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MITOCHONDRIAL F0F1-ATPASE PROTON PUMP MAY ACTIVELY PARTICIPATE IN THE PROCESS OF CYTOCHROME C RELEASING DURING APOPTOSIS.

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Backgrounds: Nitric oxide (NO) is a well-known inducer of apoptosis, but there have been many unknown issues in its apoptotic signal pathway. Recent studies have clarified that the release of cytochrome C (cyt-C) from mitochondria triggers the subsequent activation of caspase-cascade and leads to apoptosis. Bax, Bcl-2 and others are considered to regulate this process. However, the mechanisms of cyt-C releasing have not been elucidated yet. The present study was aimed to clarify the possible implication of mitochondrial functional molecules such as F0F1-ATPase and PT pore in cyt-C releasing process of NO induced apoptosis, using the rat normal gastric mucosal cell line, RGM1. Materials and Methods: RGM1 cells were cultured in a high glucose medium for 24hrs to maintain intracellular ATP level. Thereafter, the cells were exposed to pure NO donor (NOC18) under the presence or absence of various inhibitors of mitochondrial function, oligomycin as a F0F1-ATPase inhibitor, antimycinA as a respiratory chain complex III inhibitor and cyclosporineA as a PT pore inhibitor. The followings were studied, cell viability by MTT assay, Bax,Bcl-2,and cyt-C proteins by Western blotting, and caspase-3-activity and intracellular ATP levels. Results: NO induced apoptosis of RGM1 cells in a dose- and time-dependent manner. The apoptotic process was preceded by the upregulation of Bax and the cleavage of Bcl-2. The release of cyt-C from mitochondria to cytosol and the subsequent activation of caspase-3 occurred with the progress of apoptosis. Oligomycin could completely inhibit apoptosis by blocking the release of cyt-C and activation of caspase-3, although the up-regulation of Bax persisted. Oligomycin decreased the level of intracellular ATP, but its value in a high glucose medium was still higher than that in a low glucose medium. Antimycin A also showed an inhibitory effect on apoptosis, but the effect was incomplete. By contrast, cyclosporineA did not show any effect during the NO-induced apoptosis of RGM-1 cells. Conclusions: These results suggest that cyt-C may be actively released from mitochondria by the mechanism which is related to the mitochondrial functions such as F0F1-ATPase proton pump. Mitochondria may exert great influence on the sensitivity to apoptosis.

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HUMAN NEUROENDOCRINE TUMOR CELLS RESPOND TO INTERFERON-Γ TREATMENT BY INDUCTION OF APOPTOSIS.

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Background and Aim: Interferon γ (IFNγ) has been demonstrated to exhibit antiproliferative actions by multiple distinct mechanisms in a tissue and cell type specific manner. Previous studies have suggested a substantial growth inhibitory potential of IFNγ in pancreatic neuroendocrine (NE) tumor cells, but did not analyze the molecular mechanisms. Thus, the current study was designed to delineate the antiproliferative mechanisms at the molecular level. Methods: IFNγ dependent activation of the Jak-Stat-signal transduction pathway was determined based on α –PY immunoblotting of Jak-1, Jak-2 and Stat-1, immunodetection of Interferon regulatory factor-1 (IRF-1) and reportergene assays. Anchorage-dependent and -independent growth were determined by cell number and Human Tumor Clonogenic Assay (HTCA). Cell cycle distribution and apoptosis were evaluated by flow-cytometry, analysis of DNA integrity and immunoblotting for PARP and caspase 1. Results: QGP1 cells were utilized as a representative pancreatic NE tumor cell line. Expression of functionally competent IFN γ-receptors was evidenced by IFNγ induced, rapid phosphorylation of Jak-1, Jak-2 and Stat-1, dose-dependent transactivation of a transiently transfected GAS-luciferase reportergene construct as well as induction of IRF-1 expression. Prolonged treatment with IFNγ resulted in a profound, dose-dependent inhibition of both anchorage-dependent and anchorage-independent growth. Concomitant with the onset of growth inhibition, a population of cells with subdiploid DNA content was detected in cell cycle analyses of IFNγ-treated cultures, suggesting induction of apoptosis. The presence of apoptotic cells was confirmed by evaluation of DNA fragmentation and PARP cleavage. Furthermore, immunoblotting revealed a substantial upregulation of caspase 1 in IFNγ treated cells. Conclusion: NE tumor cells express functionally competent IFNγ receptors, which mediate induction of the tumor suppressor IRF-1 and growth inhibition via initiation of apoptosis. Based on these results, the potential of IFNγ in experimental biotherapeutic treatment modalities of NE tumors should be further explored.

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ENHANCED SENSITIVITY OF PANCREATIC TUMOUR CELLS TO 5-FU-CHEMOTHERAPY MEDIATED BY ADENO-ASSOCIATED VIRUS TYPE 2 (AAV-2) INFECTION *IN VITRO* AND *IN VIVO*.

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Background Chemotherapeutic drug resistance is a major clinical problem and cause for failure in the therapy of pancreatic cancer patients. The non-pathogenic adeno-associated virus type 2 (AAV-2) is a small single stranded DNA-parvovirus and has been reported to sensitize human tumour cells to gamma irradiation and chemotherapeutic drugs in vitro and in vivo. Aims of the study To investigate the sensitisation effect of AAV-2 towards 5-Fluoruracil (5-FU)-treatment of pancreatic tumour cells in vitro and in a syngene pancreatic tumour model in rats. Methods In order to determine whether infection with AAV-2 affects the in vitro proliferation rate and sensitizes these DSL6A pancreatic tumour cells to the cytotoxic action of various 5-FU-doses, we measured the relative proliferation by WST-1 cell proliferation test and colony forming assay. Induction of apoptosis was confirmed by FACS-analysis. For the animal experiments DSL6A tumour cells were inoculated in male Lewis rats and after 6 weeks treatment started weekly with 5-FU (5mg/kg/body weight) intraperitoneally and +/- AAV-2 infection (108 infective units) intratumourly. Tumour volume and animal performance status was determined weekly. Results AAV-2 infection of DSL6A cells resulted in 40-50% reduced cell viability of 5-FU treated cells compared to cells treated only with 5-FU. No significant inhibition of proliferation was observed after infection with lower than 103 MOIs of AAV-2. Further study results revealed a significant reduction of the 5-FU concentration required for 50% growth inhibition (IC50 dosis). Animal experiments showed that 5-FU single therapy had no effect on tumour growth and survival rate. In contrast animals infected with AAV-2 and treated with 5-FU tumour growth was significantly reduced ($p<0,05$) and survival time prolonged ($p<0,01$). Conclusion Our in vitro and in vivo data demonstrate that infection with the non-pathogenic AAV-2 significantly improves the efficacy of 5-FU chemotherapy in pancreatic carcinoma cells and offers a novel strategy in cancer patients treatment.

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EVIDENCE FOR A CROSS-TALK BETWEEN THE TGFBETA-SIGNALLING CASCADE AND THE RAS/MEK/MAPK-PATHWAY IN MEDIATING TRANSDIFFERENTIATION AND INVASION OF PANCREATIC CANCER CELLS.

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Background: We examined the phenotypic and functional effects of TGFbeta on pancreatic cancer cells with a functional TGFbeta cascade and the molecular mechanisms required for mediating these effects, depending on the mutational status of the ki-ras, TGFbetaRII and Smad4 genes. Methods: ki-ras mutated cell lines with functional (PANC-1, COLO 357 and IMIM-PC1) or non-functional TGFbeta pathways (MiaPaca2 and IMIM-PC2) were treated with TGFbeta (10 ng/ml), with or without the selective MEK1 inhibitor PD 098059. Phenotypic alterations were studied by electron and phase contrast microscopy as well as by measuring expression of differentiation markers. Migration and invasion were analysed using 2-chamber assays and ERK-2 activation was determined using immune complex kinase assays. Results: In TGFbeta responsive pancreatic cancer cell lines but not in cell lines with impairment of the TGFbeta pathway TGFbeta led to a reversible and time-dependant epithelial-mesenchymal transdifferentiation (EMT), which was associated with sustained activation of ERK2 and increased tumor cell migration and invasion. Treatment with a selective MEK1 inhibitor strongly reduced or abolished the TGFbeta induced morphological alterations as well as the concommitant changes of differentiation markers and the invasive and migratory potential. Conclusions: In pancreatic cancer cells with ki-ras mutations and an intact TGFbeta signaling pathway, TGFbeta-treatment causes an epithelial-mesenchymal transdifferentiation associated with increased tumor cell migration and invasion. A cross-talk with the Ras/MEK/MAPK-signalling cascade appears to be essential for mediating these effects of TGFbeta.