394 Quercetin enforces death receptor-ligands induced apoptosis in chronic lymphocytic leukemia

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Background: Chronic Lymphocytic Leukemia (CLL) is the most frequent form of leukemia in adult population (22−30% of all leukemia cases). A significant percentage of cases (up to 37%) does not respond to chemotherapeutic treatment or become resistant (up to 76%). Cell death evasion and progressive accumulation of B-cells are the most relevant events in CLL pathogenesis; therefore, targeting apoptotic pathways has been suggested as novel therapeutic approaches. One drawback of this therapeutic strategy is the resistance to death receptor (DR) induced cell death in CLL patients. Here, we investigated the ability of quercetin, a natural flavonoid, to sensitize primary cells from CLL patients to apoptosis triggered by anti-CD95 and recombinant TRAIL (rTRAIL).
Material and Methods: Mononucler cells were isolated from peripheral blood of 29 patients affected by CLL. All clinical samples were obtained with written informed consent. Cell viability was measured after 24−48 h stimulation with quercetin, rTRAIL and anti-CD95. Markers of DR-induced apoptosis were assayed, such as caspase-3 and -9 activation, Annexin V positivity, PARP degradation.
Results: Quercetin significantly potentiated sensitivity to anti-CD95 and rTRAIL treatment with an increase in cell death of about 10- and 17-fold respectively compared to DR mono-treatments. Cell death was due to apoptosis because reduced cell viability paralleled with increased caspase-3 and -9, degradation of PARP, and increased percentage of cells positive to Annexin V.
Conclusions: We demonstrate that quercetin is able to enhance apoptosis in cells isolated from CLL patients when associated to rTRAIL or anti-CD95. Overall, the present work demonstrates that resistance to DR-mediated cell death in leukemic cells can be ameliorated or bypassed by the addition of quercetin. In our view, this represents an important issue which stimulates further studies in the direction of therapeutic use of the molecule.

395 HDAC2 mediates therapeutic resistance towards intrinsic and extrinsic induction of apoptosis in pancreatic cancer cells

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Background: Pancreatic ductal adenocarcinoma (PDAC) is one of the most malignant diseases with a dismal prognosis and no effective conservative therapeutic strategies, implicating the need to detect novel therapeutic targets and therapies. Histone deacetylases (HDACs) significantly contribute to the carcinogenesis of solid tumours and HDAC inhibitors (HDACi) are promising therapeutics. However, the molecular pathways engaged by specific HDAC isoenzymes in cancer are ill defined.
Material and Methods: Expression of HDAC2 in PDAC was investigated using immunohistochemistry of tissue microarrays and by oncomining. The function of HDAC2 was analyzed using RNA interference in several pancreatic cancer cell lines. Results were reproduced using the more selective class I HDACi valproic acid (VPA). Proliferation and viability was measured using BrdU and MTT assays, respectively. Apoptosis was analyzed using Hoechst stains, Caspase 3/7 assays and PARP western blots. Transcriptome profiles were obtained using Affymetrix microarrays. Gene expression was validated by qRT-PCR and western blot. Chromatin immunoprecipitations were used for analysis of transcriptional regulation.
Results: In this study we demonstrate that HDAC2 is highly expressed in human and murine PDAC. We show that HDAC2 confers resistance towards the topoisomerase II inhibitor etoposide in PDAC cells. Correspondingly, the class I selective HDACi VPA synergizes with etoposide to induce apoptosis of PDAC cells. Similarly, depletion of HDAC2 by RNAi or VPA resulted in a marked sensitization towards the tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) in a HDAC2 isoenzyme specific manner. Transcriptome profiling of HDAC2 depleted PDAC cells revealed upregulation of the BH3-only protein NOXA. We show that the epigenetically silenced NOXA gene locus is opened after HDAC2 depletion and that NOXA upregulation is sufficient to sensitize PDAC cells towards etoposide-induced apoptosis, but not to TRAIL-dependent apoptosis. For the extrinsic apoptotic pathway, an increased expression of the TRAIL receptor 1 (DR5) in some cell lines, accelerated cleavage of the BH3-only protein Bid and increased caspase activation was observed in HDAC2-depleted and TRAIL-treated cells.
Conclusions: Our data characterize a novel molecular mechanism that links the epigenetic regulator HDAC2 to the regulation of the pro-apoptotic BH3-only protein NOXA as well as to the extrinsic apoptotic pathway. Targeting HDAC2 will therefore be a promising strategy to overcome therapeutic resistance of PDAC against DNA damage inducing chemotherapeutics or TRAIL.

396 GLUT1 and CAIX expression profiles in breast cancer correlate with MCT1 overexpression

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Background: Upregulation of glucose conversion into lactate, even in the presence of oxygen (Warburg effect), has been described as a possible adaptive mechanism to overcome intermittent hypoxia in pre-malignant lesions. Monocarboxylate transporters (MCTs) emerge as important contributors to cancer cell adaptation due to their function, on one hand, of lactate export, allowing continuous glycolysis, and, on the other hand, of tumour intracellular pH regulation and induction of extracellular acidosis, by co-transporting lactate and a proton. So, the main aim of the present work was to determine if glycolytic and acid-resistant tumours, with upregulation of GLUT1 and CAIX, present a higher expression of MCTs, supporting the involvement of these transporters in the metabolic adaptations of cancer cells. Additionally, the clinico-pathological value of GLUT1 and CAIX was evaluated.
Material and Methods: We analysed the immunohistochemical expression of GLUT1 and CAIX, in a large series of invasive breast carcinoma samples (n = 124), previously characterized for MCT1, MCT4 and CD147 expression.
Results: GLUT1 expression was found in 46% of the cases (57/124), while CAIX expression was found in 18% of the cases (22/122). Importantly, both MCT1 and CD147, but not MCT4, were associated with GLUT1 and CAIX expression. Also, GLUT1 and CAIX correlated with each other. Concerning the clinico-pathological values, GLUT1 was associated with high grade tumours, basal-like subtype, absence of progesterone receptor and presence of vimentin and Ki67 expression. Additionally, CAIX was associated with high tumour size, high histological grade, basal-like subtype, absence of estrogen and progesterone receptors and presence of basal cytokeratins and vimentin expression. Finally, patients with CAIX positive tumours had a significant shorter disease-free survival.
Conclusion: In the present study, we investigated the expression of the key hypoxia regulated proteins GLUT1 and CAIX. Importantly, they were positively associated with the major lactate transporter, MCT1, especially in a subset of aggressive breast carcinomas (basal-like), where these proteins are more frequently expressed. Since this subtype of tumours does not have a specific molecular therapy, the development of therapeutic approaches targeting these particular metabolic features could be a promising strategy to be explored in the treatment of basal-like breast tumours.

397 Development of a doxycycline-dependent caspase 3 death switch model to assess the immune response to rapid and synchronous tumour cell apoptosis in vivo

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Background: Increasingly it is being recognised that tumour cell death can act as a potential source of antigen for T-cell stimulation and promote anti-tumour activity.
Whilst apoptosis is generally considered non-immunogenic, recent evidence suggests that certain therapies such as radiation (RT), can induce a more immunogenic form of apoptotic cell death. To further define the immunogenic potential of RT-induced cell death compared to apoptosis we have developed a doxycycline-dependent caspase-3 “death switch” model, which can be used to induce rapid and synchronous apoptosis *in vivo*.
Material and Methods: We produced a “death switch” variant of the syngeneic murine B16 ova (ovalbumin expressing) melanoma. The death switch line was generated by stably transfecting the cells with a transcriptional transactivator ($rtTA2^S$-M2) to produce a “Tet-On” line and a response element containing reverse caspase 3 (revC3), under the control of an inducible promoter. In the presence of the tetracycline-analogue, doxycycline (Dox), the rtTA can bind to the inducible promoter, leading to its activation and subsequent expression of revC3, resulting in apoptosis. Cell death was confirmed by a number of assays. H-$2K^b$/SIINFEKL pentamers were used to measure the T cell response to ova and IHC to investigate the infiltration of immune effector cells into the tumours after induced apoptosis.
Results: Dox induced apoptosis was verified by Annexin V/Propidium Iodide staining; fluorometric analysis of caspase 3; and western blotting for cleaved caspase 3 and cleaved parp. Up to 80% apoptosis was observed at 24H which could be almost completely inhibited by the pan-caspase inhibiter Q-VD. Death