BFB model for amplification in breast cancer. Fine mapping of borders of amplified regions, which including her2/neu, will open new goals for target therapy of advanced beast cancer.

546 POSTER

Prognostic value of CCN3 in osteosarcoma and Ewing's sarcoma

K. Scotlandi[1], B. Perbal[1], M. Zuntini[1], D. Zambelli[1], F. Nardi[1], N. Lazar[2], P. Bacchini[3], S. Navarro[4], A. Llombart-Bosch[4], P. Picci[1]. *[1]Istituto Ortopedico Rizzoli, Lab. Ricerca Oncologica, Bologna, Italy; [2]Université Paris 7 – D. Diderot, Laboratoire d'Oncologie Virale et Moléculaire UFR de Biochimie, Paris, France; [3]Istituto Ortopedico Rizzoli, Servizio Anatomia Patologica, Bologna, Italy; [4]Fundación Instituto Valenciano de Oncología, Unit of Molecular Biology, Valencia, Spain*

Patients with osteosarcoma or Ewing's sarcoma, the two most common bone tumor, still suffer from the paucity of prognostic markers that could distinguish patients before therapy and drive treatment choices. Herein, we assessed the prognostic value of CCN1−3 genes, a group of genes involved in fundamental biological processes as well as in mesenchymal differentiation. Expression of CCN1−3 was detected by means of quantitative PCR in a series of newly diagnosed osteosarcoma or Ewing's sarcoma. In osteosarcoma, CCN1 and CCN2 expression was found statistically associated with genes involved in the commitment of mesenchymal stem cells toward osteoblasts and in the early phases of osteoblastic differentiation (RUNX family genes; cadherin 4, 11, and 13; jun and fos; collagen I and SPARC). CCN3 is highly expressed in osteosarcoma and its level of expression did not correlate with any specific osteoblastic differentiating genes. While neither differentiation genes nor CCN1 and 2 expression were statistically associated with survival, high expression of CCN3 significantly correlated with worse prognosis in osteosarcoma. CCN3 overexpression also showed a prognostic adverse relevance also in Ewing's sarcoma, either at gene and protein levels. Therefore, assessment of CCN3 expression levels at diagnosis may represent a useful molecular tool to early identify subgroups of patients with different prognosis either in osteosarcoma and in Ewing's sarcoma.

This work was supported by the European Commission (grant number: PROTHETS LSHC-CT-2004–50303306) and by the Italian Association for Cancer Research (AIRC grant to Katia Scotlandi).

547 POSTER

Prognostic significance of p53 and Ki67 in Ewing's sarcoma tumours

A. Llombart-Bosch[1], S. Navarro[2], P. Bacchini[3], F. Bertoni[3], J.A. Lopez-Guerrero[4], I. Machado[2], N. Savelov[5], A. Ruíz[2]. *[1]Fundación Instituto Valenciano de Oncología, Department of Pathology University of Valencia, Valencia, Spain; [2]University of Valencia, Department of Pathology University of Valencia, Valencia, Spain; [3]Istituti Ortopedici Rizzoli, Department of Pathology Istituti Ortopedici Rizzoli, Bologna, Italy; [4]Fundación Instituto Valenciano de Oncología, Unit of Molecular Biology, Valencia, Spain; [5]Blockin Cancer Center, Department of Pathology Blockin Cancer Center, Moscow, Russian Federation*

Background: Ewing's sarcoma (ES) is a heterogeneous neoplasm in which several genetic alterations involving cell cycle regulators have been described. Around 10% of ES suffer p53 alterations, which may be demonstrated by immunohistochemistry and/or mutational analysis. Many studies have demonstrated the prognostic significance of p53 in ES, but no large series of cases have been analyzed to validate its clinical use. Ki67 is a molecule that is detected in growing cells. Several papers have revealed the prognostic implication of Ki67 in sarcomas, but which needs to be confirmed in the case of ES. The aim of the present study is to evaluate the prognostic significance of p53 and Ki67 in a large series of ES.
Material and Methods: Paraffin-embedded material from 226 ES subtyped as follows: 66% classic ES, 10% large cell ES, 10% PNET, 3% clear cell ES, 3% atypical ES, 6% spindle cell ES and 1% hemangioendothelial ES. Seven tissue arrays (TA) were performed and immunohistochemical expression of p53 (clon DO7, DAKO) and Ki67 (MIB1, DAKO) was determined using a 1:50 dilution of each antibody.
Results: Follow-up was available from 132 patients with a median of 49 months (range: 1–306 months). 22% of cases expressed Ki67 in more than 5% of tumor cells. Expression of Ki67 was correlated with the progression of ES, being higher in recurrence (30%) and in metastasis (42%) than in primary tumors (20%) ($p = 0.042$). In the case of p53, 31% of ES expressed the protein but no relationship with progression was observed. Log rank test for progression-free (PFS) and overall survival (OS) showed the following results: Antibody: Ki67 ($\leqslant$5%, >5%), %PFS: 68 vs. 31, p: 0.001, %OS: 68 vs. 37, p: 0.009 Antibody: p53 (negative, positive), %PFS: 62 vs. 59, p: 0.366, %OS: 60 vs. 78, p: 0.040. In contrast to the expected, immunostaining of p53 was correlated with a better OS, suggesting that a mutational analysis of these cases should be performed in order to detect those with real mutant behaviour.
Conclusion: Ki67 immunostaining defines a subgroup of ES with a poor outcome and should be taken into consideration in the pathological staging of ES patients.
*Supported with grants FIS P1040822 Madrid, Spain and PROTHETS (Prognosis and therapeutic targets of Ewing's family of tumors) Contract number: 503036 of the FP6 from the European Commission.

548 POSTER

Micronuclei in exfoliated bladder cells of gynecological cancer patients receiving pelvic radiotherapy

M. Nalca Andrieu[1], G. Gumus Akay[2], D. Koc Baser[2], B. Yalcin Burhan[2], A. Dizman[3], I. Bokesoy[4]. *[1]Ankara University School of Medicine, Radiation Oncology, Ankara, Turkey; [2]Ankara University School of Medicine, Medical Biology, Ankara, Turkey; [3]Ankara Ahmet Andicen Cancer Research Hospital, Radiation Oncology, Ankara, Turkey; [4]Ankara University School of Medicine, Medical Genetics, Ankara, Turkey*

Background: The micronucleus (MN) assay in human exfoliated cells has been widely used to detect the genotoxic effects of environmental mutagens, infectious agents and hereditary diseases. This study clarifies the usefulness of the MN assay in exfoliated bladder cells to show normal (not related to the cancer) tissue damage of pelvic radiotherapy.
Materials and Methods: We measured the MN yield in exfoliated bladder cells of 20 gynecological cancer patients received pelvic radiotherapy. These patients were non-smokers, had no urinary tract disease and previous chemotherapy or radiotherapy. They received pelvic irradiation with 2 Gy daily fractions for 5 weeks. Urine samples were taken from the patients before commencement of radiotherapy (0 Gy), 24 hours after completion of the first fraction (2 Gy) and at the end of the therapy (50 Gy). In addition, to determine whether exfoliated bladder cells could be used as a sign of a genomic instability in cancer patients, baseline MN yields of the patient group before the therapy were compared with the healthy control group.
Results: We have detected significant difference between results of three different time periods ($P < 0.01$). The yield of MN after radiation doses of 50 Gy (2.93 ± 2.29) has increased when compared with the 0 Gy (1.37 ± 1.13) ($P < 0.01$) and 2 Gy (2.1 ± 1.92) ($P < 0.05$). There was no significant difference between the MN frequencies of 0 Gy (1.37 ± 1.13) and the control group (1.29 ± 0.74).
Conclusion: Exfoliated bladder cells which can be taken non-invasively could be used to show normal tissue damage after cumulative doses of pelvic radiotherapy but it is not an indicator of a genomic instability in gynecological cancers.

549 POSTER

Malignant mesothelioma (MM): prognostic risk factors and immunohistochemical markers in correlation with pathological changes and prognosis

W. Morzycki[1], G. Yeung[2], S. Husein[3], N. Juneja[4], R. Dewar[5], T. Younis[6], K. Virik[6], M. Davis[6], D. Bethune[7], Z. Xu[4]. *[1]Nova Scotia Cancer Centre, Medical Oncology, Halifax, Canada; [2]Dalhousie University, Internal Medicine Residency Program, Halifax, Canada; [3]Dalhousie University Medical School, Medicine, Halifax, Canada; [4]Queen Elizabeth II Health Sciences Centre, Pathology, Halifax, Canada; [5]Nova Scotia Cancer Center, Epidemiology, Halifax, Canada; [6]Nova Scotia Cancer Center, Medical Oncology, Halifax, Canada; [7]Queen Elizabeth II Health Sciences Centre, Thoracic Surgery, Halifax, Canada*

Background: Malignant mesothelioma (MM) is known to be a fatal malignancy that is related to asbestos exposure. A number of clinical prognostic factors have been described in the last 20 years, including histological cell type, performance status and clinical stage. However, much of the data have been conflicting probably because many of the studies have been of small size from a single centre. We conducted a population based study in Nova Scotia, Canada to examine the potential prognostic factors, as well as the protein expression of EGFR, VEGFR, and SV40 in MM and their impact on patient's survival.
Methods: All cases of MM diagnosed in the province of NS between 1990–2005 were identified through the Nova Scotia Cancer Registry. Clinical and laboratory data, including known prognostic factors such as WBC, LDH, platelet count and hemoglobin level, were abstracted through a retrospective chart review. Tissue microarray (TMA) with immunohistochemical (IHC) staining for EGFR, VEGFR and SV40 was performed. Survival, with Kaplan Meier analysis, and a multi-factorial model will be performed to detect prognostic factors.

Results: A total of 136 cases of MM were identified, with an overall incidence of 1.3/100,000. Men had a higher incidence rate compared to women (1.47/100,000 vs 0.2/100,000, respectively), and a higher median age at diagnosis (70 vs 64 years, respectively).69% of all patients had documented asbestos exposure and 78% had a positive smoking history. The distribution of MM per anatomical site was 87% pleural, 12% peritoneal and 1% unspecified (testicular). The most common presenting symptoms were shortness of breath (61%), chest pain (43%) and cough (27%). Of all cases reported, 62 had pathology evaluable for analysis. Histological type was epithelioid in 42 (68%); sarcomatoid in 12 (19%) and biphasic in 8 (13%). EGFR expression was seen in 44 (71%); VEGFR and SV40 in 14 (22%). A survival analysis for potential prognostic factors including EGFR, VEGFR, SV40, WBC, LDH, platelet count and hemoglobin level, will be presented.
Conclusion: In this large cohort, the majority of MM tumors expressed EGFR, while a small proportion expressed VEGFR or SV40. Survival analysis for prognostic factors, as well as the correlation between EGFR, VEGFR, SV40, and other variables will be presented.

550 POSTER

Demethylation of the human telomerase catalytic subunit gene promoter restored telomerase activity in tamoxifen-resistant breast cancer cells

W.C. Park[1], J.H. Joo[1], S.S. Kang[2], B.J. Song[1], J.S. Kim[1], S.J. Oh[1], H.M. Jeon[1], S.S. Jung[1]. *[1]The Catholic University of Korea, Department of Surgery, Seoul, Korea; [2]Kwandong University, Department of Surgery, Seoul, Korea*

Background: Estrogen can activate telomerase activity (TA) of breast caner cells through direct or indirect mechanisms and TA is regulated by many transcriptional factors that actually regulate the expression of the human telomerase catalytic subunit (hTERT), the rate-limiting factor in TA. In the previous study with tamoxifen-resistant T47D:A18 breast caner cells (T47D:A18/4-OHT), we showed that the TA was highly increased and deregulated by estrogen not in parental cells but in tamoxifen-resistant cells. It is also reported by others that strong and positive correlation between hTERT promoter methylation, hTERT expression, and TA. We performed this study to see whether the methylation of hTERT gene promoter is associated with deregulated and highly increased TA in T47D:A18/4-OHT cells.
Materials and Methods: We established tamoxifen-resistant cells from parental T47D:A18 human breast cancer cells by long-term treatment with 1 μM of 4-hydroxytamoxifen. The genomic DNA was isolated from cells, and bisulfite modification and methylation-specific PCR was performed using primers specific for unmethylated and methylated alleles of hTERT. In case of detecting hypermethylation of hTERT in T47:DA18/4-OHT cells, we planed to treat with a demethylating agent, 2.5 μM of 5-aza-2′-deoxycytidine (5azadC). The semiquantitative TRAP assay was performed for measurement of TA.
Results: Methylation of hTERT gene promoter was not detected in parental T47D:A18 cells, but in tamoxifen resistant T47D:A18/4-OHT cells. The elevated and deregulated TA in T47D:A18/4-OHT cells were restored to basal level of parental T47D:A18 cells, and regulated by estrogen after the treatment of 5azadC.
Conclusions: In tamoxifen-resistant breast cancer cells (T47D:A18/4-OHT cells), deregulated TA is thought to be associated with the hypermethylation of hTERT gene and could be restored to basal level with demethylation of hTERT gene promoter. This epigenetic change could be considered not only as a mechanism of the development of tamoxifen resistance, but as a target to overcome tamoxifen resistance in breast cancer cells.

551 POSTER

Clinical significance of MDR1/ABCB1 single nucleotide polymorphism (SNP) in the breast cancer patients receiving neoadjuvant chemotherapy

H.J. Kim[1], B. Keam[1], S.A. Im[1], H.S. Han[1], H.S. Ham[1], D.Y. Oh[1], W.S. Han[2], T.Y. Kim[1], I.A. Park[3], Y.J. Bang[3]. *[1]Seoul National Univ. Hospital, Internal Medicine, Seoul, South Korea; [2]Seoul National Univ. Hospital, Surgery, Seoul, South Korea; [3]Seoul National Univ. Hospital, Pathology, Seoul, South Korea*

Background: Variations in the expression and activity levels of the multidrug-resistance MDR1/ABCB1 encoded P-glycoprotein (P-gp) have an impact on the therapeutic efficacy of many drugs. C3435T and G2677T polymorphisms of the MDR1/ABCB1 gene correlate with cellular expression levels of P-gp, a membrane-bound efflux pump which removes a chemotherapeutic drug including docetaxel and doxorubicin from the cells. The aim of this study was to investigate the clinical significance of SNPs in MDR1/ABCB1 genes in breast cancer treated with neoadjuvant chemotherapy (CTx).
Methods: One hundred stage II or III patients (pts.) (median age 45; range 25−63) were treated with 3 cycles of neoadjuvant CTx consisted of docetaxel 75 mg/m^2 iv and doxorubicin 50 mg/m^2 iv D1 every 3 wks before curative surgery. The objective tumor response was evaluated by RECIST. Pathologic CR (pCR) was defined as complete disappearance of invasive carcinoma in both breast and axillary lymph nodes after 3 cycles of CTx. Whole blood samples were obtained before CTx. DNA was extracted from the PB MNCs and C1236T, G2677T/A, C3435T polymorphisms of the MDR1 gene were genotyped by PCR-restriction fragment length polymorphism (RFLP). We evaluated the correlation between the clinico-pathologic prognostic factors, polymorphisms and clinical outcomes.
Results: Out of 100 pts, blood samples were available from 92 pts (ER− and PR−: 53.2%, HER2+: 27.2%). The overall radiologic response rate (RR) was 70.6% (CR 7.6%, PR 63.0%) and 8 patients (8.7%) achieved a pCR. Although statistically insignificant, clinical RR is higher in CT or TT allele than wild type CC allele on C1236T site (73.4% vs. 53.8%, $p = 0.17$). There was no significant difference of RR according to C3435T and G2677T/A SNPs. Genetic linkage was observed between C1236T and C3435T. No polymorphism predicted severe CTC toxicity. Two-year RFS rate was higher in CT or TT group compared with CC wild type (96% vs 48%, $p = 0.002$). Multivariate analysis will be presented.
Conclusions: The C1236T MDR1 polymorphism correlated with the prolongation of RFS in this study. More research is warranted to identify the molecular biological characteristics of C1236T that lead to altered P-gp.

552 POSTER

Identification of predictive markers for tumour response to neo-adjuvant chemotherapy (NCT) treatment for locally-advanced breast cancer (LABC)

V. Ganju[1], A. Strickland[1], S. Hart[2], J. Fox[2], J. Stuckey[3], A. Baldey[3], M. Schneider-Kolsky[4], B. Susil[5], D. Wu[6], P. Rogers[7]. *[1]Monash Medical Centre-Moorabbin, Medical Oncology, Melbourne, Australia; [2]Monash Medical Centre-Moorabbin, Surgical Oncology, Melbourne, Australia; [3]Monash Medical Centre-Moorabbin, Radiology, Melbourne, Australia; [4]Monash University, Anatomy, Melbourne, Australia; [5]Monash Medical Centre-Moorabbin, Pathology, Melbourne, Australia; [6]Monash University, Biostatistics, Melbourne, Australia; [7]Monash University, Centre For Womens Health, Melbourne, Australia*

Background: The aim of this study was to use expression profiling to identify genes that are predictive for successful response to NCT in women with LABC.
Material and Methods: Following informed consent, 47 patients with locally advanced breast cancer suitable for NCT were recruited. Participants were randomly assigned to receive either sequential FEC100 (×4) and Docetaxel 100/m2 (×4), or the reverse sequence. Serial assessment of response was performed with Physical Exam, Mammography, U/S, Serial Tumour biopsy and PET scans. A total of 39 Affymetrix arrays (U133 Plus 2.0) were performed on RNA isolated from core biopsies taken pre-chemotherapy. Analysis of array data was undertaken with R language and Bioconductor using an empirical Bayes linear model (Limma) to identify genes that were associated with a successful clinical response to either treatment regimen. P-values were adjusted using false discovery rate for multiple testing.
Results: A small number of genes were identified with significant predictive value for reduction in tumor volume (as assessed by ultrasound) in response to both Docetaxel (3 genes) and FEC100 (7 genes) treatment. Pre-treatment PET "standardized uptake volume" (SUV) inversely correlates with the likelihood of complete pathologic response. Gene array was able to identify of a group of 12 genes for which the expression profiles correlated with the pre-treatment PET SUV values. The next step in this work will be to validate the genes identified using quantitative PCR, and evaluate their predictive value in a prospective study.
Conclusion: We have identified a number of genes that may be useful markers to predict response to NCT in breast cancer. Validation studies are ongoing.