

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.

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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 μ M 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 μ M UDCA. After 3 days of treatment with UDCA (600 μ M) alone, proliferation decreased by 60%. This inhibition was concomitant with the inhibition of ERK phosphorylation by about 70%. 10 μ M 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.

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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.

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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.

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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 postoperative 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