6125 POSTER

Two Polymorphisms of the Endothelin Axis in Colorectal Cancer

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Background: Endothelin (ET) is a vasoconstricting peptide that mediates its effects through the endothelin receptors (ETA and ETB). This sytems gains increasing importance in cancer. Previous studies have associated the ET-1 isoforms and ETA receptor with mitogrenic effecs on tumour and stromal cells, tumour cell survival, invasion and metastastis as well as modulation of tumour-infiltrating immune cells. The ET-1 +1383A/4A polymorphism results in altered ET-1 levels while the role of the ETA receptor rs 5333 polymorphism remains unknown. In this pilot study we asked whether these 2 polymorphisms influence relapse status in patients with colorectal cancer (CRC).
Material and Methods: DNA was extracted from formalin-fixed paraffin-embedded tissue samples from 89 patients with CRC. Genotyping was performed using real time PCR. The +1383A/4A polymorphism was genotyped with a taqman assay on 68 samples while the rs5333 polymophism was genotyped on 89 samples using a sybr green approach. Statistic analysis was performed using pasw18.
Results: Regarding the ET-1 +138 3A/4A polymorphism, the majority of patients (45.4%) were homozygous for 3A. The homozygous 4A genotype was observed in 20.1% and heterozygosity in 34.3% of patients. The 3A allele frequency was lower in patients who remained disease free compared to patients who relapsed (0.525 *vs* 0.654, respectively). Accordingly, the 4A allele was more frequent in patients who remained disease free compared to those who relapsed (0.475 *vs* 0.346, respectively). However, statistical significance was not reached.
Regarding the ETA receptor rs5333 polymorphism, the majority of patients were TT (60%) while only 9% of patients were CC. The remaining 31% comprised the heterozygotes. Both T and C allele frequencies were similar between patients who relapsed and patients who remained disease free (T: 0.737 *vs* 0.755 and C: 0.262 *vs* 0.245).
Conclusions: The ET-1 and the ETA receptor polymorphisms studied are unrelated to disease relapse in patients with colorectal cancer.

6126 POSTER

Thymidylate Synthase as Biomarker in Rectal Cancer Patients After 5-FU-based Radiochemotherapy − Evaluation of the Prognostic Capacity in Pre-treatment Biopsies and Resected Adenocarcinoma

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Purpose: Fluorouracil (5-FU) remains the backbone of neoadjuvant radiochemotherapy (RCT) as well as adjuvant therapeutic strategies in multimodal treatment of rectal cancer patients. Due to its central role as the major target of 5-FU thymidylate synthase (TS) is a promising biomarker in rectal cancer. We assessed TS in 208 patients with regard to its predictive/prognostic capacity for disease free DFS and overall cancer specific survival (CSS).
Patients and Methods: 167 patients cUICC stages II (28%) and III (72%) received preoperative 5-FU based RCT followed by total mesorectal excision (TME) A comparison group n = 41 received postoperative RCT after primary TME. All patients were treated after standardized protocols within phase-II/-III trials of the German Rectal Cancer Study Group. TS levels from pre-treatment biopsies and corresponding resection specimens were assessed by immunohistochemical staining for their impact on DFS and CSS. Additionally, a TS gene polymorphism (28 bp repeat) was analysed in respect to intracellular protein expression levels and prognostic significance.
Results: Patients with low TS expression in pre-treatment biopsies showed a correlation with impaired CSS (p = 0.015). After neoadjuvant RCT there was evidence of lymph node metastases ypUICC stage III in 32.6%. Complete histopathologically confirmed tumour regression TRG 4 was achieved in 16 patients (9.5%). During follow-up (median 57 months) patients with low intratumoral TS expression and positive nodal status were at high risk for local and/or distant metastatic recurrence (p = 0.040). Analysis of the 28bp repeat revealed a correlation of *3/*3 genotype with high TS expression in pretherapeutical biopsies (p = 0.05).
Conclusion: TS represents a prognostic biomarker in locally advanced rectal cancer indicating an unfavourable outcome for patients with low TS expression and might help to adapt adjuvant therapy regimens by stratifying patients according to their risk for cancer recurrence.

6127 POSTER

What About Risk Factors KRAS, BRAF and PI3K in a French Translational Study OMIT of 325 Patients Traited With Cetuximab Based-regimen in Real Practice

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Background: The OMIT (Drugs and Emerging Therapeutics Observatory) is a French structure created in 2003 by the Regional Health Agencies of Western France (Bretagne and Pays de la Loire). This network gathers clinical data from 50 public and private institutions. Its medical staff (oncologists, surgeons, pharmacists, biologists, etc) has a global reflection on drugs management in cancer patients and represents a task force for French Health Authorities.
K-RAS mutation status is a strong predictive marker of cetuximab efficacy in advanced colorectal cancer. However, a subset of wild-type K-RAS patients do not respond, suggesting the existence of additional markers of resistance, such as other EGFR downstream signaling molecules like B-RAF and PI3K.
Methods: K-RAS, B-RAF and PI3K mutation status were retrospectively analysed in a large group (n = 341) of mCRC patients treated with cetuximab based-regimen.
The aim of this study was to determine their role as prognostic markers.
Statistical analysis used the χ^2 test, linear regression analysis, and the Kaplan–Meier method.
Results: The sex ratio (male to female) of the population was 1.69 and the mean age was 63.8 IC95% [62.7–65.0]. 75% of the patients received the cetuximab-based regimen as second or third-line therapy. Mutations in MAP kinase pathway were found in 39% (Kras: 36%; BRAF: 3%) and PI3K mutations in 9%. 6.5% of the patients had simultaneous PI3K and KRAS mutations. Median Progression Free Survival (PFS) and Overall Survival (OS) were significally lower (p = 0.0031 & p = 0.0253) in patients with tumours presenting K-Ras mutation. The OS was significantly lower only in patients with codon 13 K-RAS mutant tumours while the PFS was significantly lower only in patients with codon 12 K-RAS mutant tumours. B-RAF and Pi3K mutation status did not affect the PFS and OS. However, in patients with K-Ras wild type tumours, the presence of B-RAF mutation or PI3K mutation leads to significantly decreased PFS (B RAF p = 0.0078; PI3K p = 0.05) and OS (B-RAF p = 0.079; PI3K p = 0.0029).
Conclusion: Our study confirms that K-RAS is a predictive biomarker of the response of metastatic colorectal cancers to anti-EGRF monoclonal antibody cetuximab. K-RAS codon 12 and 13 mutation status affect differently the response to cetuximab. In patients with wild-type K-RAS, B-RAF and Pi3K mutation status are relevant to predict cetuximab efficacy.

6128 POSTER

Frequency and Importance of KRAS Mutations in Inoperable Cholangiocarcinoma Patients Referred for Systemic Therapy

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Background: The purpose of the present study was to investigate KRAS in an unselected population of cholangiocarcinoma patients eligible for oncologic treatment. There are no curative options for inoperable cholangiocarcinoma. Systemic therapy with chemotherapy has proven to increase survival and, furthermore, combination chemotherapy is superior to monotherapy. There is, however, plenty of room for improvement and this may be achieved by adding biologic agents. In other gastrointestinal cancers supplemental effect is seen when adding EGFR inhibiting treatment to chemotherapy, but the effect depends on KRAS wild-type. The frequency of KRAS mutations in cholangiocarcinoma patients eligible for oncologic treatment is largely unknown and this is a prerequisite for designing marker driven clinical trials.
Material and Methods: From October 2008 to December 2010 all patients with inoperable cholangiocarcinoma referred to our department of oncology were included. DNA was isolated from tumour tissue and analysed with quantitative PCR for the 7 most frequent activating mutations in the KRAS gene. Data about chemotherapy and participation in clinical trials were extracted from patient records and case report forms. Date of death was verified in a central register.
Results: During 27 months 148 patients were referred with inoperable cholangiocarcinoma. 109 patients received chemotherapy and most of

these were enrolled in registered clinical trials (n = 61). KRAS analysis was successful in 125 cases. Main reasons for no KRAS analysis were clinical deterioration (n = 13), inadequate or insufficient tumour material (n = 6) or other/not specified (n = 4).
All patients but one were Caucasian, most were women (n = 91) and the median age was 68 years (range 26−83). Nineteen patients had KRAS mutant tumours, and all mutations were found in exon 12. The median overall survival was 6.6 months. It was independent of KRAS status (wild type 7.0 months and mutant 8.3 months, p = 0.75). Median survival for patients without KRAS analysis was 2.3 months.
In the 109 patients receiving chemotherapy, 16 (14.7%) had tumours with KRAS mutations.
Conclusion: KRAS mutations were found in 14.7% of a cholangiocarcinoma population eligible for chemotherapy. The number differs from surgical cohorts and this must be taken into account when designing studies testing the influence of KRAS status on treatment effect.

6129 POSTER

Analysis of Surfactant on Primary(COLO-320) and Metastatic(COLO-741) Human Colon Cancer Cells Treated With A-Lactalbumin or Sulindac

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Background: Colon cancer is the second most frequent reason in the cancer-related deaths in the world. During cancer therapy, the correct time and correct medicine is crucial for different patients. In addition, the primary and metastatic colon cancer therapy may also be different because of cancer cell behavior. Our aim is investigating the surfactant has efficiency on the medicine during treatment of cancer cells.
Materials and Methods: Colo-320 and Colo-741 lines were used in this study. The cells were cultured in RPMI-1640 media including %10 FCS, %1 L-glutamine and %1 penicillin-streptomycine. The cells were cultured in 24 wells of tissue culture plate. After subculturing of cells, they were cultured 24 hours. After 24 hours of culture, the cells will be treated with either α-lactalbumin or sulindac or α-lactalbumin+surfactants or sulindac +surfactants. After 48 hours of treatment, culture mediums from all groups were collected for cytotoxicity analysis, the cells from all groups were fixed in %4 paraformaldehyde for 30 minutes for histochemical analysis. Cell cytotoxicity were evaluated with ELISA. Cell death was investigated using TUNNEL assay.
Results: The Colo-320 cells were semi-adhesive cells; the Colo-741 cells were attachment cells. After treatment with sulindac of Colo-320 cells, the number of alive cells was less when compared with other groups. It was also observed that the number cells in Colo-741 cells which were treated with only α-lactalbumin or only sulindac groups had less cell amount than the other groups. We are still evaluating the affect of surfactants during treatments of colon cancer.
Conclusions: Our hypothesis suggests that both primary and metastatic cells will be affected when surfactants during treatment. However, the addition of surfactants during treatment protocols may cause differences in drug interactions with cells. In farther researchs. Simultaneously, Surfactants might be used to treat with different medicines of other cancer types. Researchs are still going on about this issue in our faculty.

6130 POSTER

Survivin Expression in Rectal Cancer During Preoperative Radiochemotherapy and Its Impact on Metastasis and Patients' Survival

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Background: Valid molecular markers need to be implemented in clinical trials to fulfill the demand of a risk-adapted and more individualized multimodal therapy of locally advanced primary rectal cancer. In the present study the expression of the inhibitor-of-apoptosis (IAP) protein Survivin was evaluated in pre-treatment biopsies and corresponding post-treatment resection specimens, and was correlated to histo- pathological tumour characteristics and clinical follow-up.
Material and Methods: 116 patients with stage II/III rectal cancer treated with 5-FU/Oxaliplatin based neoadjuvant radiochemotherapy (RCT) at a single university medical centre within the German Rectal Cancer Trials were investigated. Survivin expression in pre-treatment biopsies and surgical resection specimens were determined by immunohistochemistry by two independent institutions and correlated with histopathologic parameters, tumour recurrences, disease-free and cancer-specific overall survival.
Results: In pre-treatment biopsies, a higher Survivin expression correlated with advanced ypT (p = 0.026) and ypUICC (p = 0.05) stage as well as decreased disease- free survival (p = 0.038) after preoperative RCT. High post-treatment Survivin levels were associated with advanced ypT stage (p = 0.03) and residual lymph node metastases (p = 0.04). Moreover, neoadjuvant RCT resulted in a significant down-regulation of Survivin expression (p < 0.0001). A failure of RCT-induced down-regulation was associated with development of distant metastases (p = 0.0056) and cancer-related death (p = 0.026), and was significantly correlated with disease-free (p = 0.011*/0.02**) and cancer- specific survival (p = 0.0017*/0.01**) in uni* and multivariate** analyses.
Conclusions: Survivin expression in rectal cancer displays a marker with prognostic validity. These results underline the usefulness of Survivin to monitor individual response to RCT in rectal cancer, and encourage anti-Survivin strategies in multimodal rectal cancer therapy within future randomised clinical trials.

6131 POSTER

Evaluation of BRAF Mutational Status in Wild Type (WT) KRAS Metastatic Colon-Rectal Cancer (MCRC) Patients (pts) Treated With Cetuximab (C) − a Single Institution Experience

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Background: KRAS mutations in pts with mCRC have since emerged as the major negative predictor of efficacy in pts receiving anti-EGFR therapies such as C. Nevertheless, the occurrence of KRAS mutation only accounts for approximately 35−45% of nonresponsive pts. Mutations in BRAF have been recently shown to impair responsiveness to these agents, with no response observed in BRAF mutated pts.
Materials and Methods: From 11/2008 to 01/2011, 95 mCRC pts were tested for KRAS mutations and 30 pts resulted KRAS WT, all of them were treated with C+ chemotherapy (CHT). We now retrospectively analyze the clinical outcome of 20 pts according to their BRAF mutational status, in order to evaluate if BRAF mutation influence the clinical outcome. fivepts were excluded from the analysis due to the impossibility to evaluate BRAF status for little DNA. Genomic DNA was extracted from formalin-fixed, paraffin embedded (FFPE) tumour samples using a commercial kit (Invisorb Spin Tissue Mini Kit, Invitek, Berlin, Germany). V600E mutation of the BRAF gene was evaluated through an allele specific multiplex PCR (CRC Kit 2, Experteam, Venezia, Italy). Median age was 69 years (42−81), all pts underwent surgery for colon (16/20, 75%) or rectal (5/20) cancer, 8/20 (40%) received adjuvant CHT, with FOLFOX4 (50%) or De Gramont regimen.
Results: All pts received C with Irinotecan16/20, 80%) or FOLFIRI (4/20) as 2nd or 3rd line treatment. Five pts (25%) resulted as BRAF mutated (BRAF+). No response has been observed among BRAF+ pts, whereas PR or SD was obtained in 6/15 (40%) BRAF− ones. Median TTP was significantly longer in BRAF− vs BRAF+ (12 vs 8 months).
Conclusion: Our single institution experience confirms the recent data about the hypothesis that BRAF mutational status could be a predictive factor for response to anti-EGFR therapy in KRAS WT mCRC pts. Further evaluation of the RAS/RAF pathway or the analysis of polymorphism of the EGFR in BRAF− pts who didn't obtained a response to anti-EGFR therapy is needed to better understand the lack of response in KRAS WT pts.

6132 POSTER

Efficacy of Panitumumab Plus FOLFIRI Versus FOLFIRI Alone in Patients With Wild-Type (WT) KRas Metastatic Colorectal Cancer (mCRC) Treated With Prior Oxaliplatin or Bevacizumab Regimens: Results From 20050181

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Background: First-line treatment regimens for mCRC often contain oxaliplatin and the anti-vascular endothelial growth factor antibody,