magnetic particles-labeled with antibodies against epithelial markers (cytokeratin 7/8 or EpCAM) were used. Finally the disseminated tumor cells are detected using anti-cytokeratin 7/8/18/19 antibodies followed by microscopic visualization. Once detected, cells were citogenetically characterized by means of FISH, to detect described chromosomic aberrations for each tumor type using commercially avilable kits (Vysis®).
Results: The described methodology was able to detect epithelial cells disseminated in the blood of aproximately 50% of the patients, with cell numbers ranging from 1 to 16 cells, in 6−10ml of peripheral blood, and with an exceptional case of 100 disseminated cells in a prostate cancer patient. The neoplasic nature of the identified cells was verified through cytogenetic characterization, evidencing that both metastatic colorectal carcinoma and bone metastatic breast carcinoma cells show amplification of the ZNF217 (20q13) gene, which is implicated in the development and progression of the cancer. For disseminated prostate carcinoma cells ProVysión panel containing probes for LPL (8p22) and c-myc (8q24), demonstrated that these genes' loss and gain respectively characterised these patients' tumors. In patients bearing bone metastatic from lung cancer, LaVysion kit identified in the disseminated tumor cells amplifications in c-myc (8q24), and EGFR (7p12), whose amplification predicts good responses to Gefitinib (Iressa) or Erlotinib (Tarceva), and the 5p15 region. Finally, UroVysion kit, for the detection of aneuploidies in chromosomes 3, 7, and 17 and loss of the 9p21in disseminated urothelial carcinoma of the bladder cancer in blood.
Conclusions: The optimised methodology allows the detection and phenotipical and genotipical characterization of disseminated tumor cells in routine peripheral blood samples, offering additional information to empirically evaluate clinical prognosis and select the most efficient chemotherapy treatment, and providing a new tool for the post-surgical monitorisation of patients with solid tumors.

515 POSTER

Zoledronic Acid (ZOL) treatment may improve survival in patients with lung cancer and high baseline N-telopeptide levels: a multivariate Cox regression analysis

P. Major[1], V. Hirsh[2], A. Lipton[3], R.J. Cook[4], R.E. Coleman[5]. *[1]Juravinski Cancer Centre, Oncology, Hamilton Ontario, Canada; [2]McGill University Health Centre, Medicine and Oncology, Montreal Quebec, Canada; [3]M.S. Hershey Medical Center, Oncology, Hershey Pennsylvania, USA; [4]University of Waterloo, Statistics and Actuarial Science, Waterloo Ontario, Canada; [5]University of Sheffield Weston Park Hospital Cancer Research Centre, Statistics and Actuarial Science, Sheffield England, United Kingdom*

Introduction: In recent analyses, high N-telopeptide (NTX) levels were reported to be an indicator of poor prognosis in patients (pts) with bone metastases. ZOL can reduce NTX levels in this setting, and exploratory analyses have suggested that ZOL treatment correlates with improved survival in pts with NSCLC and high baseline NTX. Therefore, we conducted a multivariate analysis of baseline variables and treatment to examine their correlation with survival outcomes in pts with NSCLC and high baseline NTX levels in a placebo (PLA)-controlled, randomized clinical trial of ZOL.
Material and Methods: Pts with solid tumors and bone metastases were randomized to either ZOL or PLA for up to 21 months. Survival was assessed in the subset of NSCLC pts who had high baseline NTX levels (⩾64 nmol/mmol). The effects of >20 baseline variables and treatment group on survival were evaluated in univariate and multivariate Cox regression analyses, and significant covariates ($P < 0.05$) were included in a reduced model. The relative risk (RR) of death and associated 95% confidence interval (CI) were calculated for each.

Variable	RR	95% CI	P
ZOL vs PLA	0.565	0.381, 0.840	0.0047
Narcotics (Y/N)	1.757	1.110, 2.780	0.0161
Impaired PS (Y/N)	1.941	1.158, 3.255	0.0119
[Leu] (% incr v med)	0.977	0.960, 0.995	0.0112

Results: Among NSCLC pts, the association between ZOL treatment and survival was significantly different for pts with high v low NTX ($P = 0.020$), with RR = 1.34 ($P = 0.205$) and RR = 0.67 ($P = 0.034$) for the normal and high NTX pts, respectively. Pts with NSCLC and high baseline NTX levels (n = 144; 65% men, 35% women) had a median age of 64 yrs, ~76% had experienced ?1 SRE, ~80% required narcotic medication, 15% had some impairment of performance status (PS) and most pts had lymphopenia (median, 14% lymphocytes [Leu]). Among these pts, variables that correlated significantly with survival outcomes included treatment group, FACT-G score, race, narcotic use, PS, and [Leu]. After a full multivariate analysis, 4 significant covariates emerged for the reduced model (Table).
Conclusions: This multivariate analysis determined the following variables to independently correlate with improved survival in NSCLC pts with high baseline NTX: no narcotic use, no impairment of PS, higher lymphocyte count, and ZOL treatment. This retrospective analysis suggests that ZOL treatment is an independent variable for improved survival compared with PLA in pts with NSCLC and high NTX levels, warranting further study in prospective trials.

516 POSTER

Cap43/NDRG1 is a molecular marker of angiogenesis and prognosis in cervical adenocarcinoma

S. Nishio[1], K. Ushijima[1], N. Nishida[1], T. Yamaguchi[2], H. Tsuda[3], T. Kasamatsu[4], Y. Sasajima[5], M. Kage[2], M. Kuwano[6], T. Kamura[1]. *[1]Kurume University School of Medicine, Obstetrics and Gynecology, Kurume, Japan; [2]Kurume University Hospital, Pathology, Kurume, Japan; [3]National Cancer Center Hospital, Basic Pathology, Tokorozawa, Japan; [4]National Cancer Center Hospital, Gynecologic Oncology, Tokyo, Japan; [5]National Cancer Center Hospital, Diagnostic Pathology, Tokyo, Japan; [6]Kurume University, Research Center of Innovative Cancer Therapy, Kurume, Japan*

Background: Cap43/NDRG1 is a nickel- and calcium-inducible gene that has been recognized to play a significant role in metastasis and invasion, as well as in the primary growth of malignant tumors, possibly through its ability to induce differentiation. The majority of studies until now have suggested a negative correlation between Cap43/NDRG1 expression and cancer progression. However, this plausible role of Cap43/NDRG1 in preventing cancer progression has been shown to depend on the tissue of origin and the tumor type. The aim of this study was to investigate the association between Cap43/NDRG1 expression and angiogenesis (microvessel density) and other clinicopathological factor in cervical adenocarcinoma.
Methods: A retrospective review was conducted of the records of 100 women with FIGO clinical stage I-II cervical adenocarcinoma who underwent surgery. We evaluated Cap43/NDRG1 and CD34 expression in the resected specimens by immunohistochemistry.
Results: A significant association was found between the expression level of Cap43/NDRG1 in the tumor specimen and the microvessel density, histologic grade of the tumor, tumor diameter, stromal invasion, lymph vascular space invasion and lymph node metastasis. Kaplan-Meier plots demonstrated a clear influence Cap43/NDRG1 expression on the survival time. The median overall survival time was 54.1 months in patients with tumors showing low Cap43/NDRG1 expression, as compared with only 36.4 months in patients with tumors showing high Cap43/NDRG1 expression (log-rank test; $p = 0.0018$).
Conclusions: These results suggest that increased expression of Cap43/NDRG1 may be associated with angiogenesis and might be a poor prognostic factor in patients with cervical adenocarcinoma.

517 POSTER

Comparison of allelic polymorphisms of insulin receptor substrate-1 and leptin receptor in breast and endometrial carcinomas

J. Ulybina[1], D. Vasilyev[2], E. Imyanitov[1], L. Berstein[2]. *[1]NN Petrov Institute of Oncology, Lab. of Molecular Oncology, St-Petersburg, Russian Federation; [2]NN Petrov Institute of Oncology, Lab. of Oncoendocrinology, St-Petersburg, Russian Federation*

Background: Obesity and diabetes mellitus are among cornerstone endocrine risk factors for several malignancies. These two pathologies, however, are unequally associated with endometrial cancer (EC) and breast cancer (BC), with the prevalence of former in obese and diabetic population (Calle et al., 2003). The cause for such difference is not currently known, and it seems reasonable to assume that the explanation is in polygenic nature of the two malignancies. The aim of the present study was to evaluate the distribution of polymorphic genetic variants of insulin receptor substrate-1 (IRS1 Gly972Arg) and leptin receptor (LepR Lys109Arg and Gln223Arg) in BC patients in comparison to EC patients. Polymorphisms mentioned above are considered to be associated with higher incidence of diabetes or with excessive body weight (Salopuro et al., 2005) as well as with risk of BC (Slattery et al., 2006; Snoussi et al., 2006); similar investigations in relation to EC have not been performed.
Methods: The study included 407 females (average age around 60 years): 105 healthy women, 192 patients with EC and 110 with BC. Additionally, we included a separate group of those who underwent glucose oral loading test (n = 80) in the study. Genomic DNA was extracted from peripheral blood leukocytes. Genotyping of IRS1 and LepR polymorphisms was performed by allele-specific real time PCR.

Results: The overall IRS-1 genotype distribution was comparable within healthy women population, EC and BC patients. The frequency of the rare Arg972 IRS-1 variant was not significantly increased in EC [0.05] and BC [0.04] groups compared with healthy females [0.03]. LepR allele frequencies did not show differences between EC and BC patients either. It is interesting to mention, however, that in BC cases the frequency of LepR Gln/Arg223 genotype was higher [0.63] and Gln/Gln223 genotype lower [0.21] than in healthy females [0.49, $p < 0.05$] and [0.34, $p < 0.05$] respectively. We demonstrated the tendency to more frequent Arg972 IRS-1 allele in individuals with glucose intolerance. LepR genotypes distribution was not associated with glucose tolerance state or ROS-inducing glucose effect.
Conclusion: An inclination of EC patients to higher than in BC incidence of excessive weight and diabetes can not be explained by differences in distribution of the studied polymorphic variants. Further investigations are warranted, including the analysis of polymorphisms related simultaneously to mitochondrial status and lipid and carbohydrate metabolism.

518 POSTER

Whole genome-wide screening of cervical lymph node metastasis-associated genetic alterations in oral squamous cell carcinoma of Japanese patients

K. Sugahara[1], Y. Michikawa[2], K. Ishikawa[2], Y. Otsuka[2], M. Iwakawa[2], T. Shibahara[1], T. Imai[2]. *[1]Tokyo Dental College, Department of Oral and Maxillofacial Surgery, Chiba, Japan; [2]National Institute of Radiological Sciences, Radgenomics Project Research Center for Charged Particle Therapy, Chiba, Japan*

Background: Despite recent improvements in diagnostic and therapeutic technologies, prognosis of oral squamous cell carcinoma (OSCC) has remained dismal, as more than 50% of patients die within 5 years. Cervical lymph node metastasis (LNM) has been reported strong correlation with poor prognosis. In this study, array-based comparative genomic hybridization (CGH) with individual gene-level resolution has been carried out to precisely identify biomarkers that reflect occurrence of cervical LNM in OSCC patients.
Materials and Methods: A total of 54 patients with OSCC were included in the present study. Surgical resection of tumors from all patients has been done at the Hospital of Tokyo Dental College, Japan, between July 1999 and September 2006. Cervical LNM was confirmed by histopathological examination of resected neck tissues. Informed consent to participate in the study, which was approved by the Ethical Committees of Tokyo Dental College and of National Institute of Radiological Sciences, Japan, was obtained from each patient before surgical resection. Array-based CGH (Agilent Human Genome 44B Microarray) was carried out using primary tumor DNA from 10 each of OSCC patient with or without cervical LNM. Real-time quantitative PCR (QPCR) of selected gene loci was carried out to further investigate rest of samples.
Results: Gain at 11q13 region was the only chromosomal abnormality that reached frequency of 30% exclusively in the cervical LNM present patient group revealed by array-based CGH. Abnormality of individual genes located in this region was further investigated using the rest of samples by real-time QPCR. Two-tailed unpaired Student's t-test was applied to the analysis and it was revealed that CCND1 and FADD to be the two most strongly associated genes to cervical LNM with p-values 0.0029 and 0.0032, respectively. Area under the receiver-operating characteristic curve was then calculated to evaluate specificity and sensitivity as predictive markers. FADD was revealed to have higher score of 0.80 than CCND1 with a score of 0.70. Cervical LNM-free survival plotted by Kaplan-Meier method further confirmed superior distinguishment of patients by FADD (log rank test p-value: 0.0044) than by CCND1 (log rank test p-value: 0.2580).
Conclusions: FADD in 11q13 was revealed to be the most reliable predictive marker for the studied population. Further study with larger patient number should be conducted to validate this result.

519 POSTER

Differences in epigenetic silencing of 9p21 locus tumour suppressor genes CDKN2A/p14(ARF)/CDKN2B in HPV16 positive and negative HNSCCs

A. Baez, J. Clavell, A. Pons. *UPR School of Medicine, Otolaryngology Department, San Juan, Puerto Rico*

Extensive hypermethylation and consecutive transcriptional silencing of tumor suppressor genes have been documented in multiple types of tumors including head and neck squamous cell carcinomas (HNSCCs). The aim of this study was to determine the correlation between methylation status of multiple tumor suppressor genes, p16(INK4A), p14(ARF), p15 (INK4B) in a HNSCCs and paired serum DNA and clinicopathological parameters. We, therefore, investigated CpG island methylation of p16(INK4A), p14(ARF), p15 (INK4B) in a series of 50 pairs of primary HNSCCs and on healthy tissue to assess specificity of aberrant methylation. The samples were tested by methylation specific PCR (MSP) digested with restriction enzymes that distinguish the two species and resolved using gel electrophoresis. Gene expression was detected with real time RT-PCR while presence of p16(INK4A) gene in serum was detected using real-time PCR. Of the 50 HNSCCs examined, 34 (68%) tumors showed aberrant methylation at least on one of the genes tested. Methylation frequencies varied from 4% for p14(ARF), 50% for p16(INK4A), and 26% for p15(INK4B). Twenty-one (42%) of these HNSCCs samples were HPV-positive and 29 (58%) were HPV-negative. The frequency of methylation of the promoters was significantly different between HPV-positive and HPV-negative tumors ($p = 0.029$), being less frequent in HPV-positive HNSCCs. In addition, there was concordance between DNA methylation in tumor and paired serum DNA for p16(INK4A). Aberrant methylation of p16(INK4A), is common gene silencing mechanism in HNSCC. However, aberrant methylation of p15 (INK4B) appears to be important in the sample tested. No association between p16(INK4A), p14(ARF), p15 (INK4B) methylation and conventional clinicopathological factors was noted in this cohort. In summary, we have identified a set of aberrant methylation signatures of the 9p21 locus tumor suppressor genes CDKN2A/p14(ARF)/CDKN2B may be useful as tumor markers for the early identification of HNSCC patients.

520 POSTER

Polymorphisms in glutathione S-transferase genes and outcome of cisplatin-based chemotherapy in ovarian cancer

A.A. Moisseev[1], A.V. Khrunin[2], N.A. Pirogova[3], V.A. Gorbounova[1], S.A. Limborska[2]. *[1]N.N. Blokhin Cancer Research Centre, Chemotherapy, Moscow, Russian Federation; [2]Institute of Molecular Genetics RAS, Human Molecular Genetics, Moscow, Russian Federation; [3]N.N. Blokhin Cancer Research Centre, Statistics, Moscow, Russian Federation*

Background: Glutathione S-transferases (GST) are presumed to play an important role in cellular response to platinum drugs. Several GST genes are subjected to common polymorphisms, which can influence the outcome of anticancer chemotherapy. We evaluated prospectively the polymorphisms in GST genes among women with ovarian cancer and correlated the genetic data with efficacy and toxicity of cisplatin-based chemotherapy.
Materials and Methods: 80 women with epithelial ovarian cancer entered the study, 77 of them were available for efficacy and toxicity analysis. Before treatment initiation, patient's DNA was isolated from whole blood and tested for deletion (GSTM1, GSTT1) and single nucleotide (GSTA1 (−69 C/T), GSTP1 ($Ile^{105}Val$ and $Ala^{114}Val$) gene polymorphisms. GSTM1 and GSTT1 genotypes were determined by multiplex PCR; genotypes for GSTA1 and GSTP1 were assessed with PCR-RFLP. Chemotherapy consisted of cisplatin 100 mg/m^2 and cyclophosphamide 600 mg/m^2 every 3 weeks for 6 cycles.
Results: GSTP1 $Ile^{105}Val$ polymorphism greatly affected treatment outcome: women with Ile/Ile genotype enjoyed prolonged progression-free survival (PFS) compared with carriers of Val allele (Ile/Val and Val/Val; log rank test, $p = 0.0026$), 2-year PFS was 77% and 35%, respectively ($p < 0.05$). Median overall survival was reached in neither group, but a trend favored Ile/Ile carriers. Val/Val carriers appeared to have higher rates of clinically significant ototoxicity: 3 of 7 (43%) compared with 18% in women with other genotypes, although this association didn't reach statistical significance. Other polymorphisms didn't seem to correlate with any parameter of efficacy or toxicity.
Conclusion: Polymorphism of GSTP1 and possibly other genes may emerge as important prognostic and predictive factor in ovarian cancer chemotherapy. More studies are needed to define the role of pharmacogenomic analysis in clinical practice.

521 POSTER

The polysomal RNA from ovarian cancers can stimulate tumour antigen-specific immunity

N. Tsuda, K. Kawano, K. Ushijima, T. Kamura. *Kurume University School of Medicine, Obstetrics and Gynecology, Kurume, Japan*

Objective: The objectives of this study is to investigate whether polysomal RNA can induce tumor specific immunity to ovarian cancer cells.
Materials and Methods: We lysed the human ovarian cancer cell (SKOV3) and fractionated into 16 samples by sucrose gradient. The heavier fractions were considered as polysomal RNA which contained mRNA, ribosome RNAs, and translating nascent polypeptides. To identify which type of RNA has the strongest ability to induce cytocytic cells, we stimulated HLA-A2 positive healthy donor peripheral blood mononuclear cells (PBMC) with autologous immature Dendritic cells (iDC) pulsed with 1 μg (/10^5 dendritic