INHIBITION OF SPONTANEOUS COLON TUMORIGENESIS IN PIRC RATS (F344/NTAC-APC AM1137), MUTATED IN APC, AND MECHANISTIC STUDIES IN VITRO AND EX-VIVO, SUGGEST POTENTIAL CHEMIOPREVENTIVE ACTIVITY OF A POMEGRANATE MESOCARP PREPARATION.

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During the past decades, plant extracts have been studied as potential chemopreventive agents with the aim to block, reverse or delay carcinogenesis before tumour invasion. Among these, various pomegranate preparations have been reported to inhibit cancer cell lines and experimental colon carcinogenesis (1,2). However, while the majority of the studies have been performed with juice and aryls no studies have been performed with the mesocarp, the white part mesocarp, rich in the same ellagitannins of the juice. Aim of this study was to assess the possibility of using a pomegranate mesocarp decoction (PMD), as a chemopreventive agent for colorectal cancer (CRC), which represents a leading cause of cancer in Italy (3). To this aim, Pirc rats (F344/NTac-Apc am1137) (4), mutated in Apc, the key gene in colorectal carcinogenesis, were fed for 6 weeks with a diet supplemented with 1% PMD (providing, on the basis of the chemical PMD composition, 50 mg/kg of total polyphenols). Owing to Apc mutation, Pirc rats spontaneously develop colon tumours and preneoplastic lesions and can be thus used to test potential chemiopreventive compounds (4). The results of our study show that mucin depleted foci (MDF), the preneoplastic lesions in the colon, were significantly decreased in PMD-treated rats (MDF/colon in PMD-treated were 30 % compared with controls, $p<0.05$). No variations were observed in colon proliferation activity but a significant increase in apoptosis in the MDF from PMD-fed rats was observed. To better understand the mechanism of action of PMD, urolithin-A (u-A) and sodium butyrate (SB), two main colon metabolites of ellagitannins and soluble fiber components of pomegranate, were tested alone or in combination (USB) in HT-29 colon cell line. Compared to the respective controls, the expression of the proliferation marker PCNA was significantly reduced by u-A 25 µM, by SB 2.5 mM, and by the combination of the two (USB), tested at colon-relevant concentrations, with USB showing the strongest reduction ($p<0.001$). Activated Caspase-3 (CASP-3) expression was strongly increased by the combination of U-A and SB within 24h (about 10-fold increase, $p < 0.01$). Interestingly, we also observed that in cells treated for 72 h, the expression of the inflammatory markers i-NOS and COX-2 was reduced by all the treatments, with the USB treatment being the most effective ($p < 0.01$). Finally, colon adenoma (AD) and normal mucosa (NM) samples from Pirc rats were used for ex vivo experiments, in which the combination of u-A and SB treatment (USB), being the most effective in the in vitro experiments, was tested (u-A 25 µM and SB 2.5 mM). Significant increases in CASP-3 and BAK were observed in both AD and NM suggesting a pro-apoptotic effect of USB. Finally, a marked anti-inflammatory effect on both AD and NM samples treated with USB was observed: COX-2 protein expression was decreased of about 76,5% in AD and 69,02% in NM. In conclusion, these data indicate an anti-tumorigenesis effect of PMD in a relevant model of colon carcinogenesis and indicate that this effect is due, at least in part, to pro-apoptotic and anti-inflammatory effects of

its metabolites. All together the results suggest a beneficial effects of PMD that could be exploited in patients at risk of developing CRC.

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