whereas the response rate in triple negative patients was 44% ($p < 0.1^{-6}$). KRAS and BRAF mutations were mutually exclusive, but 6 of the 14 PIK3CA mutations were also KRAS mutated. PFS was 7.7 month (6.0−8.7 95%CI) in triple negative patients compared to 2.5 month (2.1−3.5 95%CI) in the group of patients who harboured any mutation. (HR = 0.43, 0.28−0.67 95%CI, p = 0.0001). The triple-negative patients achieved a median overall survival of 10.4 months compared to 4.8 months in patients harbouring any mutation (HR = 0.7, 0.46−1.08, p = 0.1).
Conclusion: Although the low frequency of these mutations implies a need for larger studies, the present results suggest that also BRAF and PIK3CA mutations are significantly associated with clinical resistance to third-line cetuximab/irinotecan in metastatic colorectal cancer. Consequently, these mutations may contribute as additional selection criteria when added to KRAS status.

6120 POSTER

Ursodeoxycholic acid inhibits proliferation of intestinal epithelial cells: role of EGF and ERK pathway

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Introduction: Ursodeoxycholic acid (UDCA) prevents colitis-related colon cancer which potentially could be attributed to enhanced proliferation during tissue regeneration. One of the pathways regulating epithelial cell proliferation is the EGF-MEK-ERK pathway. We investigated therefore the effects of UDCA on the growth of rodent intestinal epithelial cells *in vitro* and *in vivo* in relation to this pathway.
Materials and Methods: Two groups of six C57BL/6J mice were fed with standard diet with and without 0.4% UDCA for 3 weeks. Sections of the colon were stained with antibodies against Ki-67 protein and phosphorylated ERK protein. The normal rat intestinal epithelial cell line IEC-6 was used for *in vitro* experiments. MTT test was performed on cells treated with UDCA (0 to 800 μM for 3 days) with and without EGF addition. Western blots were made to check the effect on ERK phosphorylation. Cells were treated with different concentrations of U0126, an inhibitor of MEK kinase, for 3 days and ERK phosphorylation was monitored.
Results and Discussion: Treatment of IEC-6 cells with EGF (50 ng/ml) significantly increased the cell number (160% of control after 2 days). This increase of proliferation was abrogated in the presence of 400 μM UDCA. After 3 days of treatment with UDCA (600 μM) alone, proliferation decreased by 60%. This inhibition was concomitant with the inhibition of ERK phosphorylation by about 70%. 10 μM U0126 inhibited both proliferation and ERK phosphorylation by about 70%. Feeding UDCA to mice reduced the number of Ki-67 expressing cells by 40% in relation to the non-treated group. The treatment also decreased the amount of phosphorylated ERK in the proliferating compartment of the crypt.
Conclusion: Our results show that UDCA decreases proliferation of normal colonic epithelial cells both *in vivo* and *in vitro*. The inhibition of the EGF signalling pathway and/or decrease of ERK phosphorylation might be responsible for the proliferation inhibition caused by UDCA.

6121 POSTER

Gene expression profile related to oxaliplatin (OXA) intrinsic resistance in a panel of 14 human colorectal cancer (CRC) cell lines

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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 these multifactorial 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 < 1 mM) cells to determine genes that could play a role as a marker in oxaliplatin intrinsic resistance.
Methods: Gene expression profile was analyzed through microarray technology (Human 19K oligo; labeled with Genisphere; data analysis by Genesis 1.5.0). We analyzed changes in gene expression comparing high (LOW OXA sensitivity) versus low IC50 (HIGH OXA sensitivity) groups., the set of genes was analyzed by using two Array-tools t-test based statistical methods (NCI: class-comparisons and SAM) in order to determine their probability to be false positive markers.
Results: We obtained a gene expression profile of 198 candidate genes by using hierarchical clustering and ANOVA function (p < 0.01). According to Array-tools analysis, 16 genes were selected because they did not show any probability to be false positive markers.
Conclusions: In our model, an expression profile of 16 genes showed to be related to oxaliplatin intrinsic resistance without probability to be false positive markers. These set of genes should be validated in patients to establish their potential role in CRC treatment selection.

6122 POSTER

MicroRNA expression profile in stage II colorectal cancer

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Background: Mi(cro)RNAs are non-coding molecules which regulate gene expression by translational repression or mRNA degradation. Aberrant miRNA expression has been demonstrated in many malignancies, including colorectal cancer (CRC). Moreover, miRNAs have potential roles as diagnostic and prognostic biomarkers, and therapeutic targets. Stage II CRC has posed a significant challenge to manage due to high risk of recurrence and lack of consensus to guide adjuvant therapy. We aimed to characterise miRNA expression profiles of patients with stage II CRC and to investigate their association with clinicopathological variables.
Materials and Methods: Following ethical approval and patient informed consent, high throughput miRNA microarray was performed on a cohort of 20 tissue samples from patients with stage II CRC to profile the expression of 380 miRNAs. Differentially expressed miRNAs were validated by real-time quantitative (RQ)-PCR in an expanded cohort of 106 tissue specimens from 58 patients.
Results: On array analysis, 20 miRNAs were identified as upregulated and 13 downregulated in tumours. Five miRNAs were selected for validation in a wider cohort of CRCs by RQ-PCR; their differential expressions were confirmed: *miR-10b* ($p < 0.001$), *miR-143* ($p = 0.003$), *miR-145* ($p = 0.001$), *miR-21* ($p = 0.002$) and *miR-31* ($p < 0.001$). *Mir-31* was the most dysregulated miRNA with a fold change of over 5. Furthermore, increased *miR-31* and reduced *miR-143* expression levels were associated with disease aggressiveness.
Conclusion: This study demonstrates dysregulated miRNA expression in stage II CRC tumours. Moreover *miR-31* and *miR-143* were associated with disease aggressiveness. Dysregulation of these miRNAs is consistent with their oncogenic and tumour suppressor regulation of gene targets known to be dysregulated in CRC (*miR-21 – PTEN*, *PDCD4*; *miR-143 – KRAS*, *BCL2*). This could represent a novel means of prognostication and to guide adjuvant chemotherapy in CRC.

6123 POSTER

Circulating cytokeratin 18 fragments – M30 and M65 – as marker of postoperative residual tumour load in colorectal cancer patients

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Background: Despite radical surgery up to 50% of colorectal cancer patients subsequently develop distant metastases. Appropriate detection systems for the routine clinical use to determine the extend of pre- and prioperative haematogenic tumor cell dissemination are still missing. Soluble cytokeratin 18 (CK18; M65) and a caspase-cleaved fragment of CK18 (M30) have been used as biomarkers, corresponding to tumor cell death and apoptosis respectively. Aim of this study was to evaluate the significance of pre- and postoperative cell death measurements in serum of patients operated for colorectal cancer.
Material and Methods: M30 and M65 were quantified in serum samples pre- and postoperatively. Disseminated tumor cells in bone marrow of colorectal cancer patients, as negative prognostic factor were assessed by staining with the pan-cytokeratin antibody A45-B/B3 in bone marrow aspirates. A total of 64 colorectal cancer patients and 22 people without cancer were included into the study.
Results: Patients with colon tumors of stages UICC I and IV had significantly elevated M30 serum concentrations compared to controls. M65 measurements showed elevated levels in UICC I and IIA, compared to normal controls ($p < 0.05$). In 31 colon cancer patients, M30 and M65 determinations were performed prior to and seven days after tumor surgery. A group of 24 patients exhibited a significant decrease of M30 in response to tumor removal, in contrast to seven patients with either persistent or higher M30 levels postoperatively. M30 correlated significantly with the

increased number of recurrences within 36 months in the group with persisting levels of M30 (4/7 versus 2/24, p = 0.032). Tumor surgery led to decreased M65 serum measurements postoperatively in a subgroup of patients (19/31), in contrast to 12 patients who revealed higher M65 levels postoperatively. MRD was proven in 10% (2/19) of the first group and 50% (6/12) of the second group (p = 0.028).
Conclusions: Tumor surgery clearly has an effect on postoperative serum concentrations of the M65 antigen indicating that perioperative determination of serum concentrations of this antigen seem to constitute a marker of postoperative systemic residual tumor load in colorectal cancer patients. The difference in early and advanced tumor stages as well as in preoperative and postoperative serum concentrations of the M30 antigen seems to represent an interesting marker of residual tumor load and early tumor recurrence.

6124 POSTER

Cetuximab specific IgE antibodies can predict cetuximab-induced anaphylaxis

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Background: Cetuximab is a chimeric mouse-human IgG1 monoclonal antibody against the epidermal growth factor receptor. This drug is used for the treatment of colorectal cancer and squamous-cell carcinoma of the head and neck. Severe hypersensitivity reactions to cetuximab have been described with prevalences varying from 1.2% to 22% depending on the area of the world. An American study has shown that IgE specific for galactose-α-1,3-galactose are present in serum before therapy of most subjects who had a hypersensitivity reaction to cetuximab.
The main goal of our work was to confirm that IgE specific antibodies against cetuximab were involved in the clinical reaction observed in our hospital (prevalence of severe reaction estimated at 7%). The predictive value of cetuximab specific IgE was evaluated.
Material and Methods: IgE anti-cetuximab were measured using home made enzyme-linked immunosorbent assay (ELISA). A technical cut-off value of 10 arbitrary units IgE (AUE) was calculated from healthy blood donors. We analyzed retrospectively serum samples from 60 patients treated with cetuximab in Francois Baclesse Anticancer center.
Results: Among the 60 cetuximab-treated subjects, 14 had a hypersensitivity reaction (grade 2, 3 or 4) to the drug. IgE antibodies against cetuximab were found in 13/14 pretreatment samples. 12 out of 46 subjects without hypersensitivity reaction had IgE antibodies. Sensitivity and specificity of this test were 92.9 and 73.9% respectively. The positive predictive value was 52% and more interestingly, the negative predictive value was 97.1%.
Conclusions: Cetuximab IgE specific antibody detection could represent a valuable test and help the prescriptor to select an alternative treatment for patients with high risk of hypersensitivity reaction when available.

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6125 POSTER

Topoisomerase-1 (Topo1) as a predictive and prognostic factor in colorectal cancer chemotherapy

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Background: In the FOCUS-1 trial, analysis of primary tumour Topo1 immunohistochemistry (IHC) in 1313 patients with metastatic colorectal cancer (MCRC) receiving 5-fluouracil (FU), oxaliplatin (Ox) and irinotecan (Ir) showed higher Topo1 expression to be associated with worse outcomes with FU alone but increased benefit from Ir/Ox (Braun et al, JCO 26:2690−8, 2008). In FOCUS-2, 459 elderly/frail patients with MCRC were randomised to first-line FU, OxFU, Capecitabine (Cap) or OxCap. Topo1 expression in FOCUS2 patients was assessed to see whether the results of the FOCUS-1 study were confirmed, and whether any predictive association with Ox is affected by the fluoropyrimidine (FP) platform.
Methods: Tumour blocks were retrieved. 5 μm slices of tissue microarrays or whole sections were stained for Topo1 (Novocastra antibody) and scored as low (<10% nuclei staining) or mod/high (>10% nuclei). The Mantel-Haenszel log-rank test was used to calculate hazard ratios (HR) and 95% confidence intervals (CIs) to assess the effect on progression free survival (PFS) and overall survival (OS) when Ox is added to FU or Cap. Interactions between treatment effects and Topo1 were tested using the likelihood ratio and the magnitude of interactions was assessed using the ratio of HRs.
Results: Topo1 results were obtained for 361 (79%) patients. Overall, adding Ox to either FP improved the PFS (HR 0.78, 95%CI [0.62−0.96], p = 0.02), with a non-significant impact on OS (HR 0.88 [0.70−1.24], p = 0.27). In prognostic analysis, mod/high Topo1 was associated with worse PFS in FP-alone treated patients (HR 1.20 [0.87−1.65]), but better PFS in OxFP-treated patients (HR 0.79 [0.55−1.13]). In predictive analysis, mod/high Topo1 was associated with a benefit for adding Ox (PFS: HR 0.71 [0.55−0.91]; OS: HR 0.84 [0.65−1.10]), whilst patients with low Topo1 gained no benefit (PFS: HR 0.97 [0.65−1.46]; OS: HR 1.03 [0.66−1.60]). These results, although not independently significant, are fully consistent with the results of FOCUS-1. The interaction was more pronounced with OxFU/FU (ratios of HRs: PFS = 1.63 [0.83−3.18]; OS = 1.42 [0.69−2.94]) than with OxCap/Cap (ratios of HRs: PFS = 1.20 [0.60−2.4]; OS = 0.98 [0.47−2.06]). For comparison, the ratios of HRs for OxFU/FU in FOCUS-1 were PFS = 1.35 [0.99−1.83], OS = 1.35 [1.05−1.74].
Conclusion: FOCUS-2 is consistent with FOCUS-1, with similar magnitude of prognostic and predictive effects for Topo1 IHC in relation to FU and Ox. Less pronounced effects are seen when Cap is used as the FP platform.

6126 POSTER

Presence of K-RAS and BRAF oncogenic mutations sensitise colorectal tumours to TRAIL induced apoptosis: evidence from cell and animal models translated to the clinic

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Background: Most data on the therapeutic potential and expression of TRAIL in colorectal cancer has come from *in vitro* studies using tumour cell lines. To gain a clearer understanding about the susceptibility of patient tumours to TRAIL, we derived primary human cancer epithelial cells [1]. Increased apoptosis was observed in both primary PAP60 and MIH55 after treatment with SuperKiller TRAIL. Treating patient tumour xenograft/SCID mouse models with Killer TRAIL *in vivo* for 5 consecutive days suppressed tumour growth, although less efficiently compared to *in vitro* experiments. Sensitization to TRAIL induced apoptosis by RAS has been previously shown by our lab [2] and by others. We have presented evidence that this effect is usually mediated by TRAIL receptor DR4 and DR5 overexpression and/or redistribution in cell models [3].
Materials and Methods: Primary colorectal tumour cells, colorectal cell lines, mouse xenographs and colorectal clinical samples were either treated with recombinant TRAIL and/or analysed for the presence of oncogenic mutations and DR4, DR5 expression.
Results: We present evidence that DR5 as the most frequently upregulated DR in clinical samples of colon cancer. Furthermore, the presence of K-RAS and BRAF mutations in the tumour may directly or indirectly enhance DR expression, potentially sensitising these otherwise resistant tumours to TRAIL-based therapies [4].
Discussion: Mutations on K-RAS and BRAF oncogenes have been shown in many studies to be associated with resistance to several targeted therapeutics and combinations. TRAIL-based therapeutics, other as mono- or combination therapy could provide a promising alternative for K-RAS/BRAF bearing colorectal tumours.

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