NAAA IS CRUCIALLY INVOLVED IN PROLIFERATION, MIGRATION AND *IN VIVO* COLON TUMORIGENESIS

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Introduction: N-acylethanolamine-hydrolyzing acid amidase (NAAA) is a lysosomal enzyme highly expressed in macrophages and peripheral tissues, including the small and large intestine. NAAA is believed to primarily control the levels of palmitoylethanolamide (PEA), an endogenous lipid mediator with anti-inflammatory, neuroprotective and analgesic properties. NAAA dysregulation has been reported in a number of tumour types, however its role in colon cancer is unexplored to date. Here, we have assessed the role of NAAA in experimental colon carcinogenesis.

Methods: NAAA expression and PEA levels were measured in colonic tumours of patients at different stages of colorectal cancer (CRC) by RT-PCR and liquid chromatography mass spectrometry, respectively. NAAA mRNA expression was also evaluated in different human CRC cell lines, including Caco2and HCT116, treated or not with secretome collected from xenograft tumours. The role of NAAA was assessed *in vivo*, via pharmacological blockade by the NAAA inhibitor AM9053, in the azoxymethane (AOM) and xenograft colon cancer models as well as, in vitro, on cell growth (by BrdU incorporation), cell cycle (by flow cytometry analysis) and cell migration (by scratch assay) of human CRC cells. Finally, the mechanism of action of AM9053was evaluated on cell proliferation by using specific antagonists of the main PEA targets (i.e. PPAR-a, GPR55, TRPV1, cannabinoid receptors).

Results: NAAA expression was reduced in tumour biopsies (compared to their corresponding non-cancerous specimens) of clinically-diagnosed CRC patients at different stages as well as in CRC cells incubated with tumour secretome. Accordingly, a significative increase in PEA levels, the main NAAA substrate, was observed in biopsies of CRC patients. In vivo, NAAA inhibition by AM9053resulted in chemopreventive effects in the AOM model of colon carcinogenesis and in reduction of xenograft tumour growth. Moreover, AM9053decreased, via PPAR-a, CRC cell proliferation in vitro and this effect was associated to a blockade of the cell cycle in the S phase. AM9053treatment also caused a significant reduction of CRC cell migration.

Discussion and conclusions: Here, we have shown that NAAA is dysregulated in intestinal tumours of CRC patients as well as in mouse colon cancer tissues. Furthermore, pharmacological inhibition of NAAA, possibly through an increase of PEA levels, counteracts colon carcinogenesis in a PPAR-α-antagonist sensitive manner. Ultimately, the presented results may put forth NAAA as possible innovative prognostic marker in clinically-diagnosed CRC and as new molecular target to be explored in drug discovery.