for lymphoma patients with chemotherapy (CHOP, ABVD) and lung cancer patients with radio and/or chemotherapy – 1 injection followed by 1 injection every 5 days (total of 5 injections). Mesothelioma patient received two courses of 2 mg of Liasten every two weeks and 3 capsules Del-Immune b.i.d. Effect was measured on the basis of blood test results and survival rate.
Results: Striking decrease were found in incidence of neutropenia complications (60% versus 21%, p = 0.0032) in those patients who received chemotherapy with liasten. Postoperative cases acute conditions of chronical bronchitis, infectious pneumonia cases occurred in 25% of LC patients versus 40% in control group. Patients that received radiation therapy in combination with muramyl peptides preparations had substantial reduction of toxic side effects. There was no hospital mortality. Increased activity of T-cell immunity and plasma interferon was reported in the process of treatment. Tumor process development was consistent with the control group. There were no cases of malignant lymphoma progression. No relapses and 3.5-fold NK functional activity as well as improved activity of T3 and B-cells were reported by mesothelioma patient more than 3 years after the treatment.
Conclusions: It was possible to develop algorithm of using Liasten injections for prevention and treatment of chemotherapy-induced hematotoxic complications in malignant lymphoma and lung cancer. Because of the incurable natures of mesotheliomas, it thus seems warranted to further research the use of Liasten in combination with oral muramyl peptides preparation Del-Immune V® to extend the life expectancy of these patients.

1053 POSTER

Development of humanized monoclonal single chain antibodies, against the tumour suppressor interferon regulatory factor 1 (IRF-1), through phage display

A. Moeller[1], K.L. Ball[1]. *[1]Edinburgh University IGMM, Cancer research centre, Edinburgh, United Kingdom*

Background: Despite a potentially important role in cancer suppression, detailed knowledge of IRF-1 pathways has been hampered by a lack of biochemical tools, and in particular monoclonal antibodies, due to low immunogenicity, poor expression, and toxicity of the IRF-1 protein in *E. coli* and mammalian cells.
Materials and Methods: Preformed *in vitro*, phage display circumvents the need for immunogenicity, and was therefore particularly well suited to generating IRF-1 specific antibodies. Additionally, once antibodies capable of binding specifically to IRF-1 were selected, the phage continued to act as a genetically stable source of the antibody which could be stored over a long period. The antibody genes were extracted and moved into a variety of plasmids which allowed for higher level expression in E. coli, and *in vivo*. expression in a variety of human cancer cell lines.
Results: The single chain antibodies were raised against functional domains of IRF-1 spanning the length of the entire protein to ensure that a range of antigens were targeted. By expressing the antibodies that target functional domains *in vivo*. it may be possible to influence specific activities of IRF-1 within the cell, such as its ability to bind DNA or become transactivated.
Conclusions: In this way, single chain antibodies can be used to tease apart the IRF-1 pathway and determine the functional relevance of identified intracellular interactions.

1054 POSTER

The existence of humoral immunity to gliadin and cow's milk proteins in patients with prostatic diseases

I. Besu[1], Z. Juranic[1], M. Djordjevic[1], A. Konic-Ristic[2], I. Filipovic-Ljeskovic[3], N. Babovic[4], S. Jelic[4]. *[1]Institute of Oncology and Radiology, Experimental Oncology, Belgrade, Serbia; [2]Faculty of Pharmacy, Institute of Bromatology, Belgrade, Serbia; [3]Institute of Oncology and Radiology of Serbia, Hematology, Belgrade, Serbia; [4]Institute of Oncology and Radiology of Serbia, Medical Oncology, Belgrade, Serbia*

Background: The goal of this study was to determine the incidence of the presence of serum IgA and IgG antibodies to gliadin and IgA, IgG and IgE antibodies to cow's milk proteins by ELISA test, in patients that are having different diagnosis of malignant or nonmalignant prostatic diseases and in control group of healthy people.
Patients and Methods: Twenty-six patients with various diagnosis of prostatic diseases (carcinoma, benign prostatic hyperplasia, adenoma) were included in this research. Nine patients had prostate-specific antigen (PSA) level less than 4 ng/ml, four of them had PSA level in range 4−10 ng/ml and thirteen patients had PSA level more than 10 ng/ml. Fifty healthy people was control group.
Two kinds of antigens were used: skimmed pasteurized cow's milk powder (ICN) and crude gliadin (Sigma). Determination of IgA and IgG serum's immuno-reactivity to gliadin, or IgA, IgG and IgE to cow's milk proteins (CMP), has been performed by home made ELISA tests. The cut off value, for each test, was evaluated as the mean +2 SD of control group.
Results: Statistical analysis of obtained data reveals that the levels of anti-gliadin IgA and anti-CMP IgE were significantly higher in patients with prostatic diseases then that of controls ($p < 0.008$ and $p < 0.02$). Anti-gliadin IgG and anti-CMP IgA immunoreactivities were not significantly higher in patients, comparing to the control group. The level of anti-CMP IgG immunoreactivities in patients with prostatic diseases comparing to the control group was on the limit of statistical significance ($p = 0.0543$).
Conclusion: Results from this study, point to the non-specific association between immunity to food proteins (gliadin and cow's milk proteins) and prostatic diseases.

1055 POSTER

Expression of cancer/testis tumor antigens MAGE-A1, MAGE-A3/4 and NY-ESO 1 in medullary breast cancer

A. Juretic[1], B. Matkovic[2], V. Separovic[2], G.C. Spagnoli[3], B. Kruslin[4], R. Separovic[5], M. Gamulin[1]. *[1]Clinical Hospital Center Zagreb, Oncology, Zagreb, Croatia; [2]University Hospital for Tumors, Pathology, Zagreb, Croatia; [3]University Hospital of Basel, Surgery Division of Research, Basel, Switzerland; [4]University Hospital "Sisters of Mercy", Pathology, Zagreb, Croatia; [5]University Hospital for Tumors, Medical Oncology, Zagreb, Croatia*

The medullary breast carcinomas (MBC) account for <2% of breast invasive carcinomas. Recent publications on breast cancer classifications based on gene expression profile analyses indicate that MBC may be considered part of the basal-like carcinoma spectrum. As regards this uncommon type of invasive breast cancer we have recently published an article on the clinicopathological features of MBC in 48 patients who were operated on at our two hospitals between 1999 and 2005 (Matkovic B et al. Tumori 2008).
The present study includes immunohistochemical analyses of the expression of cancer/testis (C/T) antigens MAGE-A1, MAGE-A3/4 and NY-ESO 1 in these MBC samples. C/T genes are normally expressed in germ line cells. However, they may also become activated in a wide range of cancer types. Although a study of the expression of these C/T antigens in breast cancer was in part conducted in invasive ductal carcinomas of no special type (NOS), this has not been done with respect to special and/or relatively rare histological types of breast cancers (Hofmann O et al. PNAS USA 2008; Kavalar R et al. Virchows Arch 2001).
In the present study monoclonal antibodies "77B", "57B" and "B9.8.1" (Juretic A et al. The Lancet Oncol 2003) were used to immunohistochemically determine the expressions of, respectively, MAGE-A1, MAGE-A3/4 and NY-ESO-1 C/T antigens in MBC tissues. MAGE-A1, MAGE-A3/4 and NY-ESO-1 antigens were found to be expressed in 33% (16/49), 33% (16/49) and 22% (11/49) of patients, respectively. Immunohistochemical data concerning these C/T antigen expressions were correlated with the following MBC clinicopathological features: patient's age at diagnosis, type of operation, tumor size, axillary lymph node metastasis, adjuvant therapy, ER, PR, HER-2 expression, and patient's survival. No significant correlation between the above-stated clinicopathologic parameters and the antigen expression was identified. The only exception was the patients' survival data which indicate a possible association between the expression of these C/T antigens and decreased overall survival: MAGE-A1 P = 0.07960, MAGE-A3/4 P = 0.01088, NY-ESO-1 P = 0.11742.
The results of our retrospective study suggest that the aforementioned C/T antigens may be used in MBC as tumor markers of potential prognostic relevance. Due to the relative rarity of this type of breast cancer, further tests need to be performed on additional MBC tumor samples with respect to the expression of these C/T antigens before being able to definitely confirm this possibly original observation.

1056 POSTER

Cd274/Pdc1l1 as a genetic modifier controlling aggressiveness of T-cell lymphoblastic lymphoma

L. Gonzalez-Sanchez[1], J. Santos[1], M. Villa-Morales[1], I. Ors[1], P. Lopez-Nieva[2], P. Fernandez-Navarro[3], H. Gonzalez-Gugel[1], A.M. Roncero[4], J. Fernandez-Piqueras[1]. *[1]CBMSO (CSIC-UAM)-CIBERER, Biologia Celular e Inmunologia, Madrid, Spain; [2]CNIO, Patologia Molecular Unidad de Epigenetica del Cancer, Madrid, Spain; [3]CNE ISCIII-CIBERESP, Area de Epidemiología Ambiental y Cáncer, Madrid, Spain; [4]CBMSO (CSIC-UAM), Biologia Celular e Inmunologia, Madrid, Spain*

Background: The use of consomic and congenic mouse-strains has greatly facilitated the identification of tumour modifier genes. Using sub-congenic interspecific mice generated between SEG/Pas and C57BL/6J strains, we report a critical region on chromosome 19 (*Tlyr1c*) which does

not regulate the incidence of g-radiation-induced T-cell lymphoma but is capable of modifying the aggressiveness of tumours arising during the latency period.
Material and Methods: Mice were exposed to whole-body fractionated g-irradiation at four weekly doses for the thymic lymphoma induction. The allele expression profiles in separate stroma-enriched cell fractions and purified thymocytes were analyzed by quantitative real-time RT-PCR and western blotting. DNA sequencing was performed to identify nucleotide differences. Functional analyses were performed by immunoprecipitation and co-culture assays using transfected HEK-293-T cells and purified thymocytes.
Results: In *Tlyr1c* region, only two genes (*Cd274*/*Pdc1l1*, encoding PD-L1/B7-H1 ligand, and *Jak2*) were found exhibiting differential expression between thymus stroma cells from SEG/Pas and C57BL/6J strains. The expression of both genes increase after a single dose of g-radiation and is scarcely distinguishable in T-cell lymphomas. Several polymorphisms detected in the coding sequence of *Cd274*/*Pdc1l1* were found to be functional by co-immunoprecipitation and co-culture assays.
Conclusions: Since it is known that PD1:PD-L1 interaction can modulate survival or proliferation of thymocytes through TCR signalling, and Jak2 is a key element in the induction of PD-L1 expression, we proposed that qualitative or quantitative changes of these genes may be useful as new biomarkers for T-cell lymphomas prognosis. In particular, decreasing expression of these genes in stroma may be associated with tumour aggressiveness and significantly worse prognostic. These results improve our knowledge of the molecular mechanisms triggering T-cell lymphoblastic lymphoma development while highlighting the relevance of stroma in controlling tumoor aggressiveness.

1057 POSTER

Ligand independent assembly of purified soluble Magic Roundabout (Robo4), a tumour-specific endothelial marker

M. Yoshikawa[1], Y. Mukai[1], Y. Okada[1], Y. Yoshioka[2], S. Tsunoda[3], Y. Tsutsumi[1], N. Okada[1], A. William C[4], T. Doi[1], S. Nakagawa[1].
[1]Graduate School of Pharmaceutical Sciences, Osaka University, Osaka, Japan; [2]The Center for Advanced Medical Engineering and Informatics, Osaka University, Osaka, Japan; [3]Laboratory of Pharmaceutical Proteomics, National Institute of Biomedical Innovation, Osaka, Japan; [4]Center for Vascular Biology Research and Division of Molecular and Vascular Medicine Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, USA

Background: Magic roundabout (Robo4) is the fourth recently identified member of the Roundabout receptor family. Robo4 is predominantly expressed in embryonic or tumour vascular endothelium and is considered important for vascular development and as a candidate tumor endothelial marker. Much remains unknown about Robo4, however, such as its ligand, structure, and the details of its function. Therefore, in order to study the characteristics of Robo4, we have established an expression and purification method for obtaining soluble recombinant human Robo4 (hRobo4) and mouse Robo4 (mRobo4).
Material & Methods: The cDNAs encoding extracellular domains of Robo4 s were cloned into the pcDNA3.1D/V5-His-TOPO vector. These plasmids were transfected in mammalian 293F cells and soluble Robo4 s were expressed in their supernatants. And then, soluble Robo4 s were purified using nickel nitrilotriacetic acid (Ni−NTA) chromatography and gel-filtration chromatography. Purities of soluble Robo4 s were confirmed by sodium dodecyl sulfate -polyacrylamide gel electrophoresis (SDS-PAGE). To examine the ligand-independent multimerization of purified hRobo4 and mRobo4, we calculated the native molecular weight by analytical gel-filtration and Blue Native polyacrylamide gel electrophoresis (BN-PAGE).
Result: The expression of hRobo4 and mRobo4 was observed on 6 days after transfection. The peak Robo4 fraction was observed using imidazole for elution from the Ni−NTA column. A single peak was observed in the gel-filtration chromatography and fractions of the peak were collected. By SDS-PAGE and anti Robo4 western blotting, the single broad band was observed, which may be a result of glycosylation without residual contamination. Furthermore, based on analytical gel-filtration and BN-PAGE, the native molecular weight of Robo4 was calculated to be over 200 kDa, despite the molecular weights of the Robo4 monomers were 60 to 75 kDa in SDS-PAGE analysis.
Conclusion: We established an expression and purification method for hRobo4 and mRobo4. The multimerization analysis suggests that soluble Robo4 assembled into multimers in the absence of its ligands. These purified proteins will be useful in advanced studies to determine the importance of multimerization, identify the ligands, and Robo4-mediated signaling in angiogenesis, which may contribute to the development of novel vessel-targeting therapies.

1058 POSTER

Loss of estrogen receptor in human breast cancer cells is associated with an epithelial to mesenchymal transition

Y.A. Luqmani[1], F. Al Mulla[2], A. Al Azmi[3]. *[1]Kuwait University, Department of Pharmaceutical Chemistry Faculty of Pharmacy, Safat, Kuwait; [2]Kuwait University, Department of Pathology Faculty of Medicine, Safat, Kuwait; [3]Kuwait University, Department of Biological Sciences Faculty of Science, Safat, Kuwait*

Introduction: Loss of functional estrogen receptor (ER) is central to the development of endocrine resistant breast cancer and poses a significant clinical problem. Subsequent therapeutic intervention would benefit from increased understanding of associated molecular events participating in continued proliferation. Global gene expression was analysed in breast cancer cell lines that either over-express ER (MCF7) or in which ER is constitutively (pII) or inducibly (E2) down-regulated by transfected siRNA, to identify transcriptional response to ER blockade.
Methods: Labeled cRNA transcribed from cDNA synthesised from extracted cellular RNA was hybridised to replicate low and high density gene microarrays to compare phenotypic profiles of these cell lines; chemi-luminescence or fluorescence signals were quantified with appropriate software packages. Selected differentially expressed genes were analysed by TaqMan realtime quantitative PCR.
Results: Low density array scanning highlighted several genes that discriminated MCF7 from pII; this was confirmed and extended in the high density scans. pII cells exhibited elevated transcripts encoding proteins with motility functions, most crucially metastasis, such as urokinase plasminogen activator. Reduced ER expression was associated with loss of epithelial markers such as keratin 18/19 and increased appearance of transcripts of genes typically found in cells of mesenchymal origin; vimentin, fibronectin, cadherin 1, vascular endothelial growth factor and CD68. Differential expression of these genes was confirmed by PCR analysis, which also highlighted a similarity between pII and MDA231 cells that are *de novo* ER negative. Tetracycline-induced transient ER down-regulation in E2 cells failed to elicit the changes apparent in pII cells. Pathway analysis indicated changes in genes involved in cell-cell interaction and cell motility.
Conclusions: Our observations suggest that a change from an epithelial to a more invasive mesenchymal phenotype (a phenomenon described as EMT) may be concurrent with gradual adaptation to loss of the functional capacity of the ER transcriptional pathway, leading to an aggressive estrogen independent cancer.
Supported by Kuwait University RA grants PC02/04, GM01/05, GM01/01.

1059 POSTER

Activation of p53 stimulates transcription from the MDMX P2 promoter in tumor cells

J. Richter[1], A. Wolf[1], S. Lam[2], A. Böhnke[1], A. Teunisse[2], K. Lodder[2], S. Hauptmann[1], A.G. Jochemsen[2], F. Bartel[1]. *[1]University of Halle-Wittenberg, Institute of Pathology, Halle/Saale, Germany; [2]Leiden University Medical Center, Molecular Cellular Biology, Leiden, The Netherlands*

Aims: MDMX and MDM2 are both essential regulators of p53 activity during development and tumorigenesis. The MDM2 gene is a known p53-target gene, but transcription of the MDMX gene was assumed not to be regulated by p53. However, recently we have identified a p53-binding site in the first intron of the human MDMX gene, which indeed confers p53-induced transcription from an MDMX-P2 promoter. The putative protein translated from the MDMX-P2 transcript contains 18 additional amino acids at the N-terminus.
Methods: In this study we have analyzed the basal and stress-induced expression level of the MDMX-P2 transcript compared to the MDMX P1 and total MDMX transcripts. In comparison, the expression of other p53 target genes, i.e. MDM2 and p21 has been investigated.
Results: We found a significant increase of the MDMX- P2 transcript after cisplatin treatment of p53-wild-type tumor cell lines (OAW-42, MCF-7, U2OS, LnCap) after 24 and 48 hrs. We also found induction of the MDMX-P2 transcript in several other wt-p53 tumor cell lines after treatment with p53-activating agents, such as Etoposide. In contrast, MDMX-P2 transcript levels remained unchanged in p53-null cells (SKOV-3, SAOS, PC3). A direct involvement of p53 in the activation of the MDMX-P2-promoter was shown with the use of SAOS2 cells with inducible p53 expression.
Conclusion: Our results clearly show that the increased MDMX-P2 transcript expression upon treatment with chemotherapeutics is dependent on wt-p53. Currently, we are investigating the role of the MDX-P2-protein in the regulation of the p53-activity in response to the treatment of ovarian cancer cell lines with cisplatin.