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.

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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.