expression in breast carcinomas and correlated with the clinical outcome of the patients.
Methods: 86 specimens were tested so far for plakoglobin by means of immunohistochemistry and the expression scored separately for membrane, cytosol, and nucleus. Mean plakoglobin values were evaluated for the two groups of surviving and deceased tumor patients.
Results: In a 15 years follow-up the ratio surviving/deceased was 2.2 for membrane, 1.6 for nucleus, 1.2 for cytosol, and 1.4 for overall staining. All patients with an intense staining of either membrane or nucleus are still alive, in contrast to about 40% survival for low membrane or low nuclear staining and 12% survival for low both.
Conclusion: In conclusion, we found a close correlation of conserved plakoglobin expression in the tumor with 15 years overall survival, in particular for membraneous and nuclear staining.

229 PUBLICATION

A molecular analysis by gene expression profiling reveals BIK/NBK overexpression in sporadic breast tumors of Mexican female samples

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Background: Breast cancer is the second cause of death in Mexican women over 35 years of age. At molecular level, changes in many genetic networks have been reported as associated with this neoplasia. To analyze these changes, we determined gene expression profiles of tumors from Mexican women with breast cancer at different stages and compared these whit those of normal breast tissue samples.
Material and methods: ^{32}P-radiolabeled cDNA was synthesized by reverse transcription of mRNA from fresh sporadic breast tumor biopsies as well as normal breast tissue. cDNA probes were hybridized to microarrays and expression levels registered using a phosphorimager. Expression levels of some genes were validated by real time RT-PCR and immunohistochemical assays.
Results: We identified two subgroups of tumors according to their expression profiles, probably related with cancer progression. Ten genes unexpressed in normal tissue were turned on in some tumors. We found consistent high expression of *Bik* gene in 14/15 tumors with predominant cytoplasmic distribution.
Discussion: Recently, the product of the *Bik* gene has been associated with tumoral reversion in different neoplasic cell lines, and was proposed as therapy to induce apoptosis in cancers including breast tumors. Even though a relationship between genes, for example those from a particular pathway, can be observed through microarrays, this relationship might not be sufficient to assign a definitive role to *Bik* in development and progression of the neoplasia. The findings herein reported deserve further investigation.

Poster presentations (Mon, 31 Oct)
Molecular predictive assays (including: genetics, genomics, molecular diagnostics, prognostic factors, proteomics)

230 POSTER

Enhanced sensitivity of human lymphoblastoid cell lines with heterozygosity for a mutation in BRCA1 or BRCA2 towards the DNA-damaging agent cisplatin

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Background: heterozygous carriers of BRCA1 or BRCA2 germline mutations exhibit a high risk of developing breast and other cancers. The loss of the wild-type allele is frequently observed in the primary breast and ovarian tumours in these susceptible patients. Previous studies suggest that homozygous mutations in BRCA1/2 (BRCA−/−) result in impaired DNA damage repair and response to genotoxic damage. However, it is unclear if heterozygosity for BRCA1/2 mutations (BRCA±) have any phenotypic effect.
Material and methods: to assess whether heterozygous mutations in these genes are associated with modified sensitivity to the genotoxic anticancer agent cisplatin, we performed an *in vitro* chemosensitivity assay on human lymphoblastoid cell lines developed from a BRCA1 heterozygote carrier (GM13705), a BRCA2 heterozygote carrier (GM14622) and two BRCA1/2 competent (BRCA+/+) individuals (GM14453 and GM14454), using the MTT assay. The concentration of drug that reduced the number of viable cells to 50% (IC50) after 24 hours of exposure was calculated by logarithmic regression model. Results were derived from at least six independent sets of triplicate experiments.
Results: GM13705 (IC50: mean = 5.2 μM, s.d. = 1.9) and GM14622 (IC50: mean = 6.2 μM, s.d=1.5) cell lines were significantly more chemosensitive than the BRCA-competent GM14453 cell line (IC50: mean = 15.3 μM, s.d. = 8.0) (p = 0.0012 and 0.0026 respectively). Also, GM13705 (IC50: mean = 5.0 μM, s.d. = 1.9) and GM14622 (IC50: mean = 6.4 μM, s.d. = 1.7) cell lines were more chemosensitive than the BRCA-competent GM14454 cell line (IC50: mean = 19.1 μM, s.d. = 8.0) (p = 0.0002 and 0.0017 respectively).
Conclusions: cells containing a heterozygous mutation in BRCA1 or BRCA2 are more sensitive to the genotoxic agent cisplatin. These findings suggest that heterozigote cells are not phenotypically normal. Carriers of a single defective copy of BRCA1 or BRCA2 would have a higher risk for the induction of mutations and development of secondary tumours when exposed to DNA-damaging agents.

231 POSTER

Quantitative and qualitative analyses of plasma DNA in colorectal cancer patients as prognostic tools

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Background: A high level of cell-free circulating DNA both in plasma and in serum has been reported in several tumoral models at the time of surgery. Starting from this evidence, we would like to verify whether high levels of cell-free DNA in plasma may predict the presence of colorectal cancer.
Material and methods: We analyzed 70 patients with primary colorectal cancer. Plasma samples were obtained at the time of surgery and after 4, 10 and 16 months in patients follow-up. The cell-free circulating DNA in plasma was quantified by the Dipstick method. Tumor and plasma samples were characterized for K-Ras mutations and $p16^{INK4a}$ promoter hypermethylation. Tumor specimens were also investigated for CD31 immunohistochemical staining.
Results: In all patients the cell-free DNA levels in plasma are significantly higher at the time of surgery in comparison with healthy donors (about 25 times higher). In addition, we found that colon cancers release more DNA than tumors with a rectal location and that the levels of cell-free DNA are related to angiogenesis. The CEA value of this cohort of patients was altered in about 40% of cases. Moreover, our data show that cell-free DNA levels decreased 4 months after surgery. Ten and sixteen months after surgical intervention, cell-free DNA plasma quantities decreased progressively in tumor-free patients. By contrast, patients who developed recurrences or metastasis showed a concomitant increasing plasma DNA level. All our data are statistically significant.
Conclusions: Our preliminary data confirm that plasma DNA levels:
i. are significantly higher in all patients with colorectal cancer,
ii. decrease progressively in tumor-free patients,
iii. increase in patients with recurrence of metastasis.
Thus, we suggest that the quantification of plasma cell-free DNA may represent an useful tool for diagnostic and monitoring of colorectal cancer.

232 POSTER

Epidermal growth factor receptor as a predictor of tumor downstaging in locally advanced rectal cancer patients treated with preoperative chemoradiation

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Background: This study examined whether the expression of epidermal growth factor receptor (EGFR) can predict tumor downstaging to preoperative chemoradiation in patients with locally advanced rectal cancer.

Material and methods: Between 1993 and 2002, 183 patients with locally advanced rectal cancer (cT3/T4 or N+) were enrolled in this study. Preoperative chemoradiation consisted of 50.4 Gy of pelvic radiation with concurrent 5-fluorouracil+leucovorin bolus i.v. chemotherapy in 94 patients or oral capecitabine in 89 patients. Surgery was performed 6 weeks after chemoradiation. EGFR expression in the pretreatment paraffin-embedded tumor biopsy specimens was assessed by immunohistochemistry using an EGFR pharmDx kit (DakoCytomation). EGFR expression was determined from the intensity and extent of staining. The staining threshold for a positive result was 1+intensity in 1% of the tumor cells. EGFR immunostaining was graded as a categorical variable using an immunoreactive score (IRS) that ranged from 0 (negative staining) to 7 (strong staining) and was defined as low (IRS 0 to 3) or high (IRS 4 to 7) expression. Tumor downstaging was defined as a reduction in the pretreatment T stage by one level compared with the pathological stage. The predictive value of EGFR expression for tumor downstaging was evaluated using the chi-square test and logistic regression analysis.
Results: The median age of the patients was 59 years, and there were 111 males and 72 females. The preoperative clinical T stage was T3 in 163 patients (89%) and T4 in 20 patients (11%). Tumor downstaging occurred in 97 patients (53%). The tumor showed a pathologic complete response in 27 patients (15%). Positive EGFR expression was observed in 140 of 183 patients (76%). The grade of EGFR expression was low in 113 patients (62%) and high in 70 patients (38%). High EGFR expression was not correlated with gender, age, tumor mobility, tumor size, tumor distance from the anal verge, cT stage, cN stage, or pN stage, but was correlated with pT stage ($p = 0.043$). EGFR expression and age were marginally significant predictive factors for tumor downstaging in the univariate analysis ($p = 0.063$ and 0.081, respectively). In the logistic regression analysis, including the variables EGFR expression, age, tumor mobility, and cN stage, high EGFR expression was the only significant predictive factor for tumor downstaging (hazard ratio 0.515, 95% confidence interval 0.276 to 0.963, $p = 0.038$).
Conclusions: High EGFR expression is a significant predictive molecular marker for tumor downstaging in locally advanced rectal cancer treated with preoperative chemoradiation.

233 POSTER

Prevalence of high-risk lesions in prophylactic mastectomy specimens of 82 BRCA1 and BRCA2 mutation carriers

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Purpose: Women with a hereditary predisposition for breast cancer have a very high risk (up to 85%) of developing invasive breast carcinoma and consider prophylactic mastectomy to avoid this risk. Together with cancer-free survival, the effectiveness of prophylactic mastectomy in *BRCA1* and *BRCA2* carriers may be established by the spectrum of high-risk lesions in their mastectomy specimens. Little is known about differences between early stages of breast cancer development in *BRCA1* and *BRCA2* mutation carriers. It is unknown whether the prevalence of high-risk lesions in *BRCA1* and *BRCA2* mutation carriers is different. There may be differences in breast cancer development in *BRCA1* and *BRCA2* mutation carriers because the features of invasive breast cancer lesions are different.
Patients and methods: A prospective series of 68 *BRCA1*- and 14 *BRCA2*-prophylactic mastectomy specimens was analyzed by radiography and macroscopic inspection of 5 mm tissue slices and histological examination of suspicious lesions and random samples from each quadrant of the breast and the nipple area.
Results: Patient characteristics of the two groups were comparable for age at time of prophylactic mastectomy (36 $\pm$ 9 years), presence of previous breast cancer (35%), age at previous breast cancer (42$\pm$9 years), postmenopausal status (46%) and previous oophorectomy (23%). The earliest age of breast cancer occurrence was significantly younger (35 $\pm$ 9 years) in *BRCA1* than *BRCA2* families (44$\pm$9 years; $p = 0.02$). High-risk lesions are equally frequent among women with a *BRCA1* or a *BRCA2* mutation: all high-risk lesions 44% versus 36% ($p = 0.56$), atypical lobular hyperplasia 26% versus 21% ($p = 0.69$), atypical ductal hyperplasia 18% versus 14% ($p = 0.70$), lobular carcinoma-in-situ (LCIS) 16% versus 7% ($p = 0.38$) and ductal carcinoma-in-situ (DCIS) 9% versus 7% ($p = 0.83$).
Conclusions: The high prevalence of high-risk lesions associated with an increased risk of malignancy, substantiates the generalized nature of incipient malignant changes both in *BRCA1* and *BRCA2* mutation carriers and confirms the indication for prophylactic mastectomy. Surveillance does not detect these high-risk lesions.

234 POSTER

Cytoplasmic p27 kip-1 expression is an indicator of good prognosis in colorectal cancer patients

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Introduction: The p27kip-1 protein inhibits certain cyclin-CDK complexes in the cell nucleus, thereby preventing uncontrolled cellular proliferation. Recent data suggests that cytoplasmic p27kip1 may have an alternative function, inhibiting the activity of cytoplasmic Rho proteins which co-ordinate cytoskeletal remodelling and underlie changes in cell adhesion and migration. Our aim was to evaluate the prognostic significance of cytoplasmic and nuclear p27kip-1 in a large series of colorectal cancer patients.
Methods: Using high-throughput Tissue microarray (TMA) technology, we analysed p27kip-1 cytoplasmic and nuclear expression in a series of over 400 paraffin embedded colorectal tumor specimens. Data derived from this analysis was associated with known patient and tumor variables, and with long-term patient outcome data, in order to gain further insight into the mechanisms by which p27kip-1 may influence tumor development.
Results: 74/418 tumours expressed both cytoplamsic and nuclear p27kip-1 which was not associated with the known clinicopathological variables including tumor stage, tumor grade or the presence of vascular invasion. However, on survival analysis using the Kaplan-Meier method there was a significant correlation between p27kip-1 expression and disease specific survival ($p = 0.037$), with patients whose tumours express both nuclear and cytoplamsic p27kip1 having a good prognosis. In contrast, expression of nuclear p27kip-1 alone was observed in 217/418 (51.9%) tumours, and this did not demonstrate any correlations with clinicopathological variables or survival.
Conclusions: For tumours to metastases, cells must alter their connections to their neighbours and their substrate, and then migrate. Efficient migration requires a tight balance between activation and deactivation of Cdc42, Rac and RhoA in both time and space. Sequestration of RhoA by cytoplasmic p27kip-1 may inhibit migration by preventing cells from achieving sufficiently strong adhesion and traction to move forward. In this study cytoplasmic expression of p27kip-1 was always associated with nuclear expression. These tumours would therefore have controlled proliferation and reduced migration resulting in a less aggressive tumour and a good prognosis. These finds support recent evidence that cytoplamsic p27kip1 expression has an important biological role that can influence tumour outcome.

235 POSTER

Bromodeoxyuridine labelling index as an indicator of tumour response to neoajuvant radiotherapy in patients with rectal cancer

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Background: In clinical practice there are no certain methods to predict tumour response to neoadjuvant radiotherapy (RT). Therefore the aim of the study is an assessment of tumour proliferation rate based on Bromodeoxyuridine labelling index (BrdUrd LI) to predict tumour response to neoadjuvant RT in patients with rectal cancer.
Material and methods: Tumour samples were taken twice from each of 65 patients with rectal carcinoma qualified to neoadjuvant RT: before RT and during surgery. Tumour fragments were incubated with BrdUrd for 1 hour at 37°C, and after fixation and staining the cell preparations were analysed with flow cytometer. The BrdUrd LI was calculated as a percentage of BrdUrd-labelled cells in a sample which incorporated BrdUrd. S-phase fraction (SPF), DNA ploidy, and apoptosis were also evaluated. Patients were treated according to two RT schedules: I, short RT for 5 days with 5 Gy/fraction and surgery about one week after RT, or II schedule: short RT (5×5 Gy) with longer interval, 4–5 weeks before surgery. Tumour response after RT has been evaluated by a patologist on the basis of tumour material taken during surgery.
Results: Thirty-one patients were treated according to schedule I, in which the mean interval before surgery was 8 days (range 2–14). In 34 patients schedule II was applied, in which mean break was 32 days (range 17–45). Mean BrdUrd LI before RT was 7% (range 1.0–24.2%) and the mean value did not differ between the two schedules. After RT, tumours treated according to both schedules showed statistically significant growth inhibition (reduction of BrdUrdLI and percentage of SPF cells) in comparison with the values obtained before RT. Because the interval