Results: On the whole, larger values of MN frequency and surviving fraction were observed in SAS/mp53 cells than in SAS/neo cells, and Q cells showed lower MN frequencies than total cells. Without wortmannin, SAS/neo tumor cells, especially Q cells within SAS/neo tumors, showed large potentially lethal damage repair (PLDR) capacities, compared with total or Q tumor cells within SAS/mp53 tumors that showed little PLDR capacity. Wortmannin treatment inhibited the PLDR in SAS/neo tumors very effectively, but showed no apparent effect on either total or Q tumor cells within SAS/mp53 tumors.

Conclusion: PLDR in vivo was thought to be a p53-dependent event whether in total or Q tumor cell populations and might reflect the nonhomologous end-joining process for DNA double-strand break repair.

614 POSTER

Increased sensitivity in the detection of isolated tumor cells (ITC) in bone marrow by automated screening analysis

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The presence of isolated metastatic cells in bone marrow (BM) of breast cancer patients at the time of diagnosis indicates occult hematogenous tumor cell dissemination and predicts an increased risk for subsequent distant disease. However, manual microscopic evaluation of an adequately high number of BM cells leads to a substantial risk of decreasing read-out, and might, therefore, be unreliable. Purpose of the present study was to evaluate, whether the sensitivity of this method can be improved by using digital microscopy. In a retrospective series of 244 breast cancer patients with stage I-III disease, we analyzed BM aspirates, which were previously stained with monoclonal anti-cytokeratin antibody A45-B/B3. Only well preserved slides were included. All samples, screened manually between 1995 and 1999, were now reevaluated by two independent observers, after pre-screening with the MDS digital microscope (Applied Imaging Inc, USA), without knowledge of the initial results. By manual screening, ITC were detected in 40 patients (16%), compared to 88 patients (36%) using digital microscopy (P

615 POSTER

Correlation between sensitivity to capecitabine in xenograft models and mRNA expression levels of pyrimidine-metabolizing enzymes in tumor tissues

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Background: Analysis of factors predicting sensitivity to anticancer drugs is important and useful not only for selecting potential responders but also for developing new combinations. The oral fluoropyrimidine capecitabine (Xeloda®) is sequentially activated by three enzymes, carboxylesterase, cytidine deaminase (CD) and thymidine phosphorylase (TP), and generates 5-FU selectively within tumor tissues. We have demonstrated previously that the sensitivity of human tumor xenografts to capecitabine correlates significantly with the ratio of TP to dihydropyrimidine dehydrogenase (DPD) in tumor tissues. On the other hand, thymidylate synthase (TS) and other pyrimidine-metabolizing enzymes are also reported as predictive factors for 5-FU sensitivity.

Methods: In the present study, we analyzed the correlation between the sensitivity of xenografts to capecitabine, 5'-deoxy-5-fluorouridine (5'-DFUR, Furtulon®) and 5-FU and mRNA levels of the following pyrimidine-metabolizing enzymes: TP, DPD, CD, TS, orotate phosphoribosyl transferase (OPRT), uridine phosphorylase (UP), uridine kinase (UK), UMP kinase (UMPK), ribonucleotide reductase (RNR), thymidine kinase (TK), and TMP kinase (TMPK). mRNA levels in the tumor tissues of 80 xenograft models were determined by real-time RT-PCR.

Results: Significant correlations were demonstrated between mRNA levels of several enzymes: TS, OPRT, UK, UMPK, RNR, TK and TMPK. However, mRNA levels of these enzymes did not correlate significantly with those of TP, DPD, UP and CD. Furthermore, mRNA levels of TP, DPD and CD showed wide variation between xenograft models when compared with levels for other enzymes. Antitumor activity was assessed in 50 xenograft models for capecitabine and 24 models for 5'-DFUR and 5-FU after 3 weeks of treatment at maximum tolerated doses. The antitumor activity of capecitabine and 5'-DFUR correlated significantly with mRNA levels of TP and with the TP:DPD ratio, whereas the activity of 5-FU correlated significantly with OPRT, TMPK, UMPK and CD. In a multiple regression analysis, only TP and DPD were independent predictive factors for sensitivity to capecitabine and 5'-DFUR and only UMPK was predictive for sensitivity to 5-FU.

Conclusion: The predictive factors for sensitivity to capecitabine and 5'-DFUR in xenograft models are TP and DPD, which are different from those for 5-FU. Therefore, there is a possibility that responders to capecitabine and 5'-DFUR would be different from those responding to 5-FU.

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Expression of hMLH1 and hMSH2 proteins in normal tissues and cancer predisposition in hereditary non-polyposis colon cancer

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Backround: Germline mutations of hMLH1 and hMSH2 genes are associated with hereditary non-polyposis colon cancer (HNPCC), a cancer susceptibility syndrome characterised by increased predisposition to colon cancer, but also endometrial, stomach, small bowel, ovary, hepatobiliary and urinary tract cancer. Skin and brain tumours develop in HNPCC subtypes of Muir-Torre and Turcot's syndrome, respectively. The reasons why a higher incidence of cancer exists in these tissues are not sufficiently explained. We tested a hypothesis that a higher incidence of cancer in the specific tissues is related to their constitutive level of hMLH1/hMSH2 protein expression.

Material and methods: hMLH1 and hMSH2 proteins were studied in paraffin-embedded archival normal samples of various tissues. A total of 89 specimens was analyzed. Indirect immunohistochemical technique was used. The intensity of nuclear staining was graded as 0-3. H-scores were calculated. The results were statistically analyzed.

Results: Tissues with the highest mean H-scores were as follows: A) epithelia of large bowel (hMLH1, 114, hMSH2, 129), stomach (109, 147), small bowel (111, 127); B) endometrium (114, 134); C) ureter (106, 120), ovarian mesothelium (113, 125), liver (93, 112); skin (98, 120), brain astrocytes (92, 118). D) The H-scores for squamous epithelium of eosophagus, uterine cervix, and oral cavity, for bronchioli epithelium, prostate glands, breast tubuli, bone marrow, kidney tubuli, gallbladder and thyreoid gland epithelia ranged from 70 to 100. E) The lowest scores were found in peripheral nerves, brain microglia, lung alveolar epithelium, and kidney glomeruli (below 50). Mean H-score values for group A were hMLH1 112 (SD18.7), hMSH2 133 (SD 25.5), B 114 (SD 29.7), 134 (SD 28.1), C 101 (SD 12.7), 121 (SD 13.4), D 82 (SD 12.2), 93 (SD 13.2) and E 72,9 (SD 22,4), 82 (SD 24,9), respectively. The values for groups A, B and C were higher than for groups D and E. The differences were highly statistically significant ($p < 0.0001$), both comparing groups and comparing particular tissues between each other.

Conclusions: Tissues predisposed to an increased risk of cancer in hMLH1/hMSH2 mutation carriers express constitutively a higher level of hMLH1/hMSH2 proteins, reflecting their higher need of DNA mismatch-repair. It is possible, that this fact is co-responsible for increased risk of cancer in these tissues along with other biological and environmental factors.

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617 POSTER

Apoptotic morphology of adherent cells in vital phase-contrast microscopy compared to scanning electron microscopy images

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Background: Volume regulation is essential for the preservation of cellular functions, but also includes the regulation of cell shrinkage during apoptosis. Our aim was to analyze the geometry and especially the microscopy optical halo around P31 mesothelioma cells for its relevance in the detection and study of morphological changes in cisplatin-induced apoptosis.

Materials and methods: A phase-contrast microscopy image analysis method to study cell geometry of adherent cells perfused with 10 mg/L cisplatin in culture medium. The method was compared to image analysis and semi-quantitative estimation of scanning electron microscopy images of cells treated the same way.

Results: Phase-contrast light microscopy cell shape changes, as well as the optical halo widening induced by 10 mg/L-cisplatin, correlated well to scanning electron microscopy demonstration of apoptotic morphology with cell membrane blebbing and sprouting.

Discussion: We conclude that image analysis of vital phase-contrast microscopy of adherent single cells is a useful tool to follow early apoptotic changes induced by cancer chemotherapeutics or other agents.

618 POSTER

Urokinase-type plasminogen activator is transcriptionally repressed during12-o-tetradecanoylphorbol-13-acetate-dependent differentiation of hl-60 cells

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Background: Urokinase-type plasminogen activator (uPA) is a key regulatory enzyme in a cascade of proteolytic events important for cell migration, tissue restructuring and tumor cell invasiveness. To gain insight on the mechanism of egression of differentiated myeloid cells from bone marrow, transcriptional regulation of uPA gene expression and invasiveness have been investigated during TPA-dependent differentiation of HL-60 cells.

Methods: Human promyelocytic leukemia, HL-60 cell line was obtained from the American Type Culture Collection (CCL 240). Total RNA was prepared by a modification of the method of Karlinsey et al. and Northern blot hybridization was assayed by the method of Virca et al. Nuclear extracts were prepared by the method of Lim et al. with a modification of the method of Gorski et al. The binding activities of nuclear protein factors on DNA sequence elements were determined by DNA mobility shift assay.

Results: uPA mRNA was decreased by TPA and sodium butyrate in HL-60 cells, but vitamin D, retinoic acid and DMSO did not affect. TPA repressed uPA gene expression in time- and dose-dependent manner, whereas PAI-1 was gradually induced. uPA mRNA level of control was almost reduced by pretreatment of actinomycin-D and cycloheximide enhanced uPA mRNA level. In DNA mobility shift assay using oligonuleotide containing GATA-1 binding site or PEA3/AP1 site on the uPA promoter, one specific DNA-protein complex was identified in nuclear extract prepared from control cells, respectively. In nuclear extract prepared from TPA-treated cells, the binding activity of GATA-1 and PEA3/AP1 were vanished. TPA-dependent repression of uPA mRNA was restored by pretreatment of staurosporin and PD98059, whereas SB203580 and tyrphostin were not effect. In DNA mobility shift assay, the binding activity of GATA-1 and PEA3/AP1 were restored by the pretreatment of staurosporin and PD98059. Motility and invasiveness of HL-60 cells were increased to 30 fold and 20 fold after the TPA treatment, respectively.

Conclusion: Reduction of binding activity of GATA-1 and PEA3/AP1 are related to transcriptional repression of uPA gene during TPA-dependent differentiation of HL-60 cells, and uPA activity may be not related to invasion in HL-60 cells. [This work was supported in part by Korea Research Foundation Grant (KRF-005-D00004)].

619 POSTER

Depending on the microenvironment, nitric oxide may switch to another way of cell death in cervical carcinoma cell lines treated with staurosporine

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Background: Apoptosis is a highly regulated cellular process that can be activated as a result of aberrant proliferation or differentiation, abrogation of cell fates, including proliferation, differentiation, cell survival and apoptosis, contributes to neoplastic transformation. Most chemotherapeutic agents target tumor cell proliferation, leading to the induction of an apoptotic response. Microenvironment of the cancer cell may direct the effect of these agents. Depending on the microenvironment, nitric oxide can be converted to various other reactive nitrogen species (RNS) such as nitrosonium cation, nitroxyl anion or peroxynitrite. It has been informed in the literature that the formation of different RNSs cause differences in signal transduction and gene expression. In this case, different RNSs are induced the gain of function or switch to another function for the same protein. In this study, *in vitro* effect of NO on cell death related to microenvironment components was questioned in cervical carcinoma cell which apoptosis was induced by staurosporin.

Material and Methods: *Cells and Experimental Treatments.* The human cervical carcinoma HeLa cell lines were grown in Dulbecco's modified Eagle's medium (DMEM) with 5% fetal calf serum and 1% penicillin/streptomycin. Cells were cultured in a humidified 5%CO2 atmosphere at 37°C. HeLa cell lines were treated with L-arginine (nitric oxide donor), L-NAME (reversible inducible nitric oxide synthase inhibitor) and, apoptosis inducer staurosporine and hydrogen peroxide (H2O2) (interacts with nitric oxide and generates different metabolites). *MTT assay.* Cell survival and proliferation was studied by a quantitative colorimetric MTT assay. 100 μ l well^{-1} of a 5 mgml^{-1} stock MTT solution was added to cell in a 96well microtiter plate previously seeded at a volume of 100 μ l well^{-1}. This was incubated at 37°C until the purple formazan crystal developed. Finally the MTT-containing medium was removed and 100 μ l of isopropanol with 0.04 N HCl was added to each well. The absorbance on an ELISA plate reader with a test wavelength of 540 nm and reference wavelength of 620 nm. *Hoechst 33342 / PI assay.* The DNA-binding dyes hoechst 33342 and propidium iodide (PI) were used together in a differential dye uptake assay for microscopic identification and quantification of membrane integrity and nuclear morphology.

Results: * Nitric oxide induced a dose and time dependent cell death by apoptosis in cervical carcinoma cell lines.

* Apoptosis inductive effect of NO was more in cells which were pre-induced with staurosporine.

* Hydrogen peroxide, which interacted with and produced different metabolites of NO, changed the effect of NO an apoptosis in staurosporine-induced cervical carcinoma cell line.

Conclusion: Redox homeostasis of microenvironment may designate and change the effect of NO on cell death. Thus, in new treatment protocols the effect of microenvironment should be in consideration.

620 POSTER

Cellular potassium ion deprivation may enhance apoptosis induced by cisplatin

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Background: The anticancer drug cisplatin induces cell death by apoptosis. Apoptosis is dependent on cellular loss of potassium ions (K^+). We therefore studied K^+-fluxes and cisplatin-induced apoptosis during K+ ion deprivation of mesothelioma cells with amphotericin B (a K^+ ionophore enhancing K^+ efflux), combined with the Na^+, K^+, $2Cl^-$ cotransport blocker bumetanide (inhibiting K^+ influx).

Materials and methods: Apoptosis was detected by nucleosome formation and caspase-3 activity. To study K^+ fluxes we quantified the K^+ analogue $^{86}Rb^+$ in cisplatin-induced apoptosis of mesothelioma cells.

Results: Amphotericin B, combined with bumetanide, markedly augmented cisplatin-induced nucleosome formation and caspase-3 activity. It is suggested that amphotericin B augmented cisplatin-induced apoptosis by increasing K^+ efflux, and that amphotericin B combined with bumetanide enhanced cisplatin-induced apoptosis by reduction of K^+ influx combined with stimulation of K^+ efflux.

Discussion: K^+ flux modulation could possibly be used to enhance the antitumour efficacy of cisplatin treatment.

621 POSTER

Cell cycle arrest and induction of apoptosis by novel Cdk inhibitor MCS-C2 is associated with deregulated ubiquitination pathway in prostate cancer cells

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Background: To search for a specific inhibitor of cell cycle regulation in human cancer cells, we synthesized an analogue of toyocamycin, MCS-C2