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

E.A. Dudko[1], T.A. Bogush[1]. [1]*Russian N.N. Blokhin Memorial Cancer Research Center Russian Academy of Medical Sciences, Laboratory of Medical Chemistry, Moscow, Russian Federation*

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

S. Yang[1], H. Jo[1], H. Jang[1], M. Lee[1], K. Lee[1], S. Moon[1], J. Park[1], E. Jeong[1]. [1]*Wonkwang University School of Medicine, Deparment of Internal Medicine, Iksan-city Jeonbuk, South Korea*

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

C. Dittfeld[1], S. Peickert[1], A. Dietrich[1], M. Grade[2], T. Ried[3], L.A. Kunz-Schughart[1]. [1]*OncoRay – Center for Radiation Research in Oncology, Tumor Pathophysiology, Dresden, Germany;* [2]*Department of General and Visceral Surgery, University Medicine, Göttingen, Germany;* [3]*National Cancer Institute, National Institutes of Health, Bethesda, USA*

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

Z.M. Yenikeeva[1], S.N. Navruzov[2]. [1]*National Research Center of Oncology, Science, Tashkent, Uzbekistan;* [2]*National Research Center of Oncology, Proctology, Tashkent, Uzbekistan*

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.

Curing of mice by K-48 in low doses after chemical and irradiation influence promoted regeneration of bone marrow cellularity (karyocyte), restoration of mass of body, spleen, hemopoiesis (leukocytes and granulocytes) and immunity, and makes terms of their restoration lesser.
The anti-tumor drugs Decocine and Decovine also possess expressed radiosensitizing properties, exceeding the same action of 5-fluorouracil, that is reasoned their ability both to influence on DNA synthesis and synchronize cells in $M+G_2$ phase.
At present, clinical trials of Decocine are in process. It is revealed that it possesses ability to cure skin cancer; at it, negative affects on hemopoiesis and immunity has not been observed.
Among available synthesized compounds some new anti-tumor, immune-modulating, anti-inflammatory, anti-cirrhotic compounds are selected.

1038 POSTER

Correlation between frequency of BRAF V600E (T1796A) gene mutation and appearance of papillary thyroid carcinoma in a sample of Croatian population

D. Katalinic[1], S. Plestina[1]. *[1]Zagreb University Medical Center, University Department of Oncology, Zagreb, Croatia*

Papillary thyroid carcinoma (PTC) is the most common malignant tumor of the thyroid gland. There is several oncogenes as BRAF, RET/PTC, RAS, TRK which are involved in cancerogenesis of thyroid cells, but recently, BRAF oncogene, a serine-threonine kinase involved in the phosphorylation of MAPK signaling pathway responsible for cellular proliferation, has become a subject of great importance and interests. The BRAF gene mutations are found in 30−70% of all variants of PTC but there is no any data obout correlation between frequency of BRAF V600E (T1796A) mutation located in the exon 15 of BRAF gene (resulting in the substention of valine to glutamate at codone 600), with appearrance of PTC in a sample of Croatian examinees. We enrolled two group of patients: 59 subjects with PTC (mean age 39.6±3, range 28−56 years) and 68 healthy control subjects (mean age 40.2±2, range 25−55 years) without any history of malignancy in which the clinical evaluation including ultrasound of the neck and thyroid gland did not reveal any thyroid and neck pathology. Genomic DNA was isolated from peripheral venous blood while analysis of BRAF V600E (T1796A) gene mutation was performed using PCR-RLFP methode. The V600E (T1796A) gene mutation was detected in 21 samples in subjects with PTC (36.0%) compared to healthy group in which is mutation detected in 2 samples (2.9%). The difference was statisticaly significant ($p < 0.0001$). Our results indicate that the V600E (T1796A) mutation of the BRAF gene is genetic alteration with high frequency found in PTC amoung Croatian examinees and it could be used as a reliable genetic and preoperative marker but further investigation are needed to confirm these results.

1039 POSTER

Immunohistochemical staining of mammaglobin in breast cancer

E.A. Baker[1], L. Hall[2], J. France[2], D. Whetter[1], P. Bhaskar[1]. *[1]University Hospital of North Tees, Professorial Unit of Surgery, Stockton on Tees, United Kingdom; [2]University Hospital of North Tees, Histopathology Department, Stockton on Tees, United Kingdom*

Background: Breast tumours are heterogeneous and new tumour markers are sought to improve patient diagnosis and prognosis. Mammaglobin A appears to be a suitable marker, as it is breast-specific and elevated in up to 80% of breast tumours. This study aims to examine the relationship between mammaglobin A expression in breast cancer specimens with pathological grades/markers.
Materials and Methods: 100 breast tumour specimens were analysed by immunohistochemistry for mammaglobin A expression. Stained sections were screened under the microscope with sections regarded as positive when >10% of lesional cells stained positive. For comparison purposes histological grade, tumour type, tumour size, ER, PR, Her-2 status and the presence/absence of nodal metastasis were recorded. Controls of benign breast conditions were also included.
Results: Mammaglobin was found to be absent in benign conditions and elevated in both invasive and in situ carcinoma. There was a positive correlation between ER positive status and mammaglobin A expression (57% correlation, $p < 0.05$, Chi Squared). There was also a positive correlation between lower tumour grades 1 and 2 (62 and 55% respectively) and mammaglobin A expression, whilst a negative correlation with grade 3 tumours, with mammaglobin protein expression decreasing as tumour grade increased. No correlation was found between presence/absence of nodal metastases, PR status, Her-2 status or tumour size.
Conclusions: Since positive ER status and lower tumour grade are associated with a better prognosis for breast cancer patients, then mammaglobin A protein expression may also be associated with a better prognosis. However, long-term follow-up is required to determine this.

1040 POSTER

KDR/Flk-1 expression in the tumor tissue vascular endothelial cells in two groups of breast cancer patients

I. Nasunova[1], A.A. Lushnikova[1], A.A. Parokonnaya[1], V.D. Ermilova[1]. *[1]Cancer Research Center, Carcinogenesis Institute, Moscow, Russian Federation*

Background: Vascular endothelial growth factor (VEGF) is an endothelial cell specific mitogen which plays a role in pathogenic vascularity associated with carcinogenesis. Tumor cells and primary tumor tissues are known to express high levels of VEGF receptors. We try to evaluate VEGF receptor KDR (human homolog of Flk-1) or VEGFR2 expression in vascular endothelial cells of tumor tissue of the patients with sporadic breast cancer (BC) and pregnancy associated BC (PABC) of comparative age to suggest a bases of progression and dramatic tumor growth in PABC patients.
Material and Methods: Paraffin embedded tumor tissue sections from 12 sporadic BC patients and 12 PABC ones (250 and 300 vascular sections per each group in sum) were studied using image analyze by MatLab 7.0 algorithm. Representative images were confirmed by histopatological study. Immunohistochemical staining of tumor sections was made using pre-diluted antybodies for KDR/Flk-1, VEGF-A and DAP (Dako). KDR expression in fresh tumor tissues from three BC and three PABC patients was estimated also by RT PCR using primers for KDR encoding region (chr.4q11-q12) in comparison with GAPDH gene expression.
Results: Images obtained by Nicon digital microscope were studied for quantifying of VEGFR2 (KDR) expression revealed by immunohisto-chemistry. Preliminary automated image analysis of the receptor core number (density) in vascular endothelium sections of tumor tissue with comparative histological subtype and grade was revealed a significant difference in expression level between BC and PABC tumor tissues with receptor cores number 1.78±0.62 and 0.36±0.20 per vascular endothelium length unit in BC and PABC patients, respectively ($p < 0.002$). Over expression of KDR in PABC tumor tissues in comparison to BC ones was confirmed by RT PCR Automatic extraction of DAB-positive cores along vascular endothelium scatter plots showed more homogenous expression pattern in PABC tumors than in sporadic BC ones.
Conclusion: The data obtained suggest that VEGFR2 (KDR) expression in breast tumor vascular endothelium from PABC patients is higher than in sporadic BC tissues and it indicates on more intensive growth of the tumors and pathological evaluation of BC. Much higher VEGFR2 density and vascularity in PABC tumor tissue may induce activation of specific signal pathways, dramatic tumor progression and further angiogenesis in pregnancy – associated BC patients.

1041 POSTER

Use of tumor markers in a medicine department- a baseline and a post interventional study

I. Luis[1], M.J. Nunes da Silva[2], P. Monteiro[2], L. Pinheiro[2], J. Ribeiro[1], T. Gaspar[2], R. Dutschman[2], J. Sampaio Matias[3], M. Lucas[2], R.M.M. Victorino[2]. *[1]Hospital Santa Maria, Oncology, Lisboa, Portugal; [2]Hospital Santa Maria, Internal Medicine, Lisboa, Portugal; [3]Hospital Santa Maria, Clinical Pathology, Lisboa, Portugal*

Background: Tumour markers (TM) are potentially useful in clinical practice, but seem to have a limited role in terms of diagnostic because of their poor sensitivity and specificity. Several guidelines exist on the appropriate use of TM, however they are frequently overused. The aim of the present study was to assess the impact of informative and audit activities about the correct use of TM on the use of TM in an internal medicine department in a baseline and in a post interventional evaluation.
Materials and Methods: A baseline study was conducted in an internal medicine department, with all patients to whom TM were requested, over a three month period. Clinical data were extracted from clinical files. The appropriate or inappropriate requests were determined according international guidelines. Results of this study were presented to the clinical staff and informative actions were performed. A post-interventional study was done, using the same methodology as the baseline study.
Results: At baseline TM were requested in 19.6% of patients from the evaluated period. After the intervention this figure dropped 42.6% to 10.2%. In the baseline study the main reason for TM request was diagnosis while in the post-interventional study it screening. In both studies the majority of appropriate requests were done for screening. In both studies most of inappropriate requests were done for diagnosis. In the baseline study 17, 5% of the requests were considered appropriate and there were an increase of appropriateness (TM appropriated in the post-interventional