addition the roles of ZEB-1 and Snail, transcriptional repressors of E-cadherin, are investigated.

Materials and methods: We studied the relationship between COX-2 and E-cadherin in vitro in the cell lines MKN45, MKN28, AGS3 (all three derived from convential gastric cancers) and MKN7 (derived from EOGC). The effects of PGE-2 and the COX-2 inhibitor celecoxib on E-cadherin, COX-2, ZEB-1 and Snail expression were examined using western blot and Q-PCR. Expression of E-cadherin and COX-2 was examined using tissue microarrays (TMA) of 88 conventional gastric cancers and 106 EOGCs.

Result: Our in vitro study showed that downregulation of COX-2 by celecoxib leads to upregulation of E-cadherin expression only in conventional gastric cancer cell lines and E-cadherin repressor Snail is involved in this pathway. Surprisingly a statistically significant correlation on immunohistochemistry was found between COX-2 overexpression and normal E-cadherin expression in conventional gastric cancer (p=0.016) but not in EOGC.

Conclusions: Previously we have shown that COX-2 overexpression differs between EOGC and conventional gastric cancer. Here we show that COX-2 appears to regulate E-cadherin in gastric cancer cell lines and that this effect occurs only in conventional gastric cancer cell lines. We could not find this relation on our immunohistochemistry study. This is the first report of COX-2 and E-Cadherin acting in the same pathway in gastric cancer and our findings also further highlight the unique nature of EOGCs.

580 Poster

Lactoferricin treatment delays cell cycle progression of a human colon cancer cell line

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The aim of this study was to investigate the effects of bovine lactoferricin on the human colon cancer cell line CaCo-2, foremost with respect to cell cycle progression. In the study, we have been studying relatively low doses and their impact over time, to reconstruct physically relevant exposure. Bovine milk is a source of indigenous bioactive peptides as well as peptides encrypted in the amino acid sequence of milk proteins. The anticancer effect of milk peptides is an emerging and mostly unrevealed scientific area. A peptide derived from lactoferrin, lactoferricin, LF f(17-41), has bioactive properties in cancer prevention. A bromodeoxyuridine DNA flow cytometry method was used to study the effect of a physiologically relevant dose of lactoferricin on cell cycle kinetics and cell proliferation. Lactoferricin treatment did not affect the length of the G2 + M phase in human colon cancer cells. However, a concentration of 2 μM lactoferricin, equivalent to the quantity achieved in the intestine by milk consumption, prolonged the S phase in human colon cancer cells. A prolonged S phase may result in decreased intestinal cancer development. In normal cells, a prolonged S phase may result in improved DNA repair. A slight prolongation of the cell cycle induced by food components may in the long-term sense reduce cancer risk, as cancer development and progression is dependent on the rate of cell proliferation. To gain further knowledge of the health promoting effect of milk peptides, the subsequent step will be to study the effect of lactoferricin on proteins involved in cell cycle regulation and DNA repair. The understanding achieved from these studies may be utilized for the production of new functional foods and pharmaceutical preparations that may be used for cancer prevention and cancer treatment.

581 Poster

The effect of plant polyphenolic compounds on the proliferation and DNA methylation in MCF7 cells

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Modulation of cancer cell methylome, e.g. through the inhibition of DNA methyltransferase enzymes (DNMTs) is a widely accepted mechanism of cancer prevention. Naturally occurring polyphenols are a promising group of chemicals which can potentially affect DNA methylation in human cells. It has been reported that compounds such as epigallocatechin gallate, genistein, chlorogenic acid, caffeic acid and myricetin can lead to demethylation of aberrantly silenced tumour suppressor genes through the inhibition of DNMTs. Frequently, the resulting demethylation elicited by these compounds is low to moderate, yet a change in gene expression can be observed.

The aim of this study was to evaluate the effect of a group of dietary polyphenols: ellagic acid, rosmarinic acid, cyanidin and betanin on the proliferation of MCF7 breast cancer cells and the methylation status of RARbeta, DAPK and RASSF1A genes. The effect of these compounds on cell proliferation was assessed using the MTT assay. Methylation-specific PCR was used for the analysis of gene methylation status. The activity of poly(ADP-ribose) glycohydrolase (PARG) was measured with PARG activity kit from Trevigen.

Betanin did not considerably affect the proliferation of MCF7 cells, whereas rosmarinic acid and ellagic acid showed proliferation arrest in concentrations higher than 50 μM. Rosmarinic and ellagic acids strongly inhibited the activity of PARG in contrast to betanin and cyanidin. In gene methylation studies, MCF7 cells were exposed for 3 days to different concentrations of the compounds tested. We did not observe any differences in the methylation profile between non-treated and treated MCF7 cells for any of the studied polyphenols.

The cytotoxic effect observed for rosmarinic and ellagic acids can be related to the inhibition of PARG which leads to the cellular accumulation of poly(ADP-ribose). This polymer in certain conditions acts as a death signal. Although potentially all studied polyphenols could affect DNMTs function, it appears that they do not show any activity towards restoration of silenced tumour suppressor genes. Using quantitative techniques could probably allow more sensitive detection of discrete changes in DNA methylation, if there are any.

In conclusion, rosmarinic acid, ellagic acid, cyanidin and betanin do not affect DNA methylation in MCF7 breast cancer cells and thus cannot be considered epigenetic modifiers.

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582 Poster

Methylation of the DAPK, HIN-1 and FHIT genes in head and neck cancer

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Background: The aim of this study was to investigate the role of promoter methylation in head and neck cancer in genes for which loss or decreased expression has been reported in various cancers.

The HIN-1 (High in Normal 1) gene is expressed in the lung, tracheal epithelia and pancreas and prostate tissue. The gene is highly expressed in breast epithelium while in breast cancer its expresssion is lost. This has led to the suggestion that expression of this gene is suppressed by promoter methylation in breast cancer.

The FHIT (Fragile Histidine Triad) gene is located on 3p14.2 and codes for a tumor suppressor protein. Changes in the FHIT gene, loss of expression and promoter methylation have been reported in a great majority of the patients with esophageal cancer. FHIT gene methylation has been shown to occur in lung, breast, prostate, bladder, cervix and oral cancers. Transcriptional activation of the FHIT gene has also been associated with microsatellite instability.

The DAPK (Death Associated Protein Kinase) gene has been initially described as a mediator of the interferon gamma-induced apoptosis pathway. The gene codes for a serine/threonine kinase which plays a role in the activation of the p19ARF/p53 cell cycle control. The loss of DAPK inhibition has been reported in colon, nasopharyngeal, lung, ovarian and B or T cell cancers and associated with high metastatic capacity and invasiveness.

Materials and methods: In this study 105 patients with head and neck cancer without prior treatment were analyzed. Methylation of the promoter regions genes was analyzed by methylation-specific PCR Genomic DNA was first modified by sodium bisulfite to convert unmethylated cytosines to uracil and amplified by primer pairs specific for the methylated and unmethylated sequences. The amplified fragments were separated in agarose gels and evaluated using a gel documentation system or analyzed in an Agilent 2100 bioanalyzer.

Results: Promoter methylation was observed in % 99 of the patients for the FHIT gene, in % 74 of the patients for the HIN-1 gene and in % 57 of the patiens for the DAPK gene. In a single patient the DAPK promoter region was completely methylated. No association was found between the extent of methylation and clinical parameters.

Conclusion: Our data indicate that promoter methylation of the FHIT gene is very frequent and inhibition of the FHIT protein expression may play a significant role in head and neck carcinogenesis.