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

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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

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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

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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

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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

cells) polysomal RNA, total RNA and mRNA eluted from SKOV3 cells. We performed flow cytometry and Cr-release assay to check the peptide specificity and the cytotoxity against SKOV3-A2 cells. Western blot analysis was performed to check HER-2/neu and Heat shock protein70 (HSP70) expressions in these fractions.
Results: Polysomal RNA, as particulate Ag is phagocytosed by iDC. Polysomal RNA together with its bound proteins presented by iDC activate tumor cytolytic cells, which include CD8+ T cells. At equal amount of RNA, polysomal RNA fractions are more immunogenic than total RNA and mRNA. We found nascent HER-2 polypeptide from polysomal RNA of ovarian cancer cells expressing the indicator CTL epitope of HER2/neu (E75) to be immunogenic for functional CTL expansion and differentiation. It has been proposed that chemotherapy activates tumor antigen (TA)-specific immunity. The molecular composition of the immune activators from ovarian cancers, and their modifications by chemotherapy are unknown. Paclitaxel at sub-pharmacological doses induces formation of larger amounts of polysomal RNA. We show that paclitaxel induced polysomal RNA, chaperoned by heat shock proteins, activate immune cells such as CD8+ cells which respond by expressing perforin, IFN-gamma, and mediating lysis of tumor cells. The polysomal RNA is taken-up through scavenger receptors (CD91/Lox-1) and efficiently stimulate immature iDC to secrete IL-12.
Conclusion: These results demonstrate that polysomal RNA from ovarian cancers can induce tumor antigen-specific immunity. And Paclitaxel can intensify this CTL induction.

522 POSTER

Immunoglobulin G fragment C receptor polymorphisms and clinical outcome of EGFR-expressing metastatic colorectal cancer patients treated with cetuximab-based therapy

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Background: FcγR polymorphisms have been shown to predict clinical activity of monoclonal antibody rituximab in patients with follicular lymphoma and trastuzumab in metastatic HER-2 positive breast cancer patients. Cetuximab is a chimeric monoclonal IgG1 with demonstrated activity in metastatic colorectal cancer. In this study we tested wheter FcγR polymorphisms are associated with clinical outcome of colorectal cancer patients who received cetuximab.
Patients and Methods: Forty-five consecutive EGFR-positive advanced colorectal cancer patients receiving cetuximab-based therapy (irinotecan or oxaliplatin) were examined for the FcγR IIIa 158 valine (V)/phenylalanine (F), FcγR IIa 131 histidine (H)/arginine (R) and FcγR IIb 232 isoleucine (I)/threonine (T) polymorphisms. FcγR IIIa, FcγR IIa and FcγR IIb genotyping was performed by polymerase chain reaction followed by direct sequencing of the region of interest. Genomic DNA was used for FcγR IIa, FcγR IIIa genotyping, while FcγR IIb polymorphisms were detected on cDNA.
Results: Cetuximab and irinotecan was administered as second-line in 4 patients (9%) and after $\geqslant$3 lines of chemotherapy in 37 patients (82%). Four patients (9%) were treated with cetuximab and oxaliplatin. Our population was in Hardy-Weinberg equilibrium for the three polymorphisms. The FcγRIIIa 158 V/V genotype was associated with a higher clinical benefit rate (RR + SD; 60% v 40%) even though without statistical significance ($P = 0.46$). FcγR polymorphisms did not affect progression free survival of study cohort.
Conclusions: Despite previous data on other monoclonal antibodies, in our study the hypothetical role of FcγR polymorphisms on cetuximab activity was not demonstrated. Unknown clinical and/or molecular variables may have influenced our results. The analysis is ongoing and updated findings on an expanded number of cases will be presented.

523 POSTER

Understanding the role nitric oxide plays in tumour formation and progression

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Background: Previous work has shown that nitric oxide (NO) – a nitrogen-based free radical found within the human body – is overproduced in many human tumors. NO is produced by Nitric Oxide Synthase (NOS). Cells combat the exposure to NO by producing Glutathione-S-Transferase-pi (GST-pi), a protective mechanism. Herein, we assess the DNA damage caused by NO in cells exhibiting increased GST-pi expression.
Materials and Methods: In this study we utilized five head and neck squamous cell carcinoma cell lines that were previously adapted to high concentrations of NO by gradually introducing a nitric oxide donor into the cell culture media. The amounts of DNA damage in these adapted cell lines and their corresponding parent cell lines (cells grown in medium without nitric oxide) were determined using COMET assays. The lengths of the COMET tails were measured and compared between the NO-adapted cells and the parent cells, with the length of the COMET tail corresponding to the amount of DNA damage (i.e., increasing tail lengths correspond to greater amounts of DNA damage). Immunohistochemistry was also used to measure the expression levels of APE-1, a DNA repair enzyme.
Results: The high-NO adapted cells were found to have shorter COMET tails than their corresponding parent cells in four of the five cell lines studied. Higher levels of APE-1 expression were also found in four of the five NO-adapted cell lines.
Conclusions: The COMET assay results suggest that the elevated nitric oxide levels of the NO-adapted cells result in increased protective mechanisms that spare DNA from damage, and the immunohistochemistry findings suggest that the upregulation of APE-1 is one such mechanism that NO-adapted cells use to protect their DNA under these harsh conditions. Therefore clinically, tumors that express high levels of NOS, GST-pi, and APE-1 would be predicted to have a more aggressive behavior.

524 POSTER

UGT1A1*28 polymorphism is not associated to irinotecan-induced toxicity in pediatric patients

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Background: Hepatic uridine diphosphate glucuronosyltransferase isoform 1A1 (UGT1A1) catalyzes the glucuronidation of bilirubin and that of SN-38, the active metabolite of irinotecan. More than 50 genetic variations in the promoter and coding regions of UGT1A1 gene have been described. UGT1A1*28 polymorphism, characterized by an additional TA repeat $[A(TA)_7TAA]$ in the TATA sequence of UGT1A1 promoter, has been associated with reduced glucuronidation of SN-38 compared with the wild-type $[A(TA)_6TAA]$. Conflictive results have been reported on the association between this genotype and irinotecan toxicity, namely myelosupression and gastrointestinal toxicity. Moreover, little information exists on this association in pediatric patients. We analyzed the association between polymorphic variants in the TATA sequence of UGT1A1 promoter and the incidence of adverse events related to irinotecan in pediatric patients.
Patients and Methods: This is a retrospective analysis of 32 pediatric patients including 21 with central nervous system tumors (CNS), 4 Ewing's sarcomas, 6 rhabdomyosarcomas, and 1 neuroblastoma. Weekly irinotecan (50 mg/m^2) and cisplatin (30 mg/m^2) for CNS tumors or irinotecan (10 mg/m^2 [qdx5]x2) and oral temozolamide (100 mg/m^2 dailyx5) for systemic tumors was used. Patients were evaluated for gastrointestinal and hematological toxicity. UGT1A1*28 promoter polymorphism was genotyped by PCR and fragment size analysis. 74 children without oncological, neurological, metabolic or gastrointestinal diseases were analyzed as control population.
Results: Overall, genotype frequencies were 50% TA6/TA6, 37.7% TA6/TA7, 10.3% TA7/TA7, 0.9% TA6/TA8 and 0.9% TA5/TA7. Fifteen of the 32 patients (46.8%) displayed the TA6/TA6 genotype, 15 (46.8%) TA6/TA7, 1 (3%) TA7/TA7 and one patient had a TA8/TA6 variant (3%). The 2 patients with UGT1A1*28 polymorphism did not developed relevant irinotecan-related toxicity and did not had a greater baseline total bilirubin. The only one patient who developed irinotecan related life-threatening gastrointestinal toxicity displayed a TA6/TA7 genotype, had an underlying liver dysfunction and baseline bilirubin of 1.7. Other mutations and SNPs in the promoter and coding regions were analyzed in this particular case with negative results.
Conclusions: Severe toxicity was not increased in pediatric patients with UGT1A1*28 polymorphisms receiving low and repeated doses of irinotecan. Underlying liver disease is more important to predict irinotecan-toxicity in this particular set of patients.