369 POSTER

Selective tumor cell kill by alkyl-lysophospholipids

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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

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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

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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

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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.

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373 POSTER

Analysis of telomerase activity in physiological and pathological endometrial tissues

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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.