(median, 6 cm). Thirty-seven patients were treated with surgery. Surgical margins were negative in 19 patients, microscopically positive in 9 patients, and grossly positive in 9 patients. One patient received definitive radiation therapy. The Ki-67 expression was positive (>5%) in 9 among 38 cases (24%).
Results: The median follow-up period was 34 months (range, 7−75). Five patients developed local progressions and 9 experienced local recurrences. The 3-year disease-free survival rate and the 3-year progression-free survival rate were 54% and 55%, respectively. Positive Ki-67 expression ($p = 0.036$), tumor size more than 5 cm ($p = 0.021$), debulking surgery ($p = 0.021$), and extra-abdominal location of tumor ($p = 0.004$) were associated with poor disease-free survival with significance.
Conclusions: The current data suggests that patterns of Ki-67 expression are also a prognostic factor in addition to the gross anatomy in the sporadic desmoid tumors. Thus patterns of Ki-67 expression can be used as criteria for adjuvant therapy after surgery.

Table. Prognostic factors for disease-free survival

Variables		No. of patient	3Y DFS[a]	p-value
Location	Extra-abdominal	23	27%	0.004[b]
	Abdominal wall	11	100%	
	Intra-abdominal	4	50%	
Tumor size	<5 cm	10	90%	0.021[c]
	⩾5cm	28	37%	
Surgery	Wide excision	26	67%	0.021[c]
	Debulking	11	33%	
Surgical margin	Negative	19	69%	0.181[c]
	Positive	18	44%	
Ki-67 expression	Negative	29	66%	0.036[c]
	Positive	9	0%	

[a]disease free survival; [b]One-way ANOVA. Extra-abdominal vs abdominal wall; [c]Log-rank test

508 POSTER

Association of genetic polymorphisms with survival in Japanese pancreatic cancer patients treated with gemcitabine

H. Ueno[1], T. Okusaka[1], N. Saijo[2], J. Furuse[3], Y. Sato[4], T. Yoshida[4], E. Sugiyama[5], S. Kim[5], N. Kaniwa[5], J. Sawada[5]. *[1]National Cancer Center Hospital, Hepatobiliary and Pancreatic Oncology Division, Tokyo, Japan; [2]National Cancer Center Hospital East, Deputy Director, Chiba, Japan; [3]National Cancer Center Hospital East, Hepatobiliary and Pancreatic Oncology Division, Chiba, Japan; [4]National Cancer Center Research Institute, Genetics Division, Tokyo, Japan; [5]National Institute of Health Sciences, Project Team for Pharmacogenetics, Tokyo, Japan*

Background: Gemcitabine (Gem) is an anti-cancer drug effective against solid tumors. Nucleoside transporters including SLC29A1, cytidine deaminase (CDA), and deoxycytidine kinase (DCK) are involved in the transportation, detoxication or activation of Gem. We previously reported that a non-synonymous single nucleotide polymorphism (SNP), CDA 208G>A (A70T), affected the pharmacokinetics and toxicity of Gem [1]. Therefore, we investigated the effects of genetic polymorphisms and background factors on survival in pancreatic cancer patients treated with Gem.
Materials and Methods: The study involved 76 Japanese patients with stage IV pancreatic cancer, a subset of the patients reported previously [1], receiving Gem monotherapy and no previous radiation therapy. Plasma CDA activity was measured using Gem or cytidine as a substrate. Polymorphisms of the CDA, DCK and SLC29A1 genes were detected by dideoxy sequencing using genomic DNA obtained from peripheral blood leukocytes. The log-rank test or Cox proportional hazard models were applied for survival analyses. The ethics committees of the National Cancer Center and the National Institute of Health Sciences approved this study, and written informed consent was obtained from each patient.
Results: CDA 208G>A, which was previously reported to lead to reduced Gem clearance [1], was associated with prolonged survival (median for GG and GA; 165 and 606 days, $P = 0.042$), while an intron SNP of CDA, IVS1+37G>A, was associated with reduced survival (median for GG+GA and AA; 178 and 86 days, $P = 0.012$). A non-synonymous SNP of DCK, 364C>T (P122S), showed strong association with reduced survival (survival for CC and CT; 178 and 60 days, $P = 0.0028$). The allele frequency of DCK 364C>T was 0.061 in our study. No genetic polymorphisms of SLC29A1 showed any significant association with survival. Performance status, CA19−9, CRP, and plasma CDA activity also showed significant effects on survival ($P < 0.05$ for all). A multivariate Cox proportional hazard model suggested that CA19−9, CRP, the intron SNP of CDA, and DCK 364C>T are major factors determining the prognosis of pancreatic cancer patients receiving Gem monotherapy.
Conclusions: These observations suggest that genetic polymorphisms involved in the activation and detoxication of Gem, as well as some tumor markers, can be useful indicators of prognosis in patients with pancreatic cancer receiving Gem monotherapy.

References

[1] E. Sugiyama et al., J Clin Oncol 2007; 26: 32−42.

509 POSTER

Epidermal growth factor (EGF) +61 A/G functional genetic polymorphism influences disease-free interval in androgen blockade treated prostate cancer patients

A.L. Teixeira[1], R. Ribeiro[1], D. Cardoso[1], D. Pinto[1], A. Morais[2], F. Lobo[2], F.M. Calais-da-Silva[3], F.E. Calais-da-Silva[3], R. Medeiros[1]. *[1]Portuguese Oncology Institute, Molecular Oncology – CI, Porto, Portugal; [2]Portuguese Oncology Institute, Urology Department, Porto, Portugal; [3]Lisbon Medical Centre (Central Region), Urology Department, Lisbon, Portugal*

Background: Most patients with Prostate Cancer (PCa) will evidence sometime along the course of their disease resistance to androgen-blockade therapy (ABT), emerging an androgen-independent state. EGF activates several intracellular pathways after binding to homo- (EGFR1) or hetero-dimmer receptors (EGFR1-HER2), ultimately leading to proliferation, differentiation and tumorigenesis of epithelial cells.
The EGF-EGFR/HER2 pathway seems to assume special relevance in androgen-independent Prostate Cancer state (AIPCa). EGF role in PCa oncobiology and the high frequency of AIPCa support the rationale for studying its potential as a molecular marker in prognosis and further evaluate pharmacogenomic application in ABT. Recently, a single nucleotide polymorphism (SNP) A/G in +61 locus of EGF gene was described, in which peripheral-blood mononuclear cells from A homozygous carriers expressed significantly less EGF mRNA compared to G allele carriers (AG/GG).
Materials and Methods: In the present study, EGF+61 A/G polymorphism detection was performed through Polymerase Chain Reaction – Restriction Fragment Length Polymorphism procedures in PCa patients submitted to ABT ($N = 124$), and in healthy controls without cancer evidence ($N = 152$).
Results: In the recessive model, genotype frequencies were similar between both groups (PCa group, AA = 27.4%, AG/GG = 72.6%; Control group, AA = 37.5%, AG/GG = 62.5%), without significant risk for being diagnosed with PCa in G-allele carriers (Odds Ratio, $OR = 1.59$, $P = 0.076$). Furthermore, there is an increased risk in AG/GG carriers for being diagnosed with a high grade PCa (Gleason ⩾7), compared to the control group ($OR = 2.49$, $P = 0.008$).
Disease free interval is significantly lower in AG/GG carriers compared with AA (mean±SEM, 32.1±7.1 and 71.3±13.2, respectively, $P = 0.008$). Kaplan Meier survival curves analysis and Log Rank test (Mantel-Cox) further support the influence of EGF+61 A/G polymorphism in disease free interval cumulative probability ($P = 0.018$).
Conclusions: In this sample, G-allele carriers have an increased risk for being diagnosed with high-grade disease and for developing precociously resistance to ABT. The results suggest that this EGF functional polymorphism may contribute to the establishment of a prognostic and predictive molecular profile in AIPCa patients submitted to ABT and support the involvement of EGF in an alternative pathway for tumor progression in androgen-independent prostatic tumors.

510 POSTER

An alternative splice variant of PIK3CD is common in neuroblastoma, colorectal and ovarian cancer

K. Ejeskär, S. Fransson. *Biomedicine, Clinical Genetics, Göteborg, Sweden*

Background: Alterations of the PI3K-Akt pathway in human cancers are very common. PIK3CD encodes p110δ, a catalytic subunit of type I phosphatidyl-inositol-3′ kinase (PI3K). The gene PIK3CD resides in chromosome region 1p36.2, a region commonly deleted in a variety of human cancers indicating that there is a gene in this region with a tumour suppressor function.
Material, methods & results: We have discovered an alternative splicesite in intron 5 of PIK3CD, resulting in an extra 163 bp insertion in the mature mRNA causing a frame shift and an early termination of the protein (302 aa compared to 1045 aa in p110δ). This splice variant encodes a protein that comprises a regulatory p85-binding domain but no catalytic domain. We can by cotransfection show that the protein resulting from the splice variant of PIK3CD colocalises with p85 in aggregates in the cytoplasm, whereas p110δ colocalises with p85 evenly distributed in the cytoplasm.

PIK3CD is strongly expressed in blood and we can show by realtime RT-PCR (TaqMan) and multiplex fragment analysis that this alternative spliced variant comprises on average 45% of all PIK3CD transcripts in this tissue. A panel (20) of normal tissues was tested but no other showed high expression of this alternative PIK3CD. Intriguingly we can show that this alternatively spliced variant of PIK3CD is also common in various human primary tumors commonly displaying 1p-deletions: advanced stage neuroblastoma (56% of all transcripts; 29 stage 4 samples tested); colorectal cancer (48%; 4 samples) and ovarian cancer (86%; 3 samples). By using a TaqMan-assay specifically detecting the alternatively spliced variant of PIK3CD compared to wild-type splicing of intron 5 we have been able to show significant ($p = 0.0001$) higher amounts of alternative spliced product in aggressive neuroblastoma tumors (patient died of disease) (63% splice variant) compared to neuroblastoma tumors from patients that was cured from disease (35%); 72 tumor samples were used in this study.
Conclusions: We speculate that this variant could have a regulatory function in the PI3-pathway, considering that the p85-binding domain is intact, leading to a possible binding of p85 without resulting in a functional PI3-kinase complex. The fact that this alternative product of PIK3CD is common in tumor cells is intriguing, implicating involvement in tumor biology.

511 POSTER

Tissue microarray immunohistochemical profiling of metastatic colorectal cancer

F. Muruzabal[1], V. Gutierrez[1], R. Hernán[1], D. Hernán[1], J. Beaskoetxea[2], N. Telleria[2], L. Mendoza[3], F. Vidal-Vanaclocha[4]. *[1]Dominion Pharmakine, Histology, Bilbao-Vizcaya, Spain; [2]Dominion Pharmakine, Molecular Genetics, Bilbao-Vizcaya, Spain; [3]Dominion Pharmakine, Cell Biology, Bilbao-Vizcaya, Spain; [4]Basque Country University, Sch. Med. & Dent Dpt. Cell Biol. & Histol, Bilbao-Vizcaya, Spain*

Background: The aim of this study was to classify hepatic metastasis of colorectal cancer (HMCRC) based on tumor progression associated proteins assessed by immunohistochemistry (IHC) on Tissue Microarray (TMA).
Materials and Methods: In order to evaluate the expression of different proteins, a TMA block was constructed from 51 HMCRC patient biopsies, including 1-mm diameter tissue cores from each paraffin block. Tumor biopsies were histologically classified into encapsulated (presenting a fibrotic matrix between liver tissue and the tumor front) or invasive (with tumor cells penetrating the hepatic sinusoids). IHC on TMA slides was applied to analyse proliferation (ErbB2 and ki-67), angiogenesis (CD31 and a-actin), inflammation (IL-18Ra and VEGFR2), epithelial origin (CEA and EpCam), adhesion (CDH-1) and fibrosis (COL1A) markers. To evaluate their expression level arbitrary values between 0 and 3 were used: 0, non stained; 1. weak non-homogeneous staining; 2. weak homogeneous staining; 3. strong staining. Statistical analysis was based on non-parametric tests (Wilcoxon Signed Ranks Test, Kruskal Wallis, Mann-Whitney Test and Spearman Test), using SPSS v13 and a significance level of $p < 0.05$.
Results: In liver metastasis biopsy pairs including the central region of the metastatic tumor and its corresponding invasive front, ErbB2 and CDH-1 were found to be overexpressed in the invasive front, while VEGFR2, was only overexpressed in the central region of the metastasis. The morphological features studied showed a strong correlation with some proteins, such as the overexpression of VEGFR2 and IL18Ra in the invasive metastasis and the overexpression of CEA and CDH-1 in encapsulated tumors. Statistical analysis revealed a positive correlation of Ki-67 with S100A6 and SMA with CD31. Inversely, an opposite correlation was observed between IL18Ra and COL1A1 positivity. Finally, a strong positive correlation was observed for the staining intensity of ErbB2 and CDH-1 in colorectal cancer liver metastasis, being both negatively correlate to CD31 staining level.
Conclusions: This study shows that IHC-TMA can be reliably used to analyse multiple markers in large patient sets simultaneously. Protein expression level and distribution is related to tumor morphological features and microenviromental factors. To confirm the prognostic value of the described observations, clinical data regarding local recurrences and overall survival are being currently collected.

512 POSTER

Molecular profile of acquired docetaxel resistance in breast cancer cells

I. Brown, S.D. Heys, A.C. Schofield. *University of Aberdeen, Surgery, Aberdeen, United Kingdom*

Introduction: Docetaxel is one of the most active agents used in the treatment of breast cancer. However, tumours may be resistant, or develop resistance, to docetaxel during treatment. The mechanisms of resistance to docetaxel, whether innate or acquired, are poorly understood. The purpose of this study was to investigate the genetic pathways involved in docetaxel resistance using a unique model of docetaxel resistance, which we have developed in breast cancer cells.
Methods: We made two human breast cancer cell lines, MCF-7 and MDA-MB-231, resistant to docetaxel by exposure to increasing docetaxel concentrations. The resultant sublines were able to withstand 30 μM of docetaxel. Alterations of gene expression were determined using Affymetrix Genechip cDNA microarrays, and subsequently validated by RT-PCR and western analysis.
Results: After firstly selecting out gene changes that were common between both sets of sensitive cell lines and their resistant sublines (>2 fold), further normalisation and statistical filtering ($p < 0.01$) identified 124 probe-sets that were commonly changed in both resistant cell lines. Further statistical analyses were carried out on the gene list using ANOVA (assuming unequal variances) and the Benjamin-Hobbson false discovery rate was applied as a multiple correction factor with a significance level of $p < 0.01$. This identified a 14 probe-set, encoding 10 genes (including p-glycoprotein), which were significantly associated with resistance to docetaxel. These genes are currently being validated at the mRNA and protein level.
Conclusions: These changes, therefore, may represent common mechanisms of resistance in breast cancer cells. In addition, this is the first description, using microarray analysis, to identify the genetic pathways involved in the evolution of acquired resistance to docetaxel in a cell line model of resistance.

513 POSTER

Characteristic and outcome of young breast cancer patients with and without BRCA1 mutations

D. Gabrys[1], K. Behrendt[2], E. Grzybowska[3], R. Suwinski[1], A. Idasiak[1], P. Wojcieszek[1], K. Galwas[1], W. Pekala[3], J. Pamula-Pilat[3], H. Thames[4]. *[1]Center of Oncology Maria Sklodowska-Curie Memorial, Department of Radiation Oncology, Gliwice, Poland; [2]Center of Oncology Maria Sklodowska-Curie Memorial, Clinic of Radiation Ocology, Gliwice, Poland; [3]Center of Oncology Maria Sklodowska-Curie Memorial, Department of Tumor Biology, Gliwice, Poland; [4]The University of Texas M.D. Anderson Cancer Center, Department of Biostatistics and Applied Mathematics, Houston, USA*

Purpose: To investigate the clinical characteristic and outcomes of younger (<50 years old) breast cancer patients with BRCA1 mutation in comparison to patients without this germline mutation.
Methods and Materials: This is an ongoing study and patients will be enrolled till end of 2008. Till now we followed 480 breast cancer patients who were diagnosed before age 50 and were asked to provide a blood sample for BRCA1 mutation screening (5382insC, 300T/G, 185delAG, and 4153delA). We compared contralateral breast cancer and ovarian cancer incidence, disease free, metastases free, and overall survival, between BRCA1 mutation carriers and non-carriers.
Results: BRCA1 mutations were detected in 74 breast cancer patients; the remaining 406 women did not carry the mutation. BRCA1 related tumours showed higher grade, more frequent negative oestrogen, progesterone, HER2 receptor status. Patients with BRCA1 mutation had a higher incidence of bilateral breast and ovarian cancer. Multivariate Cox analysis for DFS (local-regional and distant failure) showed that node ratio >13%, tumour diameter, age >44 years and BRCA1 mutation negative patients significantly decreased DFS.
Conclusions: This data suggest that BRCA1 mutation carriers have better DFS and MFS compared to sporadic tumours. There is variability within BRCA1 mutation carriers with respect to lymph nodes metastases, DFS, and distant metastases.

514 POSTER

Immunodetection and cytogenetic characterization of disseminated tumor cells applied to the clinical management of patients with solid tumors

A.B. de la Hoz[1], N. Telleria[1], O. Crende[2], J. Tomé[1], L. Mendoza[1], F. Vidal-Vanaclocha[2]. *[1]Dominion Pharmakine, Molecular Genetics, Bilbao-Vizcaya, Spain; [2]Basque Country Univ. Sch. Med. and Dent., Dpt. Cell Biol. and Histol., Bilbao-Vizcaya, Spain*

Aim: The aim of the present study was to show that detection and characterization disseminated tumor cells in peripheral blood can be applied to the clinical management of patients with solid carcinomas.
Methods: A double gradient of Ficoll allowed the separation of mononuclear cells, granulocytes and epithelial origin cells from total peripheral blood. Then, for the inmunomagnetic selection of the epithelial cells