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Erucylphosphohomocholine-induced apoptosis in human glioma cells: role of the oligomycin-sensitive F0 part of mitochondrial H+-ATP-synthase

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Erucylphosphohomocholine (ErPC3), representative of a new class of antitumor agents, which target biomembranes and induce cancer cell apoptosis, has been shown to be a promising preclinical anticancer agent. However, the precise mechanisms by which ErPC3 displays its antitumor activities are unknown. In previous studies we demonstrated that ErPC3 activates the mitochondrial apoptotic pathway via the 18 kDa Translocator Protein (TSPO), which in turn causes the MPTP to open leading to collapse of the mitochondrial membrane potential, the first stage of the mitochondrial apoptosis cascade. Cyclosporin A and oligomycin protected glioma cell lines against ErPC3-induced apoptosis.

In this study, we investigated in more detail the significance of the membraneous F0 component of H^+-ATP-synthase in ErPC3-induced PTP opening and apoptosis with the aid of different inhibitors, e.g. oligomycin, applied to human glioblastoma cells, U87MG and U118MG. Furthermore, we measured cytochrome c release, apoptosis, and cellular ATP levels in this paradigm.

In these cells ErPC3-induced apoptosis was insensitive to effects of inhibitors of the mitochondrial respiratory chain and uncouplers of oxidative phosphorylation, but was suppressed by oligomycin. We showed further that release of cytochrome c and the execution of apoptosis induced by ErPC3 can be inhibited by oligomycin. However, another inhibitor of this enzyme, piceatannol, inhibiting the water-exposed F1 component, did not affect ErPC3-mediated apoptosis. In addition, we analysed a possible correlation between ErPC3, MPTP, and H^+-ATP-synthase by investigating cellular ATP levels. ErPC3 reduced cellular ATP levels in U87MG and U118MG cells, while co-administration of ErPC3 with oligomycin or cyclosporin A restored cellular ATP levels.

Together, these results suggest a role of the oligomycin-sensitive F0 component of H^+-ATP-synthase in ErPC3-induced PTP opening and apoptosis.

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Chromogranin A-derived vasostatin-1 contains a sequence homologous to ezrin-radixin-moesin binding phosphoprotein 50 (EBP50) that regulates cell adhesion

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The circulating levels of chromogranin A (CgA), a protein stored in the secretory granules of many neuroendocrine cells, are increased in patients with various neuroendocrine tumors, with important diagnostic and prognostic implications. We have shown previously that production of CgA by neoplastic cells can affect tumor growth and morphogenesis by affecting the tumor microenvironment. In vitro studies showed that CgA and its N-terminal fragment CgA 1-78, called vasostatin-1 (VS-1) can modulate, in a negative or a positive manner respectively, fibroblasts and endothelial cell adhesion. In the present study we report a novel mechanism for the regulation of cell adhesion by recombinant VS-1. We used NIH-3T3 fibroblasts as a model to investigate its mechanism of action. We observed that these cells express a large number of non-saturable binding sites for the C-terminal region of VS-1 (residues 47-78). Furthermore, the C-terminal, but not the N-terminal, region of cell-bound VS-1 was resistant to degradation by proteinase K, indicating that VS-1 was bound to a protease-resistant structure through the C-terminal domain. This domain encompasses residues 47-66, an α-helix able to interact with membrane phospholipids, and residues 69-75 (AKERAHQ) sharing sequence similarity with ezrin-radixin-moesin (ERM) binding phosphoprotein 50 (EBP-50), a protein that by one hand binds members of the ERM family, by the other hand binds transmembrane proteins. Studies with recombinant CgA 1-78 and CgA 1-65 fragments showed that the C-terminal domain containing the AKERAHQ region is critical for cell adhesion. These results suggest that VS-1 regulates cell adhesion by interacting with cell membrane through the 47-66 residues and potentially with other membrane or cytoplasmic proteins with its C-terminal domain. Based on the high sequence homology with EBP50, interaction of the C-terminus with membrane or intracellular proteins involved in the regulation of membrane-cytoskeleton interactions could be a critical mechanism.

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Novel therapy for malignant pleural mesothelioma using 3-bromopyruvate based on anti-energetic effect

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Malignant mesothelioma is a poor prognosis cancer because it is resistant to chemotherapy and radiotherapy. Here, we tested a new anti-cancer approach based on the fact that cancer cells have an impaired metabolism of the glucose, leading to the secretion of lactic acid, a phenomenon first described by Otto Warburg, more than 80 years ago. We studied the effect of 3-Bromopyruvate (3-BrPA), first in vitro, on two human mesothelioma cell lines, either sensitive (MSTO-211H) or resistant (NCI-H28) to cisplatin, and then, in nude mice developing peritoneal carcinomatosis after intra peritoneal injection of MSTO-211H cells. 3-BrPA is an analogue of pyruvate that plays key role in the energetic metabolism. It has been considered to be able to inhibit hexokinase (HK), the first key enzyme of the glycolysis. HK, associated with phospho-Bad and VDACI, form a complex on the external mitochondrial membrane inhibiting the apoptosis. The removal of HK from this complex allows Bad dephosphorylation and thus apoptosis induction.

In both cell lines, 3-BrPA slowed down the proliferation without apoptosis induction. However, resistant NCI-H28 cells massively died in response to high 3-BrPA concentrations (up to 100μM), mainly by necrosis-poisoning mechanism. In contrast, when 3-BrPA was administered immediately when the cells are seeded in the flask (i.e. on detached cells), a massive apoptotic cell death was observed in MSTO-211H in response to low concentrations of 3-BrPA (50μM), involving the mitochondrial pathway, whereas no apoptotic cell death was observed in cisplatin-resistant NCI-H28.

In vivo, 3-BrPA increased the survival very significantly ($p<0.0001$), whereas cisplatin had no demonstrable effect. Using this novel anti-energetic agent, we think it is nowadays possible, either to slow down the proliferation and perhaps to facilitate action of other strategies, or to directly provoke cell death either by the apoptotic pathway or by necrosis-poisoning mechanisms. 3-BrPA could thus constitute and interesting novel anticancer drug which could be included in clinical trials, either to allow tumour regression or to impede tumour cells adhesion and subsequent peritoneal carcinomatosis or distant organ metastasis.

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Concomitant inhibition of Bcl-xL and Mcl-1 expression by RNA interference as a novel strategy for the treatment of ovarian carcinomas

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In ovarian cancers, cisplatin exposure of sensitive cells is associated with a down-regulation of Bcl-xL expression and apoptotic cell death whereas recurrence is systematically observed when Bcl-xL expression is maintained. We thus developed a specific siRNA targeting siXL1, and evaluated its ability to induce apoptotic cell death in response to cisplatin in resistant SKOV3 cells. siXL1 led to the disappearance of Bcl-xL mRNA and protein, associated with a low rate of apoptosis. In contrast, when siXL1 was combined to cisplatin, a massive cell death was observed whereas cisplatin alone was only transiently cytostatic. Thus, the inhibition of Bcl-xL expression could constitute a chemosensitizing way for the treatment of ovarian carcinoma.

Next, we investigated siXL1 effect on survival of intraperitoneal SKOV3 tumour bearing mice. The administration of siXL1 induced a strong increased of survival rate (median survival over 150 days in siXL1 group vs. 60-70 days in control groups). The surviving mice did not present any sign of residual tumour. Moreover, this effect on survival was associated with a significant decreased in both Bcl-xL expression and proliferation in tumour nodes, 5 days after siXL1 administration. In addition, a potential effect on angiogenesis is suspected but further investigations are now required to demonstrate its importance. However, a relative heterogeneity was noted in