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Dihydroartemisinin-induced apoptosis depends on the presence of proapoptotic Bax or Bak and increases efficacy of ionizing radiation and targeted chemotherapy in vitro

T. Ontikatze[1], K.D. Bauer[2], A. Rübel[2], F. Freier[2], C. Belka[2], V. Jendrossek[1], R. Handrick[1]
[1]University of Duisburg-Essen, Molecular Cell Biology, Essen, Germany; [2] University of Tübingen, Department of Radiation Oncology, Tübingen, Germany

Introduction: Molecular signaling of apoptosis involves intermediate formation of reactive oxygen species (ROS). Properties of endoperoxides as mediators of ROS provide a rationale for their application in tumor treatment. The radical forming antimalaria drug artemisinin exerts promising cytotoxic effects. Aim of our present study was to evaluate the antineoplastic potential of the derivative dihydroartemisinin (DHA) alone and in combination with ionizing radiation or chemotherapy (TRAIL) focusing on its proapoptotic action.

Methods: Cell death in Jurkat T-lymphoma, HCT116, DU145 was analyzed by IF-microscopy, flow cytometry and immunoblotting. To elucidate molecular signaling, clones with alterations in the receptor pathway (FADD-, Caspase-8 neg.) or deficient mitochondrial pathway (lack of Bak, overexpression of Bcl-2, Bcl-xL, caspase-9DN) and DU145 reexpressing Bax as well as HCT116 clones lacking either Bax, Bak or Bax/Bak and RNAi approache were used. Activation state of proteins was analyzed by activation specific antibodies. Lipid peroxidation was measured by flow cytometry (Bodipy®).

Results: DHA induced apoptosis in Jurkat, HCT116 and DU145 in a time- and dose-dependent manner yielding 60% apoptotic cells after 24h (12.5-25μM). Cyt c release, breakdown of $\Delta\psi$m, caspase activation, PARP cleavage and DNA fragmentation were observed. Inhibition by glutathione and N-acetylcysteine support a ROS dependent mechanism. Moreover, alterations in the emission spectrum of Bodipy® suggest membrane oxidizing potential. Over-expression of caspase-9DN or of antiapoptotic Bcl-xL or Bcl-2 decreased mitochondrial alterations and DNA-fragmentation while absence of FADD or Caspase-8 did not alter apoptosis rates. While cellular levels of pro- and antiapoptotic proteins remained rather constant, the amount of Bax/Bak in an active conformation was increased by DHA. Deficiency of Bax/Bak or siRNA-mediated downregulation of Bak almost abrogated DHA-induced apoptosis.

DHA improved radiation-/TRAIL-induced apoptosis in a concentration-dependent manner, exhibiting at least additive effects.

Conclusions: Data implicate that DHA induces apoptosis via mitochondrial death pathway. While Bak expression is sufficient to mediate the effects in the Bax-/p53-deficient Jurkat model, Bax may substitute for Bak in regard to apoptosis execution in solide tumor cells. Our findings suggest that DHA may be a promising antitumor agent when it used alone or in combination with TRAIL or XRT.

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Protein tyrosine phosphatase PTPN13/PTPL1 regulates aggressiveness of breast cancer cells

M. Glondu-Lassis[1], M. Dromard[1], C. Puech[1], D. Chalbos[1], G. Freiss[1]
[1]Institut de Recherche en Cancérologie de Montpellier, INSERM U896, Montpellier, France

Background. PTPL1/PTPN13 gene presents the genetic characteristics of a tumour suppressor gene. It is located on chromosome 4q21, a region frequently deleted in ovarian and liver cancers, its expression was frequently down-regulated or silenced through promoter hypermethylation within several tumour types, and a mutational analysis of colorectal cancers identifieddifferent somatic mutations in PTPL1. At the mechanistic level, we and other have recently evidenced its ability to inhibit signal transduction driven by the tyrosine kinase receptors IGF-1R and HER2. Materials and methods. In this study, we explore the phenotypic properties of PTPL1/PTPN13 by assessing its effects on the aggressiveness of a noninvasive human breast cancer cell MCF-7 using short interfering RNA or short hairpin RNA. Results. We show that knockdown of the phosphatase significantly reduced cell-matrix adhesion on human fibronectin and collagen 4 which are components of basal lamina and/or extracellular matrix. Same result was obtained when cells were coated on Matrigel, a reconstituted basement membrane. In accordance with the negative effect of the inhibition of PTPL1 expression on cell-matrix adhesion, when coated on fibronectin, PTPL1 siRNA-transfected cells exhibited a loss of focal adhesion structures compared to control cells. This inhibition was correlated with an increase in in vitro cell invasiveness, measured in Matrigel coated Boyden Chambers, and with an enhanced outgrowth of cells embedded in Matrigel. We have then generated stable MCF-7 cells expressing a short hairpin RNA to measure longer biological effects. Two clones that exhibited 80-90% reduction in PTPL1/PTPN13 protein expression formed larger colonies in soft agar and presented a significantly increased tumour growth in nude mice compared with control clones.

Conclusions. These studies demonstrate that loss of PTPL1 expression induces phenotypic changes of breast cancer cells in agreement with its role as a potential tumour suppressor gene in this disease.

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O6-methylguanine-DNA methyltransferase (MGMT) promoter hypermethylation in neoplastic and normal mucosa in patients with colorectal carcinoma(CRC)

P. Stravoravdi[1], D. Paikos[2], S. Voyatzi[1], D. Tragiannidis[2], K. Soufleris[2], J. Pilpilidis[2], A. Tarpangos[2], J. Katsos[2]
[1]Theagenio Cancer Hospital, Research, Thessaloniki, Greece; [2] Theagenio Cancer Hospital, Gastrenterology-Oncology, Thessaloniki, Greece

Purpose: Colorectal cancer is a complex and heterogeneous disease in which genomic instability and epigenetic alterations play important roles. Hypermethylation of cytosine residues in the CpG islands of certain DNA repair genes is a distinct epigenetic alteration in CRC. The process contributes to cancer formation through the transcriptional silencing of genes. MGMT gene removes mutagenic adducts from the O6-position of guanine in DNA. Loss of its function leads to the inactivation of DNA repair and to microsatellite instability. Experimental procedures: 31pts with CRCs (18M/ 13F, mean age 67.67 years, 3 of them less than 50 years old) were included. The tumors, 13 of the right and 18 of the left colon, were staged according to Astler- Coller classification. The analysis of methylation status in the promoter region of the MGMT gene was performed on isolated genomic DNA, which was obtained from 31 paraffin-embedded colon cancer samples and from their normal mucosa. Finally, a total of 62 samples were examined using modified protocols for bisulfite treatment and methylation-specific “hot-start” PCR (MSP) followed by detection on agarose gel. Our results on promoter methylation of MGMT gene were correlated with known clinicopathological parameters. Results: MGMT promoter methylation was present in 29% of the tumors (9 out of 31 pts). Five out of 9 pts whose cancer had MGMT promoter methylation also had substantial MGMT promoter methylation in their normal mucosa. All pts with promoter methylation showed low differentiated CRCs with proximal mostly location of the tumors (6/9), while no age- related correlation was noted. Conclusion: MGMT gene is frequently methylated in CRC. It appears that the methylation plays a more important role in proximal colon cancer development than in distal colon cancer and is correlated with low differentiated CRC. In our study no patient, without promoter methylation of MGMT gene in cancer tissue, showed this epigenetic alteration in the corresponding normal mucosa. Further studies with more pts are now carried out in order to assess if the above observations may ultimately be useful in CRC studies.

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ZEB-1 can repress SEMA3F semaphorin - a tumor suppressor gene in lung cancer

J. Roche[1], J. Clarhaut[1], R. Gemmill[2], S. Ait-Si-Ali[3], J. Imbert[4], H. Drabkin[2]
[1]CNRS UMR 6187 IPBC, Faculté des Sciences de Poitiers, Poitiers, France; [2] MUSC, Division Hematology/Oncology, Charleston SC, USA; [3] CNRS FRE 2944, Inst A Lwoff and Université Paris Sud, Villejuif, France; [4] INSERM U298, TAGC and Université Méditerranée, Marseille, France

SEMA3F, a class-3 semaphorin gene, encodes a potent tumor suppressor protein with effects on both tumor and endothelial cells. Downregulation of SEMA3F is frequent in lung cancer and correlates with advanced stage disease, while the reexpression of SEMA3F in tumor cells inhibits multiple signaling components, HIF-1alpha protein translation and VEGF mRNA. Therefore, understanding how SEMA3F expression is inhibited is important. We previously defined the promoter organization of SEMA3F and found that while promoter methylation correlated with transcriptional repression, chromatin remodeling by a histone deacetylase inhibitor (HDACi) was sufficient to activate SEMA3F expression. In lung cancer, we have shown that ZEB-1, an E-box transcription repressor, is predominantly responsible for loss of E-Cadherin associated with a poor prognosis and resistance to EGFR inhibitors.

In the present study, we found that ZEB-1 is also responsible for SEMA3F repression. Levels of ZEB-1, but not Snail or Slug, significantly correlate with SEMA3F inhibition in lung cancer cell lines. Similarly, overexpression or inhibition of ZEB-1 correspondingly affected SEMA3F expression. Four conserved candidate E-box sites were identified in the SEMA3F gene. ZEB-1 binding was confirmed by chromatin immunoprecipitation assays for two of these: site 1 in the CpG-island promoter region and site 4 in the third intron. In addition, ZEB-1 binding to these sites was reduced when cells were treated with a HDACi.