and proliferation in transformated epithelial tissue and significantly contribute to cancerogenesis and progression of cervical cancer.

1034 POSTER

Multidrug resistance (MDR) transporters and vault protein LRP as tamoxifen molecular targets

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Background: Tamoxifen (TAM) is an effective antiestrogen in therapy of breast cancer. But there are other clinical important activities of TAM, one of them is overcoming the chemotherapy resistance under TAM action. Thinking over the reasons of that we have supposed TAM interaction with multidrug resistance (MDR) mechanism namely TAM binding to the MDR-transporters and vault protein LRP extruding anticancer drugs out of the cells.
Materials and Methods: TAM influence on specific monoclonal antibody (mAb) binding to Pgp, MRP1 and vault protein LRP was estimated by flow cytometry in human cell cultures Jurkat (T-lymphoblastic lymphoma) and HeLa (cervical cancer), overexpressing the MDR-markers. Mean fluorescence of mAb-labelled cells as well as the number of mAb-labelled cells were calculated over fluorescence area of isotypic controls.
Results: 1. Incubation of the cells with mAbs increased significantly their fluorescence intensity compared to the isotypic controls. 2. It was not any influence of TAM on isotypic Abs binding to the cells. 3. Incubation of the cells with 50×10^{-6} M TAM changed interaction of mAbs with the MDR-markers investigated. The mean cell fluorescence intensity in the area of specific fluorescence of mAbs and the number of mAb-labeled cells was changed but with different manner for different MDR-markers. Under TAM action the indexes for MRP1 and LRP mAbs decreased up to more than 2 times. TAM effect on mAb interaction to Pgp was different in living cells and in the cells after 0.5% Tween 20 permeabilization. For the first one, TAM increased the mean specific cell fluorescence intensity and the number of mAb-labeled cells up to more than 4 times. For the second one, the indexes decreased up to more than 2 times.
Conclusion: These data are direct evidence for the TAM interaction with the Pgp, MRP1 and LRP in tumor cells. It should decrease further binding of anticancer drugs with the MDR-markers and thereby inhibit MDR-mechanism through decrease of the MDR-drug transport out of the cells. This can be regarded as confirmation of our assumption that TAM interaction with Pgp, MRP1 and LRP may be one of the reasons for clinical overcoming chemotherapy resistance under TAM action. The conclusion is true for the MDR-drugs and the tumors expressing MDR-phenotype only and explains the TAM insufficient in increase chemotherapy efficacy in some patients.
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1035 POSTER

Autophagic cell death of the nutrient deprivation augmented by cytotoxic drugs in lung cancer cell

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Autophagy is known for its role in cellular homeostasis, development, cell survival, aging, immunity, and cancer. Autophagy has emerged as another major 'programmed' mechanism to control life and death much like "programmed cell death" is for apoptosis in several types of cancer. To be elusive that autophgic cell death on nutrient starvation in combination with cytotoxic drugs, we investigated whether its increase synergistically in two mixed conditions. When cancer cells were subjected to extreme nutrient starvation by culturing in a medium without serum and amino acids or with 2-deoxyl-D-glucose, a chemical inhibitor of glucose metabolism, cells death occurred within early time. At nutrient deprived media with cisplatin or gemcitabine treatment, Cell survival revealed a markedly decrease in percentage of living cells undergoing nutrient starved medium with each of two cytotoxic drugs compared with those drugs respectively. The staining of cells in normal media with acridine orange displayed green fluorescence with cytoplasmic and nuclear components in normal media but showed considerable red fluorescence in combined medium or cytotoxic drugs in each treated cells, suggesting formation of numerous acidic autophagolysosomal vacuoles. LC3 modification, as autophagy marker, was analyzed by western blotting. LC3 proteins have two forms: type I is cytosolic and type II is membrane-bound. During autophagy is advanced, LC3 type II increased by conversion from LC3 type I. We figured out that the autophagosome-incorporated LC3 II protein expression more increased in cell contained nutrient-deprived medium with cytotoxic drugs compare with cisplatin or gemcitabine alone. These results demonstrated that the autophagic cell death potentially increased in nutrient-deprived conditions combined with cytotoxic drugs in human lung cancer cell lines.

1036 POSTER

Response of CD133+/− subpopulations of CRC cell lines to radio- and chemotherapy

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Background: More than 40% of colorectal cancers (CRC) are located in the rectum. Preoperative 5-FU based chemoradiotherapy is recommended for locally advanced rectal adenocarcinomas. However, therapy response of individual tumors is not uniform. Complete regressions but also resistant tumors are described which may be related to a discrepancy in the presence or survival of tumor-initiating/cancer stem (TIC/CSC) cell populations leading to an individual risk of recurrence. The hypothesis of such cell populations to relate to therapy resistance and recurrence of disease is challenging because the only tool to identify or isolate such cells are surface markers with limited causal evidence. CD133 has recently been described as a potential marker to enrich TIC/CSC from primary CRC material and from the cell line HT29. The aim of our study was to investigate tumorigenic potential, radioresponse and drug efficacy in another CRC cell line which contains two distinct populations defined by their CD133 expression.
Material and Methods: The cell line HCT-116 showed two clearly distinguishable $CD133^+$ ($74.3\pm6.2\%$) and $CD133^-$ subfractions. Subpopulations were isolated via FACS and analyzed in parallel to the original, mixed cells for colony formation and therapy response *in vitro*. $SF_{2\,Gy}$ and IC_{50} values after single dose irradiation or treatment with 5-FU or Oxaliplatin (Oxa) were calculated from dose response curves. Tumorigenicity was evaluated in a subcutaneous xenograft model.
Results and Conclusions: Colony forming capacity and radioresponse of $CD133^+$ and $CD133^-$ HCT-116 subpopulations did not differ. The $SF_{2\,Gy}$ was $32.0\pm4.2\%$ for $CD133^+$ and $34.1\pm3.8\%$ for $CD133^-$ HCT-116 cells. Also, the IC_{50} values after 5-FU and Oxa treatment were comparable for HCT-116 cells with discrepant CD133 expression. The mean IC_{50} for 5-FU was $5.8\pm1.0\,\mu M$ for $CD133^+$ and $6.3\pm1.6\,\mu M$ for $CD133^-$ cells and reflected the original HCT-116. Oxa efficacy was slightly lower but revealed the observation with respect to $CD133^{+/-}$ subpopulations. Since $CD133^+$ and $CD133^-$ HCT-116 cells showed a similar xenograft formation capacity, CD133 can neither be regarded as a TIC/CSC marker in HCT-116 cells nor does it define a subpopulation with higher resistance to radio-/chemotherapy *in vitro*. The underlying reasons for differences between HT29 (literature) and HCT-116 are unknown. Extended studies including HT29 cells are ongoing.
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1037 POSTER

Cytostatic agents, radiosesitizers and immunomodulators derived from tropolone alkaloids

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Background: It has been implemented – synthesis of the new compounds obtained on the basis of tropolone alkaloids – colchicine and colcemid with a range of amino-substitutes in tropolone and heptadiene rings of the alkaloids, in the laboratory on development of anti-tumor agents of NSCO of MH of RUz. Biological properties of those are being studied and have shown to possess lowered toxicity (in 10–400 times) and high anti-tumor activity in vitro found in NCI USA.
Results: On the basis of study of toxicity and anti-tumor activity in vivo among the new derivatives 5 new agents has been selected: K-48, K-42, Decocine, Decovine, K-20, which have passed (Decocine) or are at stage of pre-clinic study. These compounds are in 13–360 times less toxic than colchicines, their anti-tumor efficiency exceeds activity of both colchicine and colcemid for 20−70%, and in a range of known cytostatics used as control. K-48 together with expressed anti-tumor activity doesn't lower immunity and hemopoiesis, that is reasoned by its ability towards CFUs increase. Cytogenetic studies has shown that K-48 in therapeutic dose doesn't cause chromosome aberrations, k-mitosis and polyploidy in bone marrow, that is peculiar to tubulin-interacting and alkylating drugs, and also lowers amount of chromosome aberrations since treatment. All it is characteristic in some degree for the ?-42 agent at per oral application.