347 POSTER

Expression of activated forms of transcription factors ATF-2, CREB and c-Myc in rat colon transversum after whole body gamma-irradiation and its contribution to pathology of intestinal form of radiation disease and biodosimetry

J. Pejchal, J. Österreicher, Z. Vilasová. *University of Defence Military Health Faculty, Department of Radiobiology, Hradec Kralove, Czech Republic*

Background: The purpose of our study is to examine the expression of phospho-ATF-2 Thr69/71 (p-ATF-2), phospho-CREB Ser133 (p-CREB) and phospho-c-Myc Thr58/Ser62 (p-c-Myc), the activated forms of transcription factors and the targets of ERK, SAPK/JNK and p38MAP kinase pathways in irradiated colon transversum in rats and to assess their ability as bidosimetric markers of irradiated enterocytes in vivo.
Materials and Methods: Male Wistar rats were randomly divided to 28 groups and irradiated with whole body γ-radiation of 0, 0.25, 0.5, 0.75, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 Gy (^{60}Co, 1.44 Gy/min). Tissue samples of colon transversum were taken 4 and 24 hours after the irradiation, immunohistochemically stained and p-ATF-2, p-CREB and p-c-Myc expression was measured in cytoplasm and nucleus using computer image analysis.
Results: We measured a significantly increased cytoplasmatic and nuclear expression of p-ATF-2 4 hours after irradiation by dose range of 0.25−1 Gy, and by dose of 10 Gy. Cytoplasmatic expression of p-ATF-2 24 hours after irradiation was significantly increased by dose range of 0.5−1 Gy, and dose of 10 Gy whereas nuclear p-ATF-2 expression increased after 0.25−1 Gy, 9, and 10 Gy irradiation.
Significantly increased cytoplasmatic expression of p-CREB 4 hours after irradiation was found by dose range of 0.25−1 Gy, and by doses of 9 and 10 Gy. The nuclear expression 4 hours after irradiation was increased in the dose range of 0.25−0.75 Gy and by dose of 9 Gy. Significantly increased cytoplasmatic expression of p-CREB 24 hours after irradiation was found by dose range of 0.25−1 Gy, and doses of 4 and 10 Gy and nuclear expression increased 24 hours after 0.25−0.75 and 10 Gy irradiation.
Significantly higher values of cytoplasmatic p-c-Myc expression 4 hours after irradiation were found by doses of 0.25, 0.75, 4, and 5 Gy and 24 hours after irradiation by doses of 0.75, 1, and 10 Gy. Values of nuclear expression of p-c-Myc 4 hours after irradiation were significantly higher by doses of 0.25−0.75, 2, 4, and 5 Gy and 24 hours after the irradiation by doses of 0.25, 0.75−3, 9, and 10 Gy.
Conclusion: The detection of p-ATF-2 and p-CREB might be considered as a perspective biodosimetric marker of irradiated enterocytes in vivo. It might be useful as a qualitative marker in the range 0.25−1 Gy during first 24 hours after irradiation. The use of p-c-Myc seems to be more controversial.

348 POSTER

The effect of NOS inhibitor L-NAME on human KB carcinoma cells overexpressing COX-2

K. Takaoka, M. Urade, H. Kishimoto, S. Hashitani, E. Segawa, Y. Zushi, K. Noguchi. *Hyogo College of Medicine, Oral and Maxillofacial Surgery, Nishinomiya Hyogo, Japan*

Background: Cyclooxygenase (COX)-2 and inducible nitric oxide synthase (iNOS) are major inflammatory mediators. It has been shown that NO produced by iNOS is important in the carcinogenic process. Recent studies have suggested that COX-2 expression is also involved in carcinogenesis, tumor cell growth, invasion and metastasis. COX-2 inhibitors have recognized widespread as an anti-tumor drugs, but elicit potentially adverse side effect. We investigated the possible relationship between COX-2 and NO using a human epidermoid carcinoma cell line KB overexpressing COX-2 protein by gene transfer and the possibility of iNOS inhibitor as an anti-tumor drug.
Materials and Methods: We isolated a COX-2 transfected clone KB/COX-2 and a neomycin-transfected clone KB/neo as the control, and were used in this study. Celecoxib was used as a selective COX-2 inhibitor and, L-NAME as a NOS inhibitor. Cell growth was assayed by MTT, and PGE2, COX-2 and NO production from the cells were assayed by ELISA. Apoptosis was measured by single stranded DNA (ssDNA) apoptosis ELISA kit. Cell motility was measured using Matrigel invasion assay. COX-2 and iNOS were detected by western blot analysis and RT-PCR.
Results: All agents inhibited cell growth of both clones to the same extent in a dose-dependent manner and induced apoptosis. The PGE2 and COX-2 production were inhibited by not only celecoxib, but L-NAME. KB/COX-2 exhibited 4-fold increase of COX-2 protein expression and produced PGE2 13 times as compared to KB/neo. On the other hand, KB/COX-2 showed higher expression and production of NO than did KB/neo, and NO production was inhibited by L-NAME. The cell motility through Matrigel was higher in KB/COX-2 than in KB/neo, and was decreased by treatment with all agents.
Conclusions: COX-2 inhibitor and NOS inhibitor can inhibit cell growth and induce apoptosis regardless of their COX-2 expression. The cell motility of human KB carcinoma cells was promoted by overexpression of COX-2 and inhibited not only by COX-2 inhibitor but also by iNOS inhibitor. COX-2 production was inhibited by L-NAME. L-NAME would be helpful in the design and development of new anti-tumor drugs.

349 POSTER

Concordance of expression subtypes in synchronous but not metachronous bilateral breast carcinomas

E. Kuligina[1], A. Sokolenko[1], A. Iyevleva[1], K. Buslov[1], A. Togo[1], D. Matsko[2], H. Baelde[3], E. Jordanova[3], C. Cornelisse[3], E. Imyanitov[1]. *[1]N.N. Petrov Research Institute of Oncology, Molecular Oncology, Saint Petersburg, Russian Federation; [2]N.N. Petrov Research Institute of Oncology, Pathology, Saint Petersburg, Russian Federation; [3]Leiden University Medical Center, Pathology, Leiden, The Netherlands*

Background: Microarray-based expression profiling of breast cancer (BC) has allowed to identify several distinct BC subtypes, e.g. luminal A and B, ERBB2+, basal, and normal-like BC variants [Sorlie et al., 2006]. Each of these BC phenotypes is characterized by expression of particular molecules, therefore a sufficiently accurate discrimination between BC variants can be done not only by cDNA arrays, but also by the analysis of limited number of subtype-specific markers.
Materials and Methods: Present study included 100 paired archival paraffin-embedded tumor samples obtained from 50 bilateral breast carcinoma (biBC) patients. ER, ERBB2, vimentin, P-cadherin, GATA3 as well as basal and luminal cytokeratins were analyzed by immunohistochemistry. MUC1, C-kit, GRB7, STAT1, CX3CL, PRNPIP expression was evaluated using real-time PCR measurement of the content of RNA transcripts.
Results: Concordance of molecular subtypes was observed in 17/23 (73%) synchronous biBC; this estimate significantly differed from the one expected for random distribution of BC expression variants ($p = 0.050$). Furthermore, when the most unfavorable BC phenotypes, i.e. ERBB2+ and basal, were analyzed together, the level of concordance reached as high as 91% ($p = 0.008$). In contrast to simultaneously arising tumor pairs, no trend towards expression concordance was observed in 27 metachronous biBC pairs ($p = 0.759$).
Conclusions: This study adds evidence to the non-random nature of BC molecular subtypes. It appears that host and environmental factors do play a role in choosing the molecular variant for BC development.

350 POSTER

A role for the Fas/FasL system in modulating genetic susceptibility to T-cell lymphoblastic lymphomas

M. Villa-Morales[1], J. Santos[1], E. Perez-Gomez[2], M. Quintanilla[2], J. Fernandez-Piqueras[1]. *[1]Universidad Autonoma de Madrid, Biologia, Madrid, Spain; [2]Instituto de Investigaciones Biomedicas Alberto Sols, CSIC-UAM, Madrid, Spain*

The Fas/FasL system mediates induced apoptosis of immature thymocytes and peripheral T-lymphocytes, but little was known about its implication in genetic susceptibility to T-cell malignancies.
Using a mouse model for gamma radiation-induced T-cell lymphoblastic lymphoma, two strains of mice exhibiting extreme differences of genetic susceptibility were analysed: C57BL/6J (Mus musculus) and SEG/Pas (Mus spretus). FasL transcriptional expression in the two strains under different conditions was determined by real-time quantitative RT-PCR. Their FasL promoters were sequenced and cloned into reporter constructs (pGL2-Basic, Promega), in order to analyse their differential activity through luciferase assays. Also, Fas and FasL cDNAs derived from the two strains were sequenced and cloned into expression vectors (pcDNA3, Invitrogen). In an effector-target manner, cells bearing C57BL/6J- or SEG/Pas-derived Fas and FasL were analysed for Fas/FasL-induced caspase-8 and 3 activation (Caspase-Glo 8 Assay, Promega, and Western Blot), as well as for apoptosis induction (TUNEL Assay). Also, apoptosis induction after the irradiation treatment was determined through TUNEL Assay in thymic T-cells derived from the two strains of mice.
Here we report that the expression of FasL increases early in all mice after gamma-radiation treatments, maintaining such high levels for a long time in mice that resisted tumour induction. However, its expression is practically absent in T-cell-lymphoblastic-lymphomas. Interestingly, there exist significant differences in the level of expression between two mice strains exhibiting extremely distinct susceptibilities that can be attributed to promoter functional polymorphisms. In addition, several functional nucleotide changes in the coding sequences of both Fas and FasL genes