abnormally elevated fecal occult blood levels (defined as greater than 2.0 mg hgb/g stool) as compared to 2 (1.5%) patients taking placebo (p=0.47). The higher mean hgb concentration in the ASA group as compared to those on placebo did not cause a higher HO positively rate: 4 (3.7%) patients had positive HO tests while on placebo, as compared to 3 (2.2%) patients on ASA (p=0.32). In contrast, subjects were significantly more likely to demonstrate a positive SENSA test while on ASA (10 patients, 7.3%) as compared to those on placebo (5 patients 3.7%)(p=0.03). Those tested with HS were significantly more likely to have positive tests while taking ASA 325 mg, but no such relationship was found with FS. 12 patients underwent endoscopy (all had colonoscopy and 6 also underwent EGD); 1 had multiple colonic adenomas (largest = 15mm), 1 had a single vascular extasia in the colon and 1 had an esophageal squamous papilloma; other endoscopic tests were normal. CONCLUSION: ASA causes a small increase in fecal blood. ASA is unlikely to lead to false positive FOB tests if done with relatively insensitive guaiac-based tests such as HO; however, testing with highly sensitive tests such as SeNSA is likely to lead to false positives. The data also raise the possibility that highly sensitive immunochemical FOB tests may detect trivial ASA-induced colonic bleeding. Supported in part by a grant from SmithKline Diagnostics, Inc., Palo Alto, CA.

● G1817

CASPASE-MEDIATED CLEAVAGE OF FOCAL ADHESION KINASE (FAK) IN NON-TRANSFORMED HUMAN INTESTINAL EPITHELIAL CELLS (IEC) UNDERGOING APOPTOSIS.

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Caspases play a critical role during the execution of apoptosis. They mediate cell death by proteolysis of select intracellular targets, one of them being Fokal adhesion kinase (FAK), a key component of the integrin-associated signaling complex. Even though *in vitro* studies using recombinant substrates showed various caspases to be candidates for mediating FAK cleavage, the sequence of events in non-transformed cells undergoing apoptosis remains elusive. We analysed the mulekular mechanisms of endogenous caspase activation, leading to cleavage of endogenous FAK in non-transformed normal human IEC undergoing apoptosis. Non-apoptotic IEC were isolated from colonic surgical specimens as described (Grossmann, et al., Am. J. Pathol.,1998;153:53–62). As IEC underwent detachment-induced apoptosis (DICD) within three hours after loss of anchorage we characterized (by western blot) the sequence of caspase activation for all members of the caspase-3 family (Grossmann et al., Am. J. Physiol., 1998; 274:G1117–1124) and the fate of endogenous FAK. Following detachment, endogenous FAK (p125) is sequentially cleaved into two fragments (p94 and p84) after 45 and 120 minutes. Inhibition of caspase-3 family members by addition of DEVD-CHO (1.5 μM) completely blocked FAC cleavage, 100-fold more effectively than inhibition of caspase-1 family members with YVAD-CHO (150 μM). The first cleavage of FAK occurs simultaneously with caspase-3 activation and displays an inhibitory profile characteristic of this caspase, whereas the second cleavage is inhibited by nmol concentrations of VEID-FMK, a selective caspase-6 inhibitor and occurs simultaneously with the cleavage of Lamin A, a substrate, targeted only by activated caspase-6. Caspase-7 activation, an early event during DICD of IEC, occurs simultaneously with PARP cleavage and 30 minutes prior even to the initiation of endogenous FAK cleavage, making this caspase an unlikely candidate for mediating FAK proteolysis. Cleavage of FAK is a late event during DICD of IEC, demonstrating the coordinative and sequential proteolytic activity of two distinct caspases, caspase-3 and caspase-6, which are consecutively activated during IEC apopotsis. FAK cleavage during apoptosis displays the remarkable property of caspases to specifically target very select but pivotal elements of a cell, doomed to die.

G1818

THE EFFECT OF CAFETIERE COFFEE ON BIOMARKERS FOR COLONIC CANCER IN HEALTHY VOLUNTEERS: A PLACEBO-CONTROLLED TRIAL.

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Epidemiologic studies suggest that coffee use might protect against colorectal cancer. Inconsistencies between studies might be related to the type of coffee. **Aim:** to study the effect of unfiltered coffee on putative biomarkers for colorectal cancer. **Methods:** 64 healthy volunteers (31M, 33F: age 42 ± 11 yr) were randomized to two groups. One group drank 1 liter of unfiltered cafetiere ('French Press') coffee daily for 2 weeks. Such coffee is rich in cholesterol-raising diterpenes. The other group, which drank no coffee, received water, milk, broth, tea or chocolate. After an 8-week washout, treatments were reversed for another 2 weeks. At the end of each intervention period fasting blood samples, 48 hr stool and rectal biopsies were collected. Differences in the change were assessed by one-sample T-test. **Results:** serum cholesterol increased by 0.48 mmol/l on coffee. There was no effect on the concentration of fecal soluble bile acids (coffee vs no coffee: 138 vs 170 umol/l), on cell proliferation in rectal biopsies as assessed by Proliferating Cell Nuclear Antigen (19.8 vs 21.0%) or on GST-activity in rectal mucosa (210 vs 211 nmol/min/mg protein). The glutathione concentration in plasma increased by 0.8 umol/l (95% CI 0.3 to 1.4) and in the rectal mucosa by 2.5 nmol/mg protein (95% CI 0.5 to 4.4). Plasma cysteine and homocysteine concentrations increased by resp. 7.7 umol/l (95% CI 2.5 to 13.0) and 1.2 umol/l (95% CI 0.5 to 1.9). **Conclusion:** Unfiltered coffee significantly increased the glutathione content of the colonic mucosa. The increase of mucosal and plasma glutathione and plasma cysteine and homocysteine do suggest an effect of unfiltered coffee on the thiol metabolism. Whether this effect can contribute to a lower colon cancer risk remains to be established.

● G1819

IDENTIFICATION OF A DIFFERENTIATION RELATED, PPARγ and Methylation Regulated Putative Metastasis Suppresser Gene Drg-1 in Human Colon Cancer

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The most life-threatening aspects of colon cancer are invasion and metastasis. Alterations in gene expression may play a crucial role in the pathogenesis of colon cancer metastasis. Using differential display, we have identified 19 genes expressed differentially between primary and metastatic colon cancer. In preliminary studies, we found that one of these genes is not expressed in metastatic colon cancer tissues or metastatic colon cancer cell lines including the SW620 cell line. A GenBank sequence homology search found that this gene is identical to a novel gene named Drg-1 (differentiation-related gene-1) cloned from a differentiated HT-29 colon cancer cell line (Belzen et al., Lab. Invest. 1997). Stable transfection of SW620 cells with Drg-1 cDNA altered cell morphology, reduced *in vitro* invasion and markedly upregulated E-cadherin expression compared to non-transfected cells. Preliminary studies with nude mice showed that over-expression of Drg-1 in SW620 cells suppressed metastases to the liver. In mechanistic studies, we found that the expression of Drg-1 gene was upregulated by troglitazone, a synthetic ligand for PPARgamma, but not by another differentiation reagent tributyrin, a prodrug of butyrate, suggesting that Drg-1 protein may be a downstream element of the PPARgamma pathway. Treatment with aza-deoxycytidine, an inhibitor of DNA methylation, also upregulated the expression of Drg-1, implying DNA methylation may down-regulate Drg-1 expression. Our hypothesis is that the Drg-1 gene suppresses colon cancer metastasis by inducing PPARgamma-dependent gene expression and altering the expression of E-cadherin and possibly other key molecules related to cancer metastasis.

● G1820

ASSOCIATION OF K-RAS MUTATIONS WITH P16 METHYLATION IN HUMAN COLON CANCER

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Background & Aims: K-ras mutations are early gene changes in colon cancer. p16, a tumor suppressor gene, can be inactivated in neoplasms by mutation, deletion or methylation. The aims of this study were to determine p16 methylation status and its possible association with K-ras mutations in human colon cancer. *Methods:* DNA isolated from eight colon cancer cell lines and 41 microdissected human colon tissue samples were analyzed. p16 methylation status was determined using 2 analytical methods. K-ras mutations were determined by DNA sequence analysis. The level of p16 expression was determined by RT-PCR and Northern blot. The DNA methyltransferase activity was determined by microassay. Parental and K-ras transformed IEC-18 cells were used to determine the potential association between K-rats mutations and p16 methylation. *Results:* Methylated p16 was found in 100% of colon cancer cell lines, 55% of colon cancers, 54% of adenomas, 25% of transitional mucosa but not in normal colonic epithelium. Thirty-eight percent of adenomas and 45% of all cancers showed both K-ras mutations and p16 methylation. Of 11 adenomas and cancers with K-ras mutation, 10 specimens showed methylated p16. In contrast, of 13 adenomas and cancers with wild type K-ras, only 3 specimens showed methylated p16 (p =0.001). Stable transformation of IEC-18 cells with K-ras increased the DNA methyltransferase activity, methylated the p16 gene and suppressed the expression of p16. Treatment with a DNA methylation inhibitor (aza-deoxycytidine) resulted in re-expression of p16 in K-ras transformed IEC-18 cells, suggesting that the expression of p16 gene was suppressed by DNA methylation. *Conclusion:* p16 methylation occurs frequently in human colonic adenomas and cancers and is closely associated with mutations of K-ras.