Type 2 Diabetes Mellitus and Colorectal Neoplasia Risk in Puerto Rican Hispanic Adults Enrolled in the Studies Epidemiology of Loss of Imprinting in Colorectal Cancer, Familial Colorectal Cancer Registry and the Type 2 Diabetes Mellitus and Colorectal neoplasia Risk in Hispanics: A case-control study. Method The case-control study included patients with incident CRC and colorectal cancer-free controls. We studied the association of type 2 DM and colorectal neoplasia (CRC or adenomas) evaluated from January 1, 2003 to December 31, 2009. Diagnosis of type 2 DM was established by previous medical diagnosis and/or anti-diabetic medications use. Unconditional logistic regression was employed to estimate the odds ratio (OR) between type 2 DM and CRC using STATA 10.0. Results A total of 422 participants (mean age 69 ± 12.1 years, 61.1% males), prevalence of type 2 DM was 26.3%. 276 patients with CRC and 146 controls were evaluated. In the cases, the prevalence of colorectal adenomas was 25.4% (70/276) and the prevalence of colorectal cancer was 74.6% (206/276). Colorectal neoplasia were more frequent in type 2 DM subjects (60.3% vs. 37.3%, p<0.05), at the stages T (21.4%), I (31.4%) and II (41.7%). Cases were mostly men (p=0.01), had lower education level (p<0.001) and reported lower use of aspirin (p=0.02) compared to the controls. Cases and controls did not differ by median age (p=0.52), first degree of family history of CRC (p=0.27), first degree of family history of diabetes (p=0.33 or obesity (p=0.73)). There were no significant associations between type 2 DM and CRC or colorectal adenomas (OR=1.10, 95% CI 0.743-0.873) or DM and colorectal adenomas (OR=1.04, 95% CI 0.45-2.42) after adjusting for gender, age, education, aspirin use, obesity and study center. Conclusion We did not observe a statistically significant association between type 2 DM and CRC. Nonetheless, a tendency towards an increase risk of colorectal neoplasia was observed among type 2 DM patients on adjusted analysis. Possible explanation for our lack of association may be related to high prevalence of type 2 DM in cases and controls and/or the high prevalence of obesity in the study sample.

Boswellia Extracts Induce DNA Methylation Changes in Colon Cancer Cells Yan Shen, Alexander Linik, Masanobu Tabagushi, Francois Balague, Keun Hur, C. Richard Boland, Ajay George

Accumulating evidence indicates that the chemopreventive effects of several dietary polyphenols are in part mediated by their ability to demethylate and subsequently reactivate methylated genes in human tumours. Boswellia extract (BE), which are derived from the plant species Boswellia serrata (commonly known as Frankincense) possess potent anti-inflammatory and anti-oxidant activities. Consequently, BE may have been successfully used to treat a wide variety of inflammatory diseases including arthritis, chronic colitis, Crohn’s disease and cancer. However, precise molecular mechanisms underlying BE-mediated chemopreventive effects remain elusive. We hypothesized that BE may modulate DNA promoter methylation in colorectal cancer (CRC) cells and eventually reactivate methylated-silenced tumor suppressor genes. Material and Methods: We treated RKO and SW480 CRC cell lines with the most active principle present in BE, acetyl-lesto-beta-boswellic acid (AKBA), at a dose range of 0-40μM. A series of assays was performed to study the effects of BE on cell viability (MTT assay), proliferation (Brdu assay), and clonogenic survival (colony formation). Genome-wide DNA promoter methylation analyses were performed using Illumina’s HumanMethylation27 BeadChip microarrays, which includes analyses of 27,000 CpG loci that span across 14,000 genes. In addition, gene expression analysis was performed using Illumina’s HumanHT-12 microarrays. A β-value of 0.1 (indicating 10% methylation change) and an increase in 2-fold gene expression was considered significant. Methylation and gene expression results were subsequently validated by methylated specific PCR and quantitative real time PCR's, respectively. 5-aza-2’-deoxycytidine (DAC) treated cells were used as positive controls for demethylation. Results: BE inhibited cell proliferation, decreased cell viability, and decreased clonogenic survival (MTT and Brdu assays) in the RKO and SW480 cell lines in a dose dependency. In contrast to global hypomethylation induced by DAC, BE treatment resulted in a more selective demethylation and simultaneous restoration of gene expression. To confirm our microarray results, we successfully validated BE-induced demethylation in a subset of tumor suppressor genes (CREB5, DRA5, and SAMD14) that showed the least decrease in gene expression for SAMD14 and SMPD3 genes. Conclusions: Our data provide novel evidence for BE-induced DNA demethylation and reactivation of gene expression in a subset of tumor suppressor genes in CRC cells. BE-induced reactivation of a subset of tumor suppressors may in part contribute to its growth inhibitory effects in CRC cells. These data provide further mechanistic insights into the chemopreventive effects of boswellia extracts.

Apigenin Inhibits mTORC2 Through Down-Regulation of RICTOR Expression in Colon Cancer Cells Zheng Guo, Yuning Zhou, B. M. Evers, Qingding Wang

Knockdown of rictor, a component of Mammalian Target of Rapamycin Complex 2 (mTORC2), blocks Akt phosphorylation and inhibits Akt activation and thus, results in inhibition of colorectal cancer growth. However, little is known about the signaling pathways that regulate rictor expression. Apigenin, a common dietary flavonoid, has proven to be one of the most promising compounds for anticancer and anti-inflammatory effect. The purpose of this study was to investigate the effects of apigenin on mTORC2 signaling. METHODS: HT29 and HCT116 colon cancer cells were treated with various dosages of apigenin. Cell growth was determined using a sulforhodamine B assay kit from Sigma. Rictor, mTOR and beta-actin expression was analyzed by Western blot. Apigenin expression was determined by real time RT-PCR. To investigate whether apigenin transcriptionally regulates rictor expression, we cloned the 5’-upstream region (1038 bp) of the human rictor gene utilizing the human Genome Walker Kit and inserted it upstream of the luciferase reporter gene. HCT116 cells were transiently cotransfected with the rictor promoter construct and the promoter activity was determined by luciferase activity assay. RESULTS: Treatment with apigenin inhibited HT29 and HCT116 cell growth. Moreover, treatment