between environmental exposure and individual susceptibility towards activation and detoxification of procarcinogens may influence levels of genotoxic agents and subsequently modify the risk of carcinogenesis. One of the most common forms of cancer is colorectal cancer (CRC). CRC affects approximately 5% of worldwide population. The majority of CRC is sporadic with unknown etiology. Exposure to procarcinogens through alimentary chain and smoking are considered as environmental factors contributing to CRC incidence. We followed associations of genetic variability in GSTM1, GSTT1, GSTP1, NQO1, CYP1B1 and EPHX1 genes with CRC risk in a case-control study.
Materials and Methods: Polymorphisms in GSTM1 (deletion), GSTT1 (deletion), GSTP1 (Ile105Val), NQO1 (Pro187Ser), CYP1B1 (Asn453Ser and Leu432Val) and EPHX1 (Tyr113His and His139Arg) were assessed by PCR RFLP based methods in groups of 649 CRC patients and 745 unrelated hospital-based controls of Czech Caucasian origin. EPHX1 (Tyr113His) variants were verified by sequencing analysis.
Results: Statistical analysis showed that variant genotype in GSTP1 (Val105Val) significantly increases the risk of CRC (crude OR = 1.48, CI = 1.02−2.15, P = 0.037). No significant association among other investigated single polymorphisms and susceptibility to CRC was found. Individuals carrying at least one variant allele in GSTP1 in combination with GSTM1 or GSTT1 deletion were under significantly increased risk of CRC in comparison with those carrying wild-type genotypes (P < 0.001 and P = 0.011, respectively). Combination of variant alleles in all three GSTs genes also significantly increased the CRC risk (P = 0.020). Combination of variant alleles in GSTP1 with altered EPHX1 also conferred increased CRC risk. Age and sex did not play a role as confounding factors.
Conclusions: Our study suggests that combinations of polymorphisms in xenobiotic-metabolizing enzymes may confer increased risk of CRC and should be further followed by larger study on related populations. Polymorphisms confirmed as risk factors may then be used for identification of subpopulations under increased CRC risk and subsequent targeting of preventive strategies.
This project was supported by GACR, no.: 310/05/2626 and 310/07/1430.

3032 POSTER

Cannabinoids induce apoptosis through CB1 and CB2 receptor activation in human colon cancer cells

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Background: Recent experimetal studies have pointed to cannabinoids as potential anticancer agents. One of the possible molecular mechanisms underlying the antitumor effect of these compounds is the ability to induce tumor cell apoptosis through activation of their cellular receptors, namely CB_1 and CB_2. The aim of this study was to determine the effect of the CB_1 receptor agonist arachinodyl-2′-chloroethylamide (ACEA) and the newly synthesized 1,8-naphthyridin-4(1H)-on-3-carboxamide derivative (compound 3g) CB_2 receptor agonist on inducing apoptosis and decreasing cell proliferation in the human colon cancer cell lines HT29 and DLD1.
Methods: mRNA and protein expression of the CB_1 and CB_2 receptors in human colorectal cancer specimens and in the HT29 and DLD1 cells were investigated by RT-PCR and Western blot analysis, respectively. The proapoptotic effect of ACEA and 3g on the two colon cancer cell lines was evaluated by means of caspase 3 activity determination and flow cytometry analysis (Annexin V test) of apoptotic cells. Tumor cell proliferation was determined by the [^{3}H]thymidine incorporation assay. The effects of the CB_1 and CB_2 agonists on ceramide and TNF-alfa production were also assessed. The HT29 and DLD1 cells were treated with 100 nmol/l ACEA and 3g.
Results: Both CB_1 and CB_2 receptors were expressed in the human colorectal cancer specimens and in the colon cancer cell lines. Treatment of the HT29 and DLD1 cells with either ACEA or 3g induced a significant increase in caspase-3 activity and number of apoptotic cells. The same treatment determined a significant decrease in tumor cell proliferation after their stimulation with 100 nmol/l epidermal growth factor. All these effects were prevented by the administration of 10μM fumonisin B1, a ceramide synthase inhibitor. Moreover, treatment of the HT29 and DLD1 cells with ACEA or 3g significantly increased the production of both ceramide and TNF-alfa.
Conclusions: Our data showed that the cannabinoid receptors CB_1 and CB_2 are expressed in human colorectal cancer. Moreover, cannabinoids can induce apoptosis in human colon cancer cells and reduce their proliferation through activation of both CB_1 and CB_2 receptors. These effects seem to be mediated by an increase in ceramide production, a known mediator of apoptosis. We also hypothesized that ceramide production is in turn stimulated by an increase in TNF-alfa production through activation of both CB_1 and CB_2.

3033 POSTER

Identification and validation of novel serum tumour markers for colorectal cancer applying proteomics approaches

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Background: The goal of this study was to identify and validate novel serum markers of human colorectal cancer as potential candidates for non-invasive detection of early colorectal neoplasm.
Materials and Methods: Two-dimensional gel electrophoresis (2-DE) and matrix assisted laser desorption/ionization-mass spectrometry (MALDI-MS) as well as a complementary shotgun proteomics approach applying nano flow two-dimensional liquid chromatography coupled to electrospray ionization MS (2-D-LC-ESI-MS) were used to analyze 16 matched colorectal cancer and adjacent normal tissue samples. Antibodies against selected proteins found to be elevated in cancer tissue, were generated and used for further validation by immunoblotting of tissue samples and immunohistochemistry. Highly sensitive immunoassays were developed for assessment of serum levels of selected proteins.
Results: In total, 735 distinct proteins were identified in colon tissue with the 2-DE/MALDI-MS approach. For a small number of these identified proteins, among them nicotinamide N-methyltransferase (NNMT) and proteasome activator complex subunit 3 (PSME3), strong elevation in colorectal cancer was confirmed by immunoblot analysis and immunohistochemistry, respectively. Highly sensitive immunoassays revealed that elevated levels of NNMT and PSME3 are found in serum from colorectal cancer patients. Employing a receiver operating characteristic curve based on the measurement of 109 colorectal cancer patients and 317 healthy controls, we obtained an area under the curve (AUC) of 0.84 for NNMT and of 0.79 for PSME3, respectively, which was superior to the established tumor marker carcinoembryogenic antigen (CEA) with an AUC of 0.77.
The 2-D-LC-ESI-MS approach led to the identification of further proteins, which were partly not identified in the 2-DE/MALDI-MS approach. Further analysis of the 2-D-LC-ESI-MS data and validation of thereof derived proteins elevated in cancer tissue is currently ongoing and the results will be presented at the conference.
Conclusions: The results of the presented study indicate that it is essential to combine different, complementary proteomics approaches to obtain a most comprehensive description of the proteome of a given tissue. It is proposed that the serum levels of NNMT and PSME3, respectively, may have a value in the early detection and in the management of colorectal cancer patients.

3034 POSTER

Antiangiogenetic-based therapy for advanced colorectal cancer patients seems to enhance the antitumor cellular immunoresponse

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Background: Multiple links have been found between angiogenesis and immunoresponse in human tumors. Vascular endothelial growth factor (VEGF) is a key agent in promoting and sustaining the immune tolerance during the cancer growth, particularly because of the indirect impairment on the functional maturation of dendritic cells (DCs) (Johnson BF, Expert Opin Biol Ther, 2007). Preclinical murine models have been shown that the block of VEGF could enhance the efficacy of cancer immunotherapy in colorectal carcinoma (Li B, Clin Cancer Res, 2006). Bevacizumab, the humanized monoclonal antibody against VEGF, is largely employed in the treatment of metastatic colorectal cancer (mCRC) pts in addition to chemotherapy (CT), and its in vivo impact on pts immune system has not been clarified.
Material and Methods: During our ongoing studies on the immunosuppressive effect of cancer treatments, we have now focused on the impact of first-line Bevacizumab-based combination therapy on 27 pts with mCRC (M/F: 20/7, median age: 55 yrs), in absence of clinically relevant infections.

Data were compared with 21 pts with mCRC who have received CT alone as first-line treatment (M/F: 16/5, median age: 56 yrs) and with 40 healthy subjects (M/F: 20/20, median age: 40 yrs). The immunological profile of our pts was evaluated by flow cytometric analysis of different PB lymphocyte and DC subsets.
Results: With respect to normal donors, a significant decrease of absolute lymphocyte number, CD4 T lymphocytes, CD19 and CD20 B-lymphocytes, NK cells and DCs was evidenced in mCRC pts treated with CT alone. Bevacizumab addition to CT didn't affect the B lymphocyte and the NK compartments. With respect to mCRC pts treated with CT alone, a statistically significant increase of CD4 T lymphocytes was observed ($p < 0.003$). At the same time, Bevacizumab administration was associated with a significant increase of absolute DC number and of their cellular subset ($p < 0.001$), with a decrease of DC humoral subset ($p < 0.002$).
Conclusions: First-line Bevacizumab-based therapy in mCRC pts seems to improve the in vivo T-cell mediated response, because of the increase of DC cellular subset. As evidenced in murine models, the VEGF blockade could have a synergistic effect in cancer immunotherapy programs for mCRC pts.

3035 POSTER

Levels of survivin splice variants correlate to degree of differentiation in colon cancer

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Background: Survivin is a member of the inhibitors of apoptosis family and plays an important role in cell division and proliferation by inhibition of apoptosis. There are five splice variants of survivin (survivin, survivin-2B, survivin-3B, survivin-ΔEx3 and survivin-2α) which appear to have different functions and take part in the regulation of the action of survivin. The aim of this study is to assess the role of these five splice variants in colon cancer.
Materials and Methods: Matched neoplastic and normal colonic tissue was obtained from 20 consecutive patients who underwent surgery for therapeutic reasons at the University Hospital of Patras. Total RNA was isolated and quantified using Ribogreen (Molecular Probes) before being reverse transcribed with Stratascript (Stratagene). Expression levels of the 5 splice variants were assessed with variant-specific primers and Taqman probes by quantitative PCR.
Results: Survivin-2α and survivin-2B, the two isoforms with pro-apoptotic function, were the isoforms most frequently expressed in normal tissue (100 and 90%, respectively). Survivin and survivin-3B were expressed in 80% whereas survivin-ΔEx3 in 60% of the normal colonic tissue. In neoplastic tissue, survivin, survivin-2B, survivin-3B and survivin-2α were expressed in all samples, whereas survivin-ΔEx3 was expressed in 95% of the samples. The levels of expression of all splice variants were significantly higher in neoplastic than in normal tissue ($p < 0.001$). Additionally, they were higher in stage B than in C although the difference was statistically significant only for surivivin-2α ($p = 0.019$). Moreover, all 5 survivin isoforms were expressed in higher levels in well compared to moderately differentiated tumors ($p < 0.05$). Furthermore, the ratios of the expression of the different isoforms were assessed. Survivin-3B/survivin-2α was higher in normal tissue than in neoplastic ($p < 0.05$). None of the ratios changed with the level of differentiation or the stage of the disease.
Conclusion: All 5 isoforms are expressed in both normal and neoplastic tissue although with different frequencies. Anti-apoptotic survivin-ΔEx3 expression exhibited the highest difference suggesting a role in colon cancer. All 5 isoforms are expressed at higher levels in neoplastic than in normal tissue. Furthermore, the higher levels of expression in well-differentiated tumors may suggest a role for survivin isoforms in the initial stages of colon carcinogenesis.

3036 POSTER

Tipification of genes related to oxaliplatin (OXA) sensitivity in a panel of 14 human colorectal cancer (CRC) cell lines by using microarray technology and real time quantitative PCR (QRTPCR)

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Background: Platinum drugs resistance is a complex process based in the alteration of genes that belong to several pathways related to drug metabolism. To clarify the multifactoriality of these mechanisms, we analyzed gene expression profile in fourteen CCR cell lines with different sensitivity to oxaliplatin. The aim of this work was to compare gene expression profile between high IC50 (IC50 > 1 mM) and low IC50 (IC50 < 1mM) cells and to validate the results by QRTPCR to determine genes that could play a role as a marker in oxaliplatin sensitivity.
Methods: Gene expression profile was analyzed through microarray technology (Human 19K oligo; labeled with Genisphere; data analysis by Genesis 1.5.0). We calculated logratio (OXA 24h treated cells vs untreated cells) for each cell line and analyze changes in gene expression comparing high (LOW OXA sensitivity) versus low IC50 (HIGH OXA sensitivity) groups. To study selected genes we used QRTPCR (Taqman®), considering as a positive validation, those genes that showed significant differences at expression level between high and low OXA sensitivity groups (ANOVA $p < 0.05$)
Results: By microarray analysis we obtained 51 candidate genes. Twelve of these genes were selected and 2 of them were positively validated after QRTPCR analysis: DUSP11 and VPS33A were upregulated in LOW OXA sensitivity group).
Conclusions: In our model, 2 genes showed expression changes between two OXA sensitivity groups. DUSP11 is a member of the dual specificity protein phosphatase subfamily that have been related to regulation of MAPK. VPS33A (vacuolar protein sorting 33A) is involved in vesicle-mediated protein transport. According to these results, both genes must be validated as a potential OXA sensitivity markers in CRC patients.

3037 POSTER

Two pathways of carcinogenesis in patients with colorectal cancer less than 45 years old

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Background: Colorectal cancer (CRC) arising from a complex series of molecular changes that involve at least in two different pathways. These include microsatellite instability (MSI) pathway and chromosomal instability (CIN) pathway. The aim of this study was the determination of predominant pathway involved in carcinogenesis of patients with CRC less than 45 years old with and without family history (FH) of CRC.
Method: In our study surgical pathology specimens of 108 patients with CRC less than 45 years old were immunostained for DNA mismatch repair proteins (MMRP) including hMLH1, hMSH2, hMSH6 and PMS2. Beta-catenin and P53 were also examined for CIN pathway.
Results: Totally 108 patients with median age of 40(20 to 45) were evaluated. Fifty seven patients were male and 51 were female. The site of tumor in 84 patients was colon and in 14 were rectum. Among 96 patients with known family history, 33(34.4%) had positive FH. The overall rate of abnormal immunostaining were MLH1 8.3%, MSH2 18.5%, MSH6 8.3%, PMS2 11.1%, P53 74.1% and beta catenin 35.2%. Meanwhile abnormal staining for hMSH2 and hMSH6 were significantly more seen in patients with positive family history ($p = 0.008$ and $p = 0.032$ respectively). Patients with positive FH for CRC had significantly more abnormal MMRP (54.5% vs. 20.6%, $p = 0.001$) and less positive p53 (54.5%vs. 81%, $p = 0.006$) than patients with negative FH. Patients with early T, N stage tumor had at least one more abnormal MMRP than advance T, N stage ($P = 0.050$ for T and $P = 0.030$ for N stage). Among different factors abnormal hMSH2 had significant association with lower cancer related death ($P = 0.060$). Patients with rectal cancer had more abnormal MMRP than patients with colon cancer but not significantly (35.7% vs. 29.8%, $p = 0.655$) and positive p53 staining for rectal and colon cancer were 71.4% and 72.6% respectively. Both in colon and rectal cancer patients with negative family history had more prevalent positive p53 (80.4% vs. 56.7%, $p = 0.022$ for colon and 81.8% vs. 33.3%, $p = 0.099$ for rectal cancer).
Conclusion: Our study indicate that even in CRC less than 45 years old, the main pathway for carcinogenesis in patients with negative family history is CIN, but in positive family history MSI is as effective as CIN.However main pathway in both colon and rectal cancer is CIN.