Results: Endometrial samples analyzed showed the following descriptives: PE (2.28 ± 0.299 UI, 88.9% positive), SE (0.475 ± 0.220, 33.3% positive), EP (1.852 ± 0.297, 78.6% positive), EH (1.247 ± 0.263, 68.8% positive) and A (1.379 ± 0.292, 92.3% positive). Comparisons among the different groups were performed. Values of telomerase in PE was statistically significant respect to SE ($p = 0.000$) and A ($p = 0.043$). ES showed significant difference with EP, ($p = 0.002$), EH ($p = 0.010$) and A (0.005). Crosstabs data revealed statiscal significance ($p = 0.011$) with a higher rate of positive samples in A and PE.

Conclusions: These data suggest that the telomerase activity is significantly increased in proliferative and endometrial adenocarcinoma with a higher rate of positive samples in both histological status revealing a greater common proliferation activity of these two situations.

374 POSTER

MUC1 mucin for the detection of epithelial-derived ovarian cancer cells in peripheral blood

A. Obermair, M. Stimpfl, B. Schmid, D. Tong, I. Schiebel, S. Leodolter, R. Zeillinger. *University Hospital Medical School Vienna, Department of Obstetrics and Gynecology, Vienna, Austria*

Purpose: Recent studies suggest the presence of epithelial derived tumor cells in the peripheral blood and in the bone marrow of patients with solid malignant tumors. However, no study evaluated the significance of disseminated tumor cells in the peripheral blood in patients with epithelial ovarian cancer.

Methods: We evaluated the expression of epithelial cell markers MUC1 (CA 15-3, EMA), CA 125, Ber-EP4 and cytokeratins (Ck7, Ck8, Ck7/8, Ck8/18/19) in seven human ovarian cancer cell lines and analyzed the cells by immunofluorescence to determine the surface as well as cytoplasmic expression of the epithelial cell markers. Furthermore, we evaluated the mRNA expression of MUC1, Ck18 and Ck19 by reverse transcriptase chain reaction (RT-PCR).

Results: All cell lines were strongly positive for MUC1 by means of RT-PCR analyses and by flow cytometry whereas all other markers were expressed inconsistently. Using immunomagnetic enrichment followed by flow cytometry, one seeded carcinoma cell per 10^6 leukocytes could be detected. A minimum number of 50 tumor cells per 20 ml blood sample had to be added to clearly distinguish real positive tumor cells from false positive signals. After RT-PCR we found faint expression of MUC1 in normal full blood samples.

Conclusion: Sensitivity and specificity decreased with the decreasing number of added tumor cells. A minimum of 50 tumor cells per 20 ml blood sample resulted in reproducible results. MUC1 gave the best results because it was expressed in every cell line tested.

375 POSTER

Matrix metalloproteinase expression in normal, inflammed and malignant mesothelial tissues

J.G. Edwards[1,4], R.A. Walker[2], J. McLaren[3], D.A. Waller[1], W.P. Steward[4], K.J. O'Byrne[4]. *[1]Departments of Thoracic Surgery; [2]Pathology, Glenfield General Hospital; [3]Obstetrics and Gynecology; [4]Oncology, Leicester Royal Infirmary, Leicester, United Kingdom*

Purpose: Matrix Metalloproteinases (MMPs) have been implicated in invasion and ang ogenesis in solid tumours. This study evaluated the expression of MMPs 2 and 9 in malignant mesothelioma (MM)

Methods: MMP expression was assessed in snap frozen, surgically resected, MM tumour specimens (5 cases), empyema specimens (EP)(3) and normal, uninflammed pleura (NP)(4). Homogenised sample supernatants, standarised for protein content, were run for 3 hours on a 10% SDS polyacrylamide gel impregnated with 1 mg/ml of denatured collagen. Gels were stained and semi-quantitative computer assisted image analysis we; used to assess MMP expression.

Results: No difference in either the intensity or pattern of MMP expression was detected in MM *vs* EP. As compared to NP, all MMPs were elevated in MM. Despite the small numbers studied, pro-MMP-2 levels were significantly elevated in MM *vs* NP ($p = 0.016$; Mann-Whitney).

Conclusions: MMP-2 and MMP-9 expression is upregulated in MM and EP compared to NP. The prognostic significance and relationship of MMP expression to angiogenesis requires further evaluation.

376 POSTER

Vascular endothelial growth factor (VEGF) in sera of patients with cervical cancer and the impact of platelets

G. Hänsgen, A. Becker, I. Hintner, J. Dunst. *Martin-Luther University Halle/Wittenberg, Department of Radiotherapy, Halle, Germany*

Purpose: VEGF is a protein with high biological activity in angiogenesis. The expression of VEGF was analyzed in the sera of 42 patients with unresectable cervical cancer, who underwent definitive radiotherapy.

Methods: 42 patient with locally advanced cervical cancer (FIGO II–IV) were analyzed. All had squamous cell cancer. VEGF concentrationes were measured with a quantitative immunoassay (Quantikine, R&D Europe).

Results: The VEGF concentration did not correlated with tumor stage.

The VEGF-levels were compared with the clinical outcome 6 months after the end of therapy. Patients with complete tumor response (CR; $n = 29$) showed a significantly lower VEGF-level (304 pg/ml ± 188) than patients with tumor symptoms (PD; $n = 13$; VEGF 892 pg/ml ± 756; $p < 0.005$). In the cases with tumor response the platelet counts were also lower (233 ± 64 Gpt/l) than in the cases with poor outcome (445 ± 344; $p < 0.0005$).

The evidence for VEGF-transport by platelets and the releasing by platelets during serum preparation was demonstrated by a correlation between serum-VEGF and the platelet counts ($r = 0.518$; $p < 0.01$).

Conclusions: A high pretreatment serum-VEGF is associated with poor response to radiotherapy in locally advanced cervical cancer. However, the serum-VEGF-concentration also correlates with the platelet count. The association between VEGF, thrombocytosis and prognosis should be further investigated

377 POSTER

Creation of a stage by stage diagnostics system of malignant tumors

S. Petrov[1,2], N. Balatenko[1], R. Khasanov[1]. *[1]Clinical Oncol. Center, Pathology, Kazan; [2]Kazan Medical University, Pathology, Kazan, Russian Federation*

Morphological diagnostics of a significant part of malignant tumors and their metastasis requires expensive additional methods application (IHC, EM, PCR in situ et al.). Due to economic and financial crisis going on at present in Russia these methods can be used only in the biggest oncological hospitals.

Aim: The purpose of our work was creation of the system of advisory help (IDO, Immunohistochemical Diagnosis in Oncology) for pathologists-oncologists of a large region of Russia, occupying several hundreds of kms and with the population of 10 million people.

Results: For this purpose in Kazan in a 1996 a well-equipped laboratory performing diverse diagnostics of the most difficult cases was created. The following factors gained had crucial significance for the successful activities: a) maintenance of strict sequence in diagnostics and usage of rational schemes; b) strict organization of work; c) existence of skilled personal, comprising a coordinated team; d) existence of efficient system of tumor samples delivery. Within this period of time diagnostics of more than 2 thousands of the most complicated tumors of various localization and histogenesis was done. We managed to accurately diagnose 96 persent of the cases. Having gained a certain experience we in a 1998 have issued the first in Russia manual on immunohistochemical diagnostics of human tumors. In a 1998 the 1st All-Russia workshop on immunohistochemistry in diagnostics of tumors was held in Kazan.

Conclusion: Thanks to the proper organization of work it becomes possible to gain good results in verification of malignant tumors.

[1] Immunohistochemical diagnosis of tumors in man (guidebook for pathologists, oncologists), Eds.: by S.V. Petrov and A.P. Kiassov, Book house Press, Kazan, 1998.

378 POSTER

Genistein inhibits initial dynamic adhesion of HT-29 cells to extracellular matrix under flow conditions

J. Haier[1], M. Nasralla[1], G.L. Nicolson[1]. *[1]Inst. f. Mol. Medicine, Huntington Beach, CA, United States*

Problem: Cell adhesion receptors on tumor cells generate cellular regulatory signals that allow them to control cell migration and invasion into host organs. Integrin-mediated signal transduction is required for adhesion to extracellular matrix (ECM) components. Shear forces under flow conditions can modify various cellular functions, including phosphorylation events and

cytoskeletal alterations. The influence of broad-spectrum protein tyrosine kinase (PTK) inhibitor genistein on integrin-mediated dynamic adhesion to ECM components was investigated.

Methods: HT-29 colon carcinoma cells were used to study dynamic cell adhesion to collagen in a parallel plate laminar flow chamber. Wall shear adhesion threshold (WSAT), dynamic adhesion rate (DAR) and adhesion stabilization rate (ASR) were determined to differentiate initial adhesion events and adhesion stabilization. These data were compared to static adhesion rates and cell spreading.

Results: Genistein interfered with early events of a2b1-integrin-mediated adhesion under flow conditions, but not with secondary adhesion stabilization and cell spreading. This drug lead to an increased rate of adhesion under static conditions, but the same treatment inhibited dynamic adhesion of HT-29 cells. DAR was significantly reduced using genistein-pretreated cells, whereas WSAT and ASR did not show differences between treated and untreated cells.

Conclusions: Genistein-sensitive PTK appear to be involved in initial events of stabilization of integrin-mediated cell adhesion to ECM. Dynamic conditions of fluid flow may have substantial influence on integrin-mediated signal transduction involved in adhesion stabilization of HT-29 cells.

379 POSTER

Signal transduccion pathways activated by antineoplasic drugs and their role in apoptosis

Isabel Sánchez-Pérez, Rosario Perona. *Instituto de Investigaciones Biomédicas del C.S.I.C.-UAM.C/Arturo Duperier, 4 Madrid 28029, Spain*

Antineoplasic agents such as cisplatin and adriamycin execute their pharmacological role by inducing apoptosis. We have studied the mechanism of apoptosis induction by cisplatin and adriamycin. Both drugs activate JNK with a late and persistent kinetic. Adriamycin activates caspase-3 before the onset in JNK activity, while cisplatin activation occurs hours after JNK activation. Induction in JNK activity is necessary for cisplatin-induced apoptosis while is dispensable for adriamycin induced cell death. Cells derived from c-jun Knock out mice were more resistant to cisplatin cell death than normal cells, while no difference was observed in response to adriamycin. Activation of JNK and cell death induction by cisplatin is mediated by the MEKK1/SEK-1 cascade. p38 is also activated by cisplatin with a similar kinetic than JNK. AP-1 complexes actuated by cisplatin include mainly c-jun/ATF-2 heterodimers. These results suggests that AP-1 dependent transcription is necessary for cisplatin induced apoptosis.

380 POSTER

Angiogenesis of tumors in childhood. Preliminary study

M. Moschovi, G.Th. Tsangaris, J. Vamvoukakis, A. Anastasiou, C. Van Vliet-Konstantinidou, F. Tzortzatou-Stathopoulou. *Oncology Unit, First Dept. of Paediatrics, Univ. of Athens, "Agia Sophia" Children's Hospital, Athens, Greece*

The growth of a tumor requires the formation of new capillaries. The propose of this study was to estimates the angiogenesis in benign and malignant tumors in childhood and to investigate how tumor angiogenesis correlates with the tumor's behavior.

Methods: Angiogenesis was studied in paraffin blocks in 42 children, aged 4–14 years. We highlighted the endothelial cells of microvessels by immunocytochemical staining using the anti-CD31 monoclonal antibody. The microvessels were carefully counted using light microscopy (200 × field), in the most active areas of neovascularization. Stained endothelial cell or clusters were considered as a single, countable microvessel. Neither red blood cells nor vessel lumens were concidered necessary for a structure to be defined as a microvessel. Microvessel density (MD) was expressed as the highest number of microvessels identified and counted within an), single 200 × field.

Results: Higher rate of MD (73.3 ± 7.4) was revealed in malignant tumors, particularly in those with metastatic disease at diagnosis. Lower rate (44.6 ± 10.2) was revealed in benign tumors and in brain tumors (p = 0.003). Namely, MD in non-Hodgkin lymphomas was 108.6 ± 24.3, in Hodgkin's disease 73.7 ± 13.7; in Wilm's tumor 98.8 ± 21.3; in neuroblastoma 71.2 ± 14.9 and in sarcoma 65.7 ± 15.6. In malignant tumors of genital system MD was 63.6 ± 17.8 but in nasopharygeal carcinoma it was 113.5 ± 2.5. Also, high MD occurred in patients with inflammatory inosarcoma and bad outcome.

Conclusions: a) Malignant tumors presented higher MD than the benign tumors. b) The MD may be a useful maker of the malignant tumor's behavior. Further studies are needed to indicate if angiogenesis can be used as a phenotyping marker of the disease.

381 POSTER

Lymphocyte subpopulations in patients with multiple primary tumors

E. Robinson, R. Segal, L. Struminger, D. Faraggi, R. El'ad-Yarum, T. Mekori. *Rambam Medical Center and Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel*

Background: Cancer patients with single tumors live longer today due to earlier detection and improved treatment methods. For this reason, we see more patients who develop a second primary tumor. The purpose of this study was to investigate the lymphocyte subsets of these patients.

Methods: We investigated the lymphocyte subsets in 88 patients from our tumor registry with at least one breast or colon cancer and a second primary of the same or another site. Mononuclear cells were obtained from heparinized blood by the standard fractionation Hypaque gradient centrifugation technique. Helper and suppressor cells were identified by using three murine monoclonal antibodies: CD_3 for mature T lymphocytes; CD_4 for helper inducer cells, CD_8 for suppressor cytotoxic cells. T cell subset distribution was evaluated using the flow cytometer.

Results: Most values of CD_3, CD_4, and CD_4/CD_8 were lower in patients than in healthy controls. The values of CD_4, and CD_4/CD_8 were lower in patients who had a second tumor in the colon rather than in the breast.

Conclusions: As tumors in patients with a second primary recur at times or the patient develops a third primary, we are following the patients prospectively to see whether those with immunosuppression have a greater tendency to develop recurrent disease ota third primary.

382 POSTER

Gain of an intracrine, proliferative loop involving FGF-2 and FGF-receptors in human non-small cell lung cancer (NSCLC) cells

W. Berger[1], U. Setinek[2], L. Elbling[1], T. Mohr[1], I. Kindas-Mügge[1], M. Micksche[1]. [1]*Inst. for Cancer Research, Appl. + Exp. Oncology, Vienna;* [2]*Inst. for Pathology and Bacteriology, KH. Baumgartner Höhe, Vienna, Austria*

Purpose: Basic fibroblast growth factor (FGF-2) as well as FGF receptor-1 (FGFR-1) expression have been related to an unfavorable prognosis in non-small cell lung cancer (NSCLC) patients. To clarify the underlying cellular mechanisms we investigated NSCLC cell lines (N = 16), surgical specimens (N = 11), and control cell lines (N = 2).

Methods: FGF-2 and FGFR-1 to -4 expression were assessed by RT-PCR, ELISA, immunoblot and immunostaining. Effects of FGF-2 and FGF-2 antagonists were tested by 3H-thymidine incorporation- and MTT-based proliferation assays.

Results: NSCLC cells expressed elevated levels of FGF-2 and FGFRs in vitro and in vivo. FGF-2 production correlated with both a short doubling time and potent anchorage-independent growth of NSCLC cell lines. In contrast to control cells, NSCLC cells did not secrete considerable amounts of FGF-2. In low FGF-2-producing NSCLC and control cell lines FGFRs were located at the plasma membranes. These cells were sensitive to the proliferative effect of recombinant FGF-2. In NSCLC cell lines with enhanced FGF-2 production, representing the majority tested, FGFR localisation was intracellular. These cells were insensitive to both the proliferative effect of exogenous FGF-2 and growth inhibition by FGF-2-neutralising antibodies. In contrast, several agents antagonising FGF2 intracellularily impaired growth of all NSCLC cell lines.

Conclusion: Data suggest that FGF-2 stimulates proliferation of NSCLC cells mainly by an intracrine proliferative loop involving intracellular FGFR.

383 PUBLICATION

Effect of the conditioned medium (CM) of bone marrow (BM) fibroblast colony forming units (CFU-F) from patients with solid tumor on the growth of normal CFU-F

N. Chasseing[1], A. Honegger[1], R. Bordenave[2], E. Hoffer[1], C. Shanley[1], L. Rumi[1]. [1]*Instituto de Biologia y Medicina Experimental, Cancer Inmunology, Buenos Aires;* [2]*Hospital I. G. Iriarte, Oncology Department, Quilmes, Argentina*

Previously, we observed a decrease in cloning efficiency of CFU-F from BM of untreated advanced lung and breast carcinoma patients (LCP and BCP). Now we measured levels of platelet derived growth factor AB (PDGF-AB) and basic fibroblast growth factor (bFGF) in the CM obtained from CFU-F cultures of LCP (n = 9), BCP (n = 6) and normal volunteers (NV, n = 6). Both