RIMONABANT KILLS COLON CANCER STEM CELLS WITHOUT INDUCING TOXICITY IN NORMAL COLON ORGANOIDS.

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In the last decades, the improvement of colon cancer management increased patient's life expectancy but the need to restrain the high malignancy forms and the occurrence of metastatic and drug resistant phenotypes remains a clinical and social priority.

The colorectal cancer (CRC) progression is typically associated with a stepwise accumulation of specific genetic alterations. The majority of both familial adenomatous polyposis and sporadic colon cancers, arise from inactivation of APC tumor suppressor gene that negatively regulates Wnt/β-Catenin pathway. The APC truncation results in loss of β-Catenin-binding domain and, thereby, into failure of β-Catenin degradation. With high frequency, CRCs with wild type APC, harbour gain of function mutations in β-Catenin gene, typically associated with loss of phosphorylation sites, required for its proteasomal degradation. Substantially, both APC and β-Catenin gene mutations, allow to hyperactivation of the Wnt-mediated signalling (Deitrick & Pruitt, 2016; Fearon, 2011).

Wnt/β-Catenin pathway is a highly conserved signalling, known to exert a key role not only in embryonic development and in normal tissue homeostasis, but also in cancer-related processes, such as proliferation, differentiation, apoptosis, and cell survival.

Although alterations in Wnt/β-Catenin signalling were found in the most of CRC, it is now clear that only Cancer Stem Cells (CSCs), a Wnt hyperactive subset of cells within the tumor bulk, retain tumorigenic ability, supporting the hierarchical organization of CRC. It is widely accepted that in high malignancy, the chemoresistance and thus the onset of relapses are mainly ascribable to CSCs (Zeuner et al., 2014).

To date, several compounds able to target Wnt pathway in CRC and other tumors, have been identified, including cannabinoids (Laezza et al, 2012; Aguado et al., 2007).

Our previous data suggested that in vitro and in vivo antitumor effects of Rimonabant, an antagonist/inverse agonist of Cannabinoid receptor CB1, are ascribable to inhibition of Wnt/β-Catenin pathway and to reduction of p300 Histone Acetyltransferase (HAT) activity. Several modulators of the Wnt/β-Catenin pathway and p300/CBP inhibitors are currently in ongoing clinical trials and they seem promising compounds able to control cancer stemness in several tumor types.

Using established 3D in vitro model, we evaluated the effects of Rimonabant in colon CSCs. Primary cells stable transfected with Wnt-TOP-GFP reporter were used to assess caspase-3 activation in both differentiated tumor cells (Wntlow) and CSCs (Wnthigh). Rimonabant strongly activates caspase-3 in both differentiated tumor cells and CSCs, and induced a conspicuous DNA fragmentation in a dose-dependent manner. Moreover, the compound was able to strongly

reduce CSCs survival in long-term cultures, allowing us to highlight for the first time the Rimonabant ability to control colon cancer stemness. Unfortunately, in primary CSCs, Rimonabant was unable to ameliorate nor Oxaliplatin neither 5-Fluorouracil effects, result probably ascribable to the high chemoresistance of CSCs.

Last but not least, encouraging results obtained, allowed us to evaluate Rimonabant toxicity against normal colon epithelium, using 3D ex vivo models of normal colon human organoids (wild type). In this model, Rimonabant did not induce DNA-fragmentation and, moreover, the organoid clonogenicity was not affected.

Described results allow us to candidate Rimonabant as a novel lead compound, able to eradicate colon CSCs population. Finally, our preliminary results on normal colon human organoids, seems to suggest a Rimonabant selectivity toward cancer cells.

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