369 POSTER

Selective tumor cell kill by alkyl-lysophospholipids

S.F. Zerp[1,2], G.A. Ruiter[1,2], H. Bartelink[1], W.J. van Blitterswijk[2], M. Verheij[1]. [1]*Department of Radiotherapy;* [2]*Division of Cellular Biochemistry, The Netherlands Cancer Institute, Amsterdam, Netherlands*

Synthetic cell membrane-permeable alkyl-lysophospholipids (ALPs) are potent inhibitors of mitogenic signaling and are capable to induce cell death in a variety of tumor types. Moreover, these compounds appear to act synergistically in concert with classical antitumor regimens, such as radiotherapy and chemotherapy. We tested the effect of two ALPs (Et-18-OCH_3 and HePC) on apoptosis induction in 4 different malignant cell lines (U937, Jurkat T, A431, B103) and 2 types of normal vascular endothelial cells (HUVEC, BAEC). In all tested tumor cell systems, both ALPs induced a steep dose- and time-dependent increase in apoptotic cell death (ED_{50} range 8–15 μM). The sensitivity of endothelial cells towards ALPs was dependent on the proliferative status of the cells. Confluent endothelial cells showed no significant levels of apoptosis at concentrations as high as 20 μM, whereas proliferating endothelial cells, such as occurs during tumor neovascularization, were highly sensitive to ALPs: more than 90% of the cells underwent apoptosis after 10 μM.

These data demonstrate a selective apoptotic effect of ALPs in various tumor cell types and proliferating endothelial cells. Confluent endothelial cells, however, remained unaffected. These findings may provide a basis for selective and efficient tumor cell kill, both directly (through apoptosis) and indirectly (through inhibition of angiogenesis).

370 POSTER

Involvement of cytoskeleton in 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: Adhesion of colon carcinoma cells to extracellular matrix (ECM) components is mainly mediated by integrins which are intracellularly linked to cytoskeletal proteins. The functional status of integrins is regulated by complex interactions with cytosolic, cytoskeletal and membrane-bound proteins. Wall shear stress also influences cellular functions. We examined the role of various cytoskeletal components in dynamic cell adhesion under flow conditions.

Methods: Dynamic adhesion of HT-29 colon carcinoma cells to collagen was investigated using a parallel plate laminar flow chamber. Cells were pretreated with cytochalasin D, nocodazole, colchicine or acrylamide to disrupt actin filaments (AF), microtubules (MT) or intermediate filaments (IF). Wall shear adhesion threshold (WSAT), dynamic adhesion rate (DAR) and adhesion stabilization rate (ASR) were determined to differentiate initial adhesion from its stabilization.

Results: Disruption of AF inhibited cell adhesion completely. Pretreatment with IF disrupting agents did not interfere with dynamic cell adhesion, whereas it partially reduced adhesion rate under static conditions. Significant DAR and ASR were found after disruption of MT, and cells demonstrated extensive crawling on collagen-coated surfaces. This was in contrast to static adhesion where this pretreatment did not result in different adhesion rates.

Conclusions: Native AF and MT seem to be involved in integrin-mediated cell adhesion to collagen under dynamic conditions of fluid flow. Our results demonstrated that AF are required to withstand shear forces. MT appeared to be necessary for adhesion stabilization, but not for initial adhesive interactions.

371 POSTER

Cross-talk between c gamma RIIA and c gamma RIIc on human natural killer cells

I. Popescu[1], M. Hirt[1], A. Calugaru[1], D. Baiu[1], M. Manciulea[1], D. Metes[2], P. Morel[2], R. Herberman[2], A. Sulica[1]. [1]*Institute of Virology, Center of Immunology, Bucharest, Romania;* [2]*University of Pittsburgh, Cancer Institute, Pittsburgh (PA), United States*

Human natural killer (NK) cells bind through their cell-surface marker Fc gamma RIIIA the physiologic ligand (IgG molecules) which may have regulatory effects on several cell functions such as NK cell activity, killing of target cells through antibody-mediated cell cytotoxicity, cytokine production, etc. Recently, we provided evidence about another type of IgG binding cell-surface structure, namely the Fc gamma RIIc. Our findings proved that this second type of receptor is present at least on some of NK cells and it is a triggering molecule transducting intracellularly an activation signal. In the present studies we attempted to explore whether functional differences could be identified between Fc gamma RIIIA (CD16) and Fc gamma RIIc (CD32). In our previous studies we presented evidence that Fc gamma RIIIA attached also the monomeric form of IgG (mIgG) besides the polymeric form (pIgG). In contrast, Jurkat transfectant cells expressing Fc gamma RIIc were found capable to interact only with pIgG. Marked differences between these two receptors Fc gamma RIIIA and Fc gamma RIIc, were observed when we obtained a down- or up-regulation of the NK cell activity induced following treatment of highly purified NK cells with either F(ab')2 fragment of anti-CD 16 monoclonal antibody (mAb) 3G8 or F(ab')2 fragment of anti-CD32 mAb KB61, respectively. The inhibitory effect triggered by the engagement of CD16 is in good agreement with our previously reported data regarding the mIgG-induced inhibition of NK cell activity mediated through Fc gamma RIIIA. No different modulation effect on NK cell activity was determined when the intact molecule of anti-CD32 mAb KB61 was used as compared with that of its F(ab')2 fragment. In contrast, the anti-CD16 antibody 3G8 containing its Fc region enhanced the NK activity expressed by effector cells isolated from about 60% of donors whereas these cells responded by inhibition following stimulation with F(ab')2 fragment of 3G8 antibody devoid of its Fc region. Evidence was also found regarding the activation of [Ca2+]i mobilisation, and signal transduction in the expression of the activation of LCK tyrosine kinase of NK cells. Consequently, we assume that the contrasting regulatory effects of the 3G8 mAb versus its F(ab')2 fragment on the NK cell activity can be partially explained by a cross-talk between the signals triggered by the entire molecule of mAb 3G8 which can cross-link Fc gamma RIIIA and Fc gamma RIIc through its combining site and Fc region.

372 POSTER

Quantification and characterization of micro tumor load: An option for monitoring adjuvant and palliative disease

L. Habets[1], O. Grotius[1], S. Doll[1], B. Lippok[1], M. Gajewsky[1], P. Braun[1], H. Knechten[1]. [1]*PZB, Aachen, Germany*

Emerging new (antibody) or expensive (taxanes) therapies require quantification and characterization of macro- or micro-disseminated disease. We could show feasebility of combining immunomagnetic enrichment (MACS) and flowcytometry (FACS) for detection of cytokeratin-positive cells in peripheral blood and bone marrow of cancer patients.

IN 70% of patient with metastasizing tumors circulating epithelial cells can be detected in numbers ranging from 1 to hundred in 20 ml blood. Further characterization (Her-2-NEU, 17-1A, MUC-1) by flowcytometry allowing quantification of antigen expression to determine targetability by MoAb therapy (Herceptin, Panorex) is possible. Sorting of even 1 cell in 20 ml of peripheral blood could be achieved by the sorting device of FACSCalibur. Recovery of gated cells was always above 90%. Sorted cells are open for cell culture or molecular techniques (PCR, FISH), Extended characterization of therapy or prognosis related antigens (UPA1, PgP, Thymidilat-synthetase) can be achieved by using doublelaser cytometry. Further optimalization of this approach could lead to: 1. Close biological monitoring of disease development and biological effects of therapy. 2. Quantification of micro tumor load in bone marrow could allow direct monitoring of therapeutic efficacy during adjuvant therapy.

Sponsored by: Metares.e.V., Megapharm, Lederle, Glaxo-Wellcome.ribosepharm, onkoworks.

373 POSTER

Analysis of telomerase activity in physiological and pathological endometrial tissues

E. Jiménez, J. García-Foncillas, M. Guzmán, E. Andión, M. Huarriz, I. Okroujnov, T. Errasti, M. Jurado, J. Schneider, A. Brugarolas. *Laboratory of Molecular Oncology, University Clinic of Navarra, Pamplona; Department of Gynecology, Univesity of the Basque Country, Spain*

Purpose: To evaluate the possible role of telomerase activity in the different physio- and pathological conditions analyzed and its potential clinical usefulness in the early detection of endometrial cancer.

Method: A total of 77 endometrial tissue samples, comprising 9 proliferative endometrium samples (PE), 12 secretor (SE), 14 endometrial polyps (EP), 16 endometrial hyperplasias (EH) and 13 adenocarcinomas (A) were analysed for telomerase activity by Telomeric Repeat Amplification (TRAP) assay followed by ELISA detection. Descriptive, crosstabs statistics were performed. Comparisons between groups was done by using Mann-Whitney test.

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