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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

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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.

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

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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.

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

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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.

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

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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

significantly affect their biological activity, as the SEG/Pas-derived Fas/FasL system drives cell apoptosis to a significantly higher extent than the C57BL/6J-system in vitro, and is far more efficient in vivo, subsequently leading to a significant increase in gamma-radiation induced-apoptosis of thymic T cells.
These results lead us to propose that germ-line functional polymorphisms affecting either the levels of expression and/or the biological activity of both Fas and FasL genes could be contributing to the genetic risk to develop T-cell lymphoblastic lymphomas.

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Genetic background and cervical development: the influence of cytochromes P450IID6 genotypes

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Background: CYP2D6, a member of the Cytochromes P450 (CYP) family, is a phase I metabolic enzyme involved in the oxidative metabolism of numerous endogenous and exogenous molecules, including pro-carcinogens molecules. The CYP2D6*4 polymorphism has been reported to be a major cause of CYP2D6 poor metaboliser phenotype, leading to the absence or decrease in the amount and activity of its protein. The aim of this study was to understand the role of CYP2D6 genotypes on the development of cervical cancer.
Material and Methods: This study included 378 patients diagnosed with cervical cancer in the Portuguese Institute of Oncology – Porto, Portugal and 334 women without history of oncology disease. DNA was extracted from peripheral blood and submitted to Polymerase Chain Reaction (PCR) followed by Restriction Fragment Length Polymorphism (RFLP), in order to identify the CYP2D6 genotypes.
Results: The genotypes frequencies of the patients group were: 74.30% GG, 21.4% AG and 4.2% AA. In the other group the genotypes frequencies were: 65.57% GG, 27.35% AG and 7.19% AA. We observed that patients carrying the A allele have protection to the development of cervical cancer (OR = 0.698; 95% CI 0.480−1.014; p = 0.059).
Conclusions: The A allele of this polymorphism is responsible of the poor metaboliser phenotype. Therefore, women with AG or AA phenotype will have less ability for metabolizing the pro-carcinogenic molecules, which justify the protective association found in this study to the development of cervical cancer. Our results suggests the influence of genetic background as a cofactor in cervical cancer, a human papillomavirus associated neoplasia.

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Apicidin overcomes TRAIL-resistance on Bcr-Abl expressing K562 cells through inhibition of PI3K/AKT mediated pathway

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Background: Tumor necrosis (TNF)-related apoptosis-inducing ligand (TRAIL) is a pro-apoptotic cytokine that is capable of inducing apoptosis in a wide variety of cancer cells but not in normal cells. Although many cancer cells are sensitive to TRAIL-induced apoptosis, Chronic myeloid leukemia (CML) develop resistance to TRAIL. Histone deacetylase (HDAC) inhibitors are emerging as a new class of anticancer agents, here we investigated histone deacetylase inhibitor apicidin can overcome the TRAIL resistance in CML cells.
Materials and Methods: The effect of combination of apicidin with TRAIL in CML-derived K562 cells was assessed by annexin V analysis. Also, activation of caspase and the changes in the amounts of DR4, DR5, PI3K NF-κB, Bcl-xL, and Bcr-Abl proteins were analyzed by immunoblots. The blocking of TRAIL receptor on apicidin-induced sensitization to TRAIL was evaluated as using neutralizing antibodies DR4 and DR5. The effects of inhibiting PI3K and AKT were also examined by treating K562 with LY294002 and AKT inhibitor IV, which are selective inhibitors of PI3K and AKT, respectively. To explore whether expression of Bcr-Abl contributes to TRAIL-resistance, the sensitization of TRAIL on the Bcr-Abl deleted K562 cells was examined.
Results: Apicidin enhanced TRAIL-induced apoptosis via caspase activation without mediating through TRAIL receptors, DR4 and DR5, although both receptors are expressed in K562 cells. Apicidin downregulated PI3K and enhanced the effect of LY294002 and AKT inhibitor IV on TRAIL induced-apoptosis. Moreover, Bcr-Abl as well as NF-κB and Bcl-xL were also decreased after treating with apicidin, and Bcr-Abl-deleted K562 cells were sensitized to TRAIL.
Conclusion: Our results demonstrated that apicidin can overcome resistance to TRAIL through downregulation of Bcr-Abl and inhibition of PI3K/AKT in K562 cells. Moreover, Inhibition of PI3K activity by apicidin resulted in diminished phosphorylated AKT, inhibition of NF-KB transcriptional activity and significant reduction of expression of NF-KB-dependent protein, Bcl-xL. These were associated with enhancement of the intrinsic sensitivity of cancer cells to cytotoxic effect of TRAIL, therefore, combination of apicidin with TRAIL may be an effective strategy for treating TRAIL-resistant Bcr-Abl expressing CML cells

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Effect of sulfinosine [(R,S)-2-amino-9-beta-D-ribofuranosylpurine-6-sulfinamide] on lung carcinoma cell lines and its role in overcoming multidrug resistance

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The acquired multidrug resistance (MDR) phenotype in cancer cells is defined as resistance to an applied drug, as well as to many structurally and functionally unrelated compounds. It often develops as a result of changes in drug influx/efflux pumps and changes in glutathione (GSH) detoxification system.
Our research was focused on studying the molecular mechanisms underlying MDR in the non-small cell lung carcinoma cell line (NSCLC) that was selected for resistance to doxorubicin (DOX). In an attempt to successfully modulate MDR, we studied the cytotoxicity of sulfinosine (a guanosine analog) on NSCLC cells, its effect on GSH level in cells and potential to alter the expression of MDR-related genes: mdr1, gst-pi and topo II alpha. In addition, we examined the effects of sulfinosine (SF) in combination with an anti-neoplastic agent curcumin.
The cytotoxic effects of SF, curcumin and their combination on sensitive (NCI-H460) and resistant (NCI-H460/R) cell lines was measured by the sulforhodamine B assay, and their interaction was analyzed with Calcusyn software. GSH level in these cells, both treated and untreated, was assessed using Glutathione Colorimetric Detection Kit. The expression of MDR-related genes was evaluated by semi-quantitative RT-PCR.
Our study showed that the cytotoxic effect of SF was dose-dependent in both cell lines. Interaction of SF and curcumin antagonized growth inhibition in the NCI-H460 cell line while their effect on NCI-H460/R was synergistic. In the NCI-H460/R compared to the NCI-H460 cell line, MDR-related genes had significantly altered expression: mdr1 and gst-pi were 7-fold and 50% increased, respectively, whereas topo II alpha was 2-fold decreased. RT-PCR gene expression analysis in the resistant cell line demonstrated that: (i) SF down regulated the expression level of mdr1; (ii) curcumin decreased the expression level of mdr1 and gst-pi; (iii) a combination of these drugs synergistically decreased the expression of mdr1 and not the expression of gst-pi mRNA.
Further studies revealed that GSH level didn't differ between these two cell lines. Still, it was significantly decreased under low concentration of SF both in NCI-H460 and NCI-H460/R.
In general, our results revealed that the MDR phenotype can be modulated by SF and curcumin, both on level of gene expression and on glutathione level. Moreover, the combined application of these two drugs exceeds the effects obtained after treatment of NCI-H460/R with only one agent.

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A-toxin enhancement of cisplatin-induced apoptosis in cisplatin-resistant mesothelioma cells

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Cisplatin (cis-diamminedichloroplatinum) is a drug used in the treatment of several solid tumors and is extensively used in the treatment of pulmonary mesothelioma. After an initial response the effectiveness of cisplatin is often hampered by inherent or acquired cisplatin resistance causing a severe problem in the treatment of these malignancies. α-toxin (α-hemolysin) from Staphylococcus aureus is a pore-forming toxin which induces apoptosis (intrinsic cell suicide) in eukaryotic cells. Disability to enter apoptosis is a key component in the development of cancer. Finding methods to overcome tumour cell drug resistance to apoptosis could greatly enhance current chemotherapy.