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

mitogen for CFU-F were quantified by ELISA methodology (RD System). For CFU-F assay 2 × 106 BM mononuclear cells were cultured in alfa medium with 20% of fetal bovine serum (FBS) at 37 C and after 7 and 14 days the CM were harvested. We evaluated the effect of the CM from LCP and BCP CFU-F cultures on the growth of normal CFU-F. 2 × 106 normal BM mononuclear cells were incubated with alfa medium contained 20% of FBS and 20% of the CM of CFU-F cultures from patients and NV. The number of fibroblast colonies was counted at day 14. All the samples of the CM were incubated in duplicate with 5 normal BM.

Results: PDGF-AB (pg/ml, X ± ES) = 7 days: LCP = 47.0 ± 10.0; BCP = 95.0 ± 20.0 and NV = 77.0 ± 22.0 and 14 days: ± 31.2 in all the groups. bFGF (pg/ml, X ± ES) = 7 days: LCP = 6.0 ± 1.0; BCP = 5.0 ± 2.0 and NV = 16.0 ± 7.0 and 14 days: LCP = 6.0 ± 0.9; BCP = 7.0 ± 1.0 and NV = 6.0 ± 0.4.CFU-F assay: (number of CFU-F/2 × 106 normal BM mononuclear cells, X ± ES) = control without CM = 23.50 ± 2.98 (n = 5); with CM of LCP CFU-F cultures (7 days) = 9.74 ± 2.03 (n = 8, p < 0.002 vs. Control) and (14 days) = 8.63 ± 1.36 (n = 8, p < 0.0003 vs. Control); with CM of BCP CFU-F cultures (7 days) = 12.50 ± 2.22 (n = 7, p < 0.01 vs. Control) and (14 days) = 12.69 ± 3.08 (n = 7, p < 0.03 vs. Control); with CM of normal CFU-F cultures (7 days) = 19.50 ± 3.43 (n = 6) and (14 days) = 20.50 ± 2.66 (n = 6).

Conclusion: results show that CM from CFU-F cultures of LCP and BCP have inhibitory effect on fibroblast progenitors proliferation in serum supplemented cultures. Inhibition is not correlated with the PDGF-AB and bFGF levels.

384 PUBLICATION

Efficient autologous inhibition of tumor growth in a murine model of renal carcinoma

H. Hugenschmidt[1], C. Wittig[1], C. Unger[1], D. Marmé[1], J. Drevs[1]. [1]*Tumor Biology Center, Freiburg, Germany*

Purpose: The murine RENCA-model simulates renal cell carcinoma growth and metastasis. Purpose of this study was to identify autologous inhibition of tumor growth in this model.

Method: 8 × 104 RENCA-cells were injected s.c. in either one or both hind feet of Balb/c mice. Animals were sacrifice, d 28 days after injection and weight, volume and vessel density of the s.c. tumors were determined. In a second experiment RENCA-cells were injected intrarenaly after flanc incision of anesthesized animals. Seven days after injection tumor nephrectomy was performed on half of the animals. On day 21 all animals were sacrificed and vessel density, weight and volume of the primary or recurrent tumor, lung metastasis count and weigth were measured.

Results: 50% of the animals with bilateral tumors had complete remissions of their established tumors, no remissions were observed in the unilateral group. On day 28, bilateral tumors were significantly smaller than unilateral tumors. In the second experiment tumor recurrence occurred in 4 of 10 nephrectomized animals. In the nephrectomy group lung metastases were reduced by 70% for animals with tumor recurrence. Lung metastasis of nephrectomized animals without recurrence was equal to controls. Recurrent tumors were of comparable size as primary tumors of the untreated group.

Conclusion: The experiments show that autologous inhibition can lead to strong antitumoral effects in the murine RENCA-model. The pronounced effects make autologous inhibition an interesting topic for further investigation of the underlying mechanism.

385 PUBLICATION

K+-efflux modulation of cisplatin-induced apoptosis and cytotoxicity to cultured mesothelioma cells

L. Marklund[1], R. Henriksson[2], K. Grankvist[1]. [1]*Umeå University, Clinical Chemistry, Umeå;* [2]*Umeå University, Oncology, Umeå, Sweden*

The efflux of potassium, plays a necessary role during the induction of apoptosis. Apoptosis with cell shrinkage due to potassium efflux is counteracted by increased inward pumping of potassium ions. Modulation of potassium pump activity could thus decrease or increase apoptosis due to the nature of the disruption of cellular potassium homeostasis and affect anticancer drug cytotoxicity.

We explored the role of enhanced potassium efflux as an explanation to cisplatin cytotoxicity and apoptosis. Amphotericin B, known to potentiate cisplatin cytotoxicity, is a potassium ionophore inducing cellular potassium efflux. Modulation by inhibition of the K+ influx pumps ATPase and Na+, K+, Cl−-cotransport on a mesothelioma cell line with activity of only theses K+ influx pumps was performed. To determine the cytotoxicity we incubated the cells with amphotericin B, ouabain a Na+, K+, ATPase blocker, bumetanide a Na+, K+, Cl−-cotransport blocker and cisplatin, alone or in combination for 1 h.

The number of surviving clones were compared with untreated controls. For apoptosis we first analysed the DNA integrity by DNA-ladder formation on agarose gels.

To quantify free nucleosomes we used a ELISA-kit.

Combination of cisplatin (16.7 umol/L) with amphotericin B (3.2 mmol/L), led to an additive reduction of the percentage of surviving clones. When 100 umol/L ouabain was added a potentiation of the cytotoxicity was seen. Ouabain however, reduced apoptosis of the amphotericin B/cisplatin combination. Bumetanide (100 umol/L) did not affect the cytotoxicity of amphotericin B and cisplatin combined but significantly increased apoptosis of the mixture.

We conclude that cisplatin cytotoxicity and ability to induce apoptosis is influenced by cellular potassium flux modulation.

386 PUBLICATION

In-vivo growth modulation of FaDu-xenografts in nude mice with 2-methoxyoestradiol and oestradiol

J. Claßen, W. Budach, M. Bamberg. *Dep. of Radiooncology, University of Tuebingen, Germany*

Introduction: Recently, significant in-vivo growth inhibition has been reported for the treatment of B16 melanoma and Meth-A-sarcoma in mice with oral 2-methoxyoestradiol (ME). As the underlying mechanism, antiangiogenic properties of ME have been postulated. We therefore investigated whether ME or oestradiol (ED) exert an antiproliferative effect on a human squamous cell carcinoma xenograft in the nude-mice model.

Methods: FaDu human squamous cell carcinoma was transplanted as single cell suspension subcutaneously (10^6 cells/0.05 ml medium) on nu/nu athymic mice. Oral treatment with ME (100 mg/kg per day, 7 days per week) or ED (600 mg/kg per day, 7 days per week) suspended in olive oil was started on the day of transplantation. Tumour volume was measured daily. Animals were killed when the tumour volume reached 1000 mm^3. The in-vivo tumour doubling time of ME or ED treated animals and controls as well as the specific growth delay were calculated.

Results: Daily oral treatment was well tolerated without obvious toxicity. However, no difference in tumour doubling time in ME or ED treated animals as compared to controls could be observed.

Conclusion: Neither ME nor ED exert a significant growth inhibition on FaDu xenografts in nu/nu-mice.

387 PUBLICATION

p53 protein expression and proliferative activity in renal cell carcinomas

A. Ioakim-Liossi[1], D. Pantazopoulos[2], P. Karakitsos[2], P. Athanassiadou[1], E. Stergiou[3], A. Karkabassi[3], P. Athanassiades[4], P. Davaris[1]. [1]*Pathology Laboratory, Cytology Department, Medical School, Athens University,* [2]*Urology Department, Medical School, Athens University,* [3]*Cytology Laboratory, "Laiko" Hospital,* [4]*Clinical Therapeutics "Alexandra" Hospital, Medical School, Athens University, Athens, Greece*

Objective: Renal cell carcinoma is an heterogeneous group of neoplasia with unpredictable biological behavior. The aim of this study was to investigate the p53 protein expression and the proliferative activity in a group of 55 renal cell carcinoma cases in relation to the histological grade of malignancy.

Patients and Methods: Fifty five unselected patients with renal cell carcinoma (RCC) undergoing radical nephrectomy in the Department of Urology were prospectively studied. All patients were operated curatively. In order to investigate the p53 protein and the proliferative activity of tumor cells an immunohistochemical ABC technique was applied in histological sections DO-7 and MIB-1 (Ki67) are used as primary antibodies correspondingly.

Results: Immunostaining of p53 suggested the presence of mutant p53 was found in 30% of cases. The p53 immunoreactivity was confined to the tumor cell nuclei. Tumors did not stain homogeneously. The percentage of MIB-1 positive cells ranged between 1% and 90%. Positive statistical correlation was seen between the tumor grade and MIB-1. In some tumors, an inverse trend of p53 protein expression and proliferative activity was observed.

Conclusion: Our results suggest that mechanisms related to cell cycle control and cell proliferation play a role in the biology of renal cell cancer. More detailed analysis is needed to elucidate the role of the tumor suppressor gene p53 and cell proliferation in renal cell carcinoma and prognosis.