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

was associated with release of danger signals including HMGB1 and Heat shock proteins. *In vivo*, Dox treatment resulted in tumour regression which was reduced in immune deficient compared to immune competent mice. Pentamers were used to measure the specific T cell response and infiltration of immune effector cells were analysed by IHC.
Conclusions: This system allows us to explore the relationship between the amount and type of cell death and the ability to prime tumour-(ova)-specific T-cell responses *in vivo;* provide important clues as to what regulates immunogenicity of cell death *in vivo*; and eventually guide therapeutic approaches which aim to induce immune responses to dying tumour cells.

398 Glycan gene expression signatures distinguish normal and malignant breast tissue; possible role in diagnosis and progression

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Background: Glycosylation is the stepwise procedure of covalent attachment of oligosaccharide chains to proteins or lipids, and alterations in this process have been associated with malignant transformation. Studies focusing on the expression of the whole glycome have now become possible and prompted us to perform a comprehensive analysis of breast carcinomas focusing on glycosylation related genes.
Material and Methods: Various data resources were used to select a set of 419 functionally relevant genes. Two expression data sets were analyzed. The first consisted of samples from 64 stage I-IV breast cancer patients and normal breast tissue from 79 healthy women. Additionally, expression data from tumour and adjacent normal tissue of 26 breast cancer patients was analyzed.
Results: The glycome mRNA expression pattern was significantly different in tumour tissue compared to normal breast tissue, demonstrating the involvement of glycosylation in malignant transformation at several levels. The N-glycan pathway seems to be affected at different stages involving both the early precursor synthesis as well as certain later modifications including β1,6 branching and addition of α1,6 fucose to the core. Such reconfiguration may have a modulating effect on signaling of integrins, cadherins, epidermal growth factor and transforming growth factor-β leading to changes in growth pattern and possibly playing a role in the epithelial-mesenchymal transition. Furthermore, expression of glycosyltransferases involved in the synthesis of glycosphingolipids implied a profound change in structural appearance of gangliosides, including differences in sialylation. These changes may result in alteration of intercellular adhesion and signaling. Transcription levels of O-glycan related genes point to an altered glycosylation of mucins which in turn may influence adhesion and immunogenic properties of carcinoma cells. The same might be achieved through alterations in Lewis antigen structures presented on the cell surface as suggested by altered mRNA levels of a variety of fucosyl, sialyl- and galactosyltransferases, indicating higher levels of type 2 structures. Altered expression of genes coding for transferases associated with synthesis and sulfation of several types of glycosaminoglycans may imply an impact on the local environment immediate to the cell surface, both in terms of adherence and change in the reservoir of chemokines and other signaling molecules.
Conclusion: In this study we have performed a comprehensive analysis of all known glycan-related genes using expression data from breast carcinomas and normal breast tissue samples. The results clearly demonstrate a unique glycan gene expression signature of malignant carcinomas of the breast significantly different from that of healthy breast tissue. Several of the alterations in the glycosylation pathways revealed by this signature are novel and warrant further investigation.

399 Endoplasmic reticulum stress mediates cell death in human hepatocellular cancer cells: an alternative apoptotic pathway induced by the pan-deacetylase inhibitor panobinostat

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Background: Panobinostat (LBH589), a pan-deacetylase inhibitor, represents a novel therapeutic option for human cancer diseases. We have previously shown that panobinostat has a potent apoptotic activity *in vitro* and causes a significant growth delay of hepatocellular carcinoma (HCC) tumour xenografts in nude mice models. We have demonstrated that treatment with panobinostat is able to induce cell death in HepG2 (p53wt) and in Hep3B (p53null) cell lines that, interestingly, is not dependent on canonical apoptotic pathways. Here we analyse the involvement of Endoplasmic Reticulum (ER) in cell death induced by panobinostat treatment.
Material and Methods: Human HCC cell lines HepG2 and Hep3B were cultured under standard conditions and treated for 6−72 hours with 0.1 μM panobinostat. Sub-G_1 events were quantified by flow cytometry after propidium iodide staining and verified by immunofluorescence of cytokeratin-18 cleavage. ER-stress factors were evaluated by quantitative RT-PCR and western blotting. Caspase-12 and caspase-4 activities have been determined by a Fluorometric Assay kit (Biovision), caspase-3/7 and -8 activities have been evaluated by Caspase Glo assay kit (Promega).
Results: Treatment of both HCC cell lines induced cell death as was shown by an increase in sub-G_1-events. The ER response involvement was clarified by IRE1-alpha, BIP and ATF-4 transcript evaluations that increased after 6 hours of treatment *in vitro* and after 1 day in xenografts specimens. Neither HCC cell line showed an expression of IRE1-beta, the IRE1-alpha homologous gene. Moreover, panobinostat caused an increase of expression for CHOP/GADD153 transcript and a stable expression of its protein level; otherwise a decrease in the level of Xbp transcript in HepG2 cells was shown. We also demonstrated the up-regulation of eIF2-alpha phosphorylated form after treatment with panobinostat in Hep3B cells. A transient increase of the phosphorylated status of JNK, ERK and p38MAPK was clearly detected. Finally, activation of caspase-12 and caspase-4 was detected and their inhibitions lead to a downregulation of caspases-3/7 and -8 activities.
Conclusion: The novel pan-DACi panobinostat induces cell death in HCC cell lines. ER-stress plays a key role to drive cells to die through the induction of three main actors of alternative death pathway: CHOP, JNK and caspase-12/-4 leading to activation of executioner caspases.

400 Met as a potential therapeutic target in basal-like breast cancer

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Met overexpression has been associated to a highly invasive and poorly differentiated subtype of breast cancer, known as basal-like breast cancer. These tumours show an aggressive phenotype and do not express hormone receptors or Erb2, which makes them insensitive to therapies currently in use for mammary tumours. Based on gene expression profiles, it has been proposed that basal-like tumours derive from mammary stem/progenitor cells. The deregulation of pathways specific to mammary undifferentiated cells may contribute to the generation of these tumours. We investigated how the MET affects function of normal mammary cells, and whether its hyperactivation favors to the formation of neoplastic lesions.
We explored the functional role of Met expression in mammary gland development by fat pad transplantation experiments. Constitutive activation of Met in the transplanted cells enhanced their proliferation ability with the formation of a hyper-branched ductal tree and dilations of the TEBs. In limiting dilutions transplants, Met activation led to a significant increase in the frequency of mammary repopulating units compared to wild-type cells.
Consistently, in vitro cultures showed that hyperactivation of Met in primary mammary cells induced the generation of colonies higher in number and larger in size than those arising from wild-type cells; moreover, Met pharmacological inhibition reduced the growth potential of mammary cells on irradiated fibroblasts, underscoring the role of Met in sustaining the clonogenic ability of mammary cells. Gene expression analysis and flow cytometry-based cell sorting revealed that Met is differentially expressed in the various mammary epithelial subpopulations: it is highly expressed in luminal progenitors ($CD24^{high}$ ER^{-}), whereas it is barely detectable in the differentiated cells of the basal $CD24^{low}$ compartment – which also includes stem cells – and in the terminally differentiated luminal cells $CD24^{high}$ ER^{+}. Interestingly, the $CD24^{high}$ ER^{-} progenitor population has been recently described as the candidate target population for basal tumour development in BRCA1 mutation carriers. Expression analysis in tumours derived from a mouse model of basal-like cancer (BRCA1/p53 ko) revealed that Met is overexpressed in a subpopulation of CD24+ ER− cells. This is in line with the observation that Met overexpression in basal-like breast tumours might play a causative role in the onset and maintenance of the transformed phenotype.

401 A new Golgi-based signalling cascade involved in tumoural cell invasion

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Background: Metastasis is the most frequent cause of death in cancer patients but the molecular mechanisms that regulate metastatisation remain to be clearly defined. We have demonstrated that KDEL receptor (KDELR) engagment by incoming traffic at the Golgi complex triggers activation of the oncogenic Src family kinases (SFKs) on the Golgi itself. The aim of this study is to determine the role of this new signalling pathway in tumoural cell invasion.