PPARγ and estrogen receptor (ER) in breast cancer cells. The present study was carried out to evaluate the transcriptional activity of PPARγ isoforms, PPARγ1 and PPARγ2, and ERα in human breast cancer cell lines treated with ligands for PPARγ and ERα, 15deoxy-prostaglandin J_2 (15d-PGJ_2) and 17β-estradiol (E2) respectively, by quantitative Real-Time PCR using homologous internal standards. ER-positive (MCF-7) and ER-negative (MDA-MB-231) breast cancer cells were treated with EC_{50} doses of 15d-PGJ_2 and 10 nM E2 alone or in combination for up to 48 hrs. The ERα, PPARγ1 and PPARγ2 mRNA expression levels were significantly up regulated ($p < 0.05$) in MCF-7 cells treated with 15d-PGJ_2 or E2 alone. The combined treatment of 15d-PGJ_2 and E2 however, significantly down regulated the ERα mRNA expression, showed no significant difference in PPARγ1 mRNA expression and up regulated the PPARγ2 mRNA expression level in MCF-7 cells. The PPARγ1 mRNA expression was significantly up regulated in MDA-MB-231 cells treated with 15d-PGJ_2 alone and in combination with E2. In contrast, no significant difference in the PPARγ1 mRNA expression level was observed in E2 treated cells. The mRNA expression of PPARγ2 was significantly down regulated in MDA-MB-231 cells treated with 15d-PGJ_2 but not with E2 treatment. Interestingly, a significant up regulation of PPARγ2 mRNA expression was observed in these cells when treated with the combination of 15d-PGJ_2 and E2. The differential expression of PPARγ1, PPARγ2 and ERα in ER-positive and ER-negative breast cancer cells suggests a complex regulation of these transcription factors in breast carcinogenesis, the mechanism of which remains to be elucidated.

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Inactivation of the FHIT gene in clear cell renal carcinomas

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FHIT is a tumour suppressor gene which is frequently inactivated in different types of cancer. Yet little is known about the mechanism of FHIT inactivation in clear cell renal carcinomas. Since genetic alterations were not frequently observed in DNA corresponding to the FHIT gene in renal tumours, to elucidate the mechanism of FHIT gene silencing we examined 22 paired samples of clear cell renal carcinoma and non-malignant renal tissue for the methylation of the FHIT 5'CpG island by methylation-specific PCR. Hypermethylation of the FHIT 5'CpG island was detected in 54.5% of clear cell renal carcinomas. Bisulfite sequencing of the FHIT 5'CpG island confirmed the results obtained by methylation-specific PCR for selected samples. We showed that expression of the FHIT gene is inversely correlated with hypermethylation of the FHIT 5'CpG island in the selected samples. Our results suggest that hypermethylation of the FHIT 5'CpG island may be responsible for inactivation of the FHIT gene in clear cell renal carcinomas.

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Profile of methylaion of tumour related genes in breast cancer in Tunisian women

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Background: It is becoming increasingly recognized that aberrant hypermethylation of gene promoter regions is an important mechanism inducing transcriptional silencing of tumor suppressor genes in various human cancer including breast carcinomas. There are several reports on methylation profiles of breast cancer patients from Western population. However, to our knowledge there is no study in Arabian populations till date. It is important to note that Tunisia belong to low incidence zone of breast carcinoma with standardized incidence of 19.6 per 100 000 women. The present study was undertaken to evaluate the DNA methylation profile of tumor-related genes in Tunisian breast carcinomas.

Methods: One hundred and nine invasive ductal carcinomas diagnosed at the Department of Pathology at Farhat-Hached Hospital of Sousse (Tunisia) were investigated for the methylation status of a panel of fifteen known tumor-suppressor and -related genes by methylation-specific polymerase chain reaction. Both specific methylated and unmethylated primers were used for PCR and the products were visualized with agarose gel electrophoresis.

Results: Of the 109 cases 23 (21%) showed methylation at 1 to 3 genes, 36 (33%) were methylated at 4 to 6 genes, and 50 (46%) were methylated in more than 6 genes. No cases were methylated at all fifteen genes and all cases showed at least one gene methylated. Hypermethylation frequencies were 78% for RASSF1A, 66% for SHP1, 61% for HIN1 and BRCA1, 47% for P16 and ER, 42% for CDH1 and APC, 40% for BLU, 35% for DAPK, 34% for RARβ2, 27% for GSTP1, 17% for TIMP3, 14% for CCND2, and 8% for hMLH1.

Conclusion: This study shows high frequencies of methylation of tumor-suppressor and -related genes in Tunisian women in comparison with Western women. These observations suggested that gene hypermethylation may be affected by ethnicity. Besides ethnicity, these epigenetic variations may also be attributed to differences in the risk factors such as life style and dietary habits. Thus, our study underscores the limitation of extrapolation of the Western data to other populations. Our findings, reported here will hopefully provide a stimulus for additional studies comparing populations with different ethnicity and risk factors.

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Do PIKE, PIK3CA and PTEN genes in Phosphoinositide-3-kinase/Akt signaling pathway play a crucial role in progression of high-grade gliomas?

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Background: High-grade gliomas are the most common primary brain tumors and associated with poor survival. Phosphoinositide 3-kinase/AKT signaling pathway is important in the development of malignant gliomas. The PIK3CA gene, encodes the p110alpha catalytic subunit of PI3K, is activated in various cancers. PIKE (CENTG1), encodes a protein that binds to phosphorylated Akt and increases its activity, is frequently amplified in glioblastomas. phosphatase and tensin homology deleted on chromosome 10 (PTEN) is an important regulator of the PI3K/Akt pathway via its ability to antagonize PI3K. PTEN function is lost in high-grade glioma due to loss of heterozygosity or mutations and loss of this gene function associated with activated AKT levels. In this study we aimed to identify the roles of the genes which were components of the PI3K/Akt signaling pathway and correlation between their expression profiles in malignant disease progression.

Materials and methods: Human brain tumor samples were obtained from patients who underwent primary therapeutic subtotal or total tumor resection performed under surgical operation. All cases signed a written informed constant statement approved by local ethics committee. Explant cell cultures were performed from brain tumor tissues of 18 (6 female, 12 male; average age 49.72±14.83) cases. Malignant lesions have been described in the medical history of cases: anaplastic oligoastrocytoma WHO grade III (7 cases), GBM WHO Grade IV (7 cases) and brain metastasis from lung cancer (4 cases). Total RNA was isolated from tumor cells. RNA of the tumor samples were reverse-transcribed with oligo dT primers and quantified by real-time reverse transcription polymerase chain reaction (RT-PCR) performed with the LightCycler instrument. U87MG glioblastoma cell line was used as positive control.

Results: The mean relative ratios of PIK3CA, PIKE and PTEN genes were found; 162.46, 13.67 and 3733.61, respectively. There was no significant association between tumor grades/age and gene expressions. The correlation between PIKE and PTEN gene expressions was found significant especially in anaplastic oligoastrocytoma ($p<0.0001$). Similar correlation was found between PIK3CA and PIKE genes in cases with brain metastasis from lung cancer ($p=0.037$).

Conclusion: Due to these expression correlations, PI3K/Akt signaling pathway genes could be used as pivotal biomarkers and build smart and effective drug combinations of molecular targeted treatments of malignant gliomas.

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Cyclooxygenase-2 dependent regulation of E-cadherin through the transcription repressors Snail and ZEB1 is limited to conventional gastric cancers cell lines

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Background: Approximately 10% of patients present with gastric cancer before the age of 45, so-called early onset gastric cancer (EOGC), and it is postulated that genetic factors may play a more important role than in conventional gastric cancer (presenting > 45 years old). EOGCs have been shown to have a different molecular pathway than conventional gastric cancers and we have shown previously that they have a strikingly low expression of COX-2 compared to conventional gastric cancer, where it is often overexpressed.

Aims: COX-2 regulation of E-Cadherin has been shown to occur in lung cancer and given that E-Cadherin is critical in gastric carcinogenesis, we examine the relationship between these two molecules in this study. In

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.

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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.