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

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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

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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

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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

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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

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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