p27, p53, MDM2, E2F1 and, Androgen receptor. Du145+Id4 cells have significantly decreased proliferation due to an S-phase arrest suggested by an E2F-1 intermediary response. The increased expression of E-cadherin, p27, p21 and p53 strengthens the hypothesis of Id4 as a tumor suppressor by regulating key cell cycle control and apoptosis associated genes. A functional androgen receptor marked a dramatic change in the androgen independent prostate cancer cell type. Id4 ectopic expression resulted in a significant decrease in Id1 and Id3, which are known contributors to metastasis and cell survival. Id4's role may be to inhibit bHLH transcription factors involved in proliferation, metastasis, apoptosis and senescence. Id4 induces apoptosis whether dependent/independent of the mutated p53 gene in DU145 cells. The presence of senescent cells in Id4 transfected cell lines suggest that Id4 may also play a role in autophagic cell death. As a transcription factor Id4 has the capability of inducing a total cellular reprogramming, by influencing a number of key cellular pathways. We conclude that the tumor suppressor function of Id4 is responsible for causing a reversal in the cancer phenotype of the cell by inducing apoptosis, senescence, S-phase mediated cell cycle arrest resulting in a molecular and morphological change.

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Berberine sensitizes TRAIL-induced apoptosis through proteasome-mediated down-regulation of c-FLIP and Mcl-1 proteins

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Berberine is an isoquinoline alkaloid used in traditional Chinese medicine and has been isolated from a variety of plants, such as *Coptis chinensis* and *Phellodendron amurense*. It has a wide spectrum of clinical applications such as in anti-tumor, anti-microbial, and anti-inflammatory activities. We showed that co-treatment with subtoxic doses of berberine and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) induced apoptosis in human renal cancer cells, Caki cells, but not in normal tubular kidney cells. Treatment of Caki cells with berberine down-regulated c-FLIP and Mcl-1 proteins in dose- and time-dependent manners. Interestingly, berberine-induced decreases in c-FLIP and MCl-1 protein levels were involved in proteasome dependent pathways, which was confirmed by the result that pretreatment with proteasome inhibitor, MG132, inhibited berberine-induced down-regulations of both c-FLIP and Mcl-1 proteins. Pretreatment with N-acetyl-l-cysteine (NAC) significantly inhibited the cell death induced by the combined treatment with berberine and TRAIL as well as recovered the expression levels of c-FLIP and Mcl-1 down-regulated by the combinatory treatment with berberine plus TRAIL, suggesting that berberine-stimulated TRAIL-induced apoptosis appears to be dependent on the generation of reactive oxygen species for down-regulation of c-FLIP and Mcl-1. Taken together, the present study demonstrates that berberine enhances TRAIL-induced apoptosis in human renal cancer cells by ROS-mediated c-FLIP and Mcl-1 down-regulations.

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Characterisation of novel, small molecule antagonists of XIAP, cIAP1 and cIAP2 generated by fragment based drug discovery (FBDD)

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The inhibitor of apoptosis (IAP) family of proteins are important regulators of cancer cell survival, making them attractive targets for cancer therapy. They are characterized by one to three baculovirus IAP repeat (BIR) domains, which are necessary for the antiapoptotic activity of most IAPs. Several small molecule BIR antagonists mimic the N-terminal sequence of SMAC (second mitochondrial activator of caspases), an endogenous inhibitor of the IAPs. These peptidomimetic compounds have the ability to sensitise and/or promote apoptosis in cancer cells and inhibit tumor growth *in vivo*. Using our fragment-based screening approach, Pyramid™, we identified a range of diverse, non-peptidomimetic chemotypes which bind to the P1′−P2′ pocket in the BIR3 domain of XIAP. Alanine-like fragments have also been identified with excellent Ligand Efficiency (LE) values, which are superior to LE of Ala-Val (natural substrate) and to LEs of published competitor compounds. Optimisation of these hits using a structure based approach led to novel series (both alanine and non-alanine) which bound with sub μM potency to both XIAP and cIAP1. The most potent compounds were characterised further in proliferation assays using two sensitive human breast cancer cell lines EVSA-T and MDA-MB-231 (with an insensitive cell line, HCT116, as a control). Anti-proliferative compounds were investigated further for their ability to induce cIAP1 degradation and to increase the levels of cleaved caspase-3 in EVSA-T cells. cIAP1 degradation occurred rapidly at low compound concentrations in all cell lines tested; whilst caspase-3 induction closely paralleled the anti-proliferative data. In conclusion fragment-based screening has enabled the identification of non-peptidomimetic ligands that inhibit this protein:protein interaction. These chemotypes represent promising start points for novel, selective IAP antagonists.

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Notch-1 fragment peptide induces autophagy and caspase-independent cell death in leukemic cell lines

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Background: Non-apoptotic cell death such as autophagic cell death or necrosis is an important physiological process. Potent inducers of non-apoptotic cell death promise to be a valuable tool for development of novel chemotherapeutic agents. In this study, we found that a 25mer peptide conjugate induces a caspase-independent cell death in multiple human leukemic cell lines.
Material and Methods: All the peptides were synthesized by using an Fmoc chemistry-based automatic peptide synthesizer. Human monocytic cells and leukemic cell lines were maintained in RPMI-1640 medium supplemented with 10% fetal calf serum. Measurements of cell viability and mitochondrial membrane potential were determined by WST-8 chromogenic assay and fluorescence-activated cell sorter using the JC-1 dye, respectively.
Results: To suppress Notch signaling which is overactivated in T-cell leukemia and glioma, we designed Tat-Notch-1 fragment peptide (Tat-NF), which is a cell penetrating HIV-1 tat-conjugate with a 13mer peptide fragment (R-R-Q-H-G-Q-L-W-F-P-E-G-F) derived from Notch-1 intracellular domain. Unfortunately, the conjugate has no inhibitory effect on the Notch signaling in malignant gliomas, whereas the gamma-secretase inhibitor blocked the signal. However, the Tat-NF rapidly killed almost the leukemic cells (Jurkat-T, CCRF-CEM, Molt-4 etc.) tested in a tumor cell-specific manner. Single alanine substitutions of the LWF motif caused a significant decrease of the peptide-inducing cell death. Pharmacological inhibition of caspase activity did not prevent the cell death, although mitochondrial membrane potential was significantly decreased. In the cells undergoing such cell death, we observed the conversion of the soluble LC3-I to the autophagic vesicle-associated LC3-II and the formation of lysosomes/autophagosomes.
Conclusions: The 13mer peptide conjugated with HIV-1 tat has an ability to induce autophagy and caspase-independent cell death without affecting the Notch signaling. These data suggest that the conjugate is useful to elucidate the molecular mechanism of non-apoptotic cell death and to develop novel chemotherapeutic agents for treating leukemia with apoptotic defects.

Biomarkers

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In vivo detection of mammary tumor and its lung metastases in the 4T1 metastasis mouse model by PET imaging using [F-18]-D-FMT (BAY 869596)

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Background: Detection and localization of metastatic lesions and their differentiation from therapy induced inflammation is still a difficult obstacle for diagnosis and subsequent treatment decisions. Positron emission tomography (PET) allows the sensitive detection of radioactive labeled molecules, which specifically accumulate in tumor tissue. This offers a promising tool to assess molecular details about the disease comprehensively and contributes to an optimized cancer patient management and therapy control. At present [F-18]-fluorodeoxyglucose (FDG) is the most frequently used PET tracer in oncology. However, FDG has limited specificity by accumulating also in inflammatory cells due to their increased glucose metabolism. To overcome this problem, amino acids have been investigated. D-[F-18]-fluoromethyl tyrosine (D-FMT, (R)-2-amino-3-(4-[F-18]fluoromethoxy-phenyl)-propionic acid) has shown good uptake into HeLa tumors in nude mice with no accumulation in sterile induced inflammation sites. The aim of this study was to investigate D-FMT in a metastasis model for its capability to detect the primary tumor as well as metastatic lesions and differentiate metastatic from inflammatory sites.
Material and Methods: 2.5×10^5 4T1 mouse mammary carcinoma cells were implanted subcutaneously in NMRI mice, which generated several