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.

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

B. Rack[1], W. Janni[1], C. Kentenich[1], P. Schaffer[1], R. Sykes[2], B. Spengler[1], S. Thieler[1], H. Sommer[1], S. Braun[3], K. Friese[1]. *[1] I. Frauenklinik der LMU Muenchen, Gynecology, Muenchen, Germany; [2] Applied Imaging Inc., Newcastle upon Tyne, GB; [3] Universitaetsklinikum für Frauenheilkunde, Leopold-Franzens-Universitaet, Gynecology, Innsbruck, Austria*

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

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

K. Mori[1], H. Yasuno[1], M. Kurasawa[1], M. Yanagisawa[1], Y. Sato[2], Y. Tanaka[1]. *[1] Chugai Pharmaceutical Co., Ltd., Product Research, Kamakura, Japan; [2] Chugai Pharmaceutical Co., Ltd., Pharmaceutical Research, Kamakura, Japan*

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

P. Plevova[1,2], E. Sedlakova[1], J. Zapletalova[3], Z. Kolar[1]. *[1] Medical Faculty, Palacky University, Dept. of Pathology and Laborat. of Molecul. Pathol, Olomouc, Czech Republic; [2] University Hospital, Dept. of Radiotherapy, Ostrava, Czech Republic; [3] Medical Faculty, Palacky University, Dept. of Biophysics, Czech Republic*

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

P. Behnam-Motlagh[1], P. Horstedt[2], K.G. Engstrom[3], R. Henriksson[4], K. Grankvist[1]. *[1] Umea University, Dept. of Medical Biosciences, Clinical Chemistry, UMEA, Sweden; [2] Umea University, Dept. of Medical Biosciences, Pathology, UMEA, Sweden; [3] Umea University, Dept. of Surgical and Preop. Sciences, UMEA, Sweden; [4] Umea University, Dept. of Radiation Sciences, Oncology, UMEA, Sweden*

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.