones with indel mutations between S1203 and R1308. Truncation of the CTD increased basal ERK and AKT phosphorylation levels, proliferation, invasion and migration compared to isogenic cells expressing wild type PTCH1. The mutant clones also showed persistent proliferation in the presence of glycolysis inhibitors and of the autophagy inducer rapamycin. Moreover, both clones displayed an enhanced autophagic flux by analysis of the LC3BI/II and p62 markers in the presence and absence of Bafilomycin A1. RNA-seq analysis revealed that the CTD truncation resulted in increase in GO terms of metabolic pathways, pathways in cancer, mTOR signalling pathway, PI3K-AKT pathway, MAPK signalling pathway and regulation of actin cytoskeleton, among others. The clones morphology changed together with a remarkable cadherin switch and loss of vimentin at RNA and protein level, suggestive of mesenchymal-epithelial transition, a process necessary for establishment of metastasis following cancer cell spread. Interestingly, one clone showed enhanced apoptosis in response to FOLFOX (5-fluorouracil/lecovorin/oxaliplatin), the most effective chemotherapy for metastatic CRC, as determined by cleaved PARP and Caspase 7 activity, compared to the parental cells. These findings reveal important tumour suppressor functions of the CTD of PTCH1 acting to suppress autophagy and mitogenic signalling, which are dysregulated by CTD mutations in a subset of colon cancer patients. Our results provide mechanistic insights of the effect of PTCH1 mutations in CRC and identify potential therapeutic vulnerabilities.