Material and Methods: Androgen-independent and -sensitive (PC3 and LNCaP) prostate cancer cells and non-neoplastic HECV cells were used in the study. Proliferation, cell cycle progression and apoptosis were analyzed by MTT assay and cytofluorimetric analysis. DNA damage was assessed by COMET assay. Invasive potential was investigated by soft agar assay and metastatic ability by adhesion assay. Interleukin 23 (IL-23) and Protease activated receptor-1 (PAR-1) expression were determined by real time PCR.
Results: 2-CADO inhibits the growth of PC3 cells, through a mechanism involving cellular uptake, by inducing apoptosis and accumulation of cells in the S-phase of the cell cycle. 2-CADO pre-treatment followed by docetaxel at subclinical dosage reduced the viability of either PC3 or LNCaP while it did not enhance docetaxel-induced cytotoxicity in adherent non-neoplastic HECV. The drugs reduced the invasive potential of PC3 cells by inducing apoptosis and blocking cell cycle progression. Down-regulation of PAR-1 gene expression resulted in a slightly lower metastatic potential, whereas up-regulation of IL-23 induced the activation of the immune system.
Conclusions: Pretreatment of PC3 cells with 2-CADO decreased the effective concentration of docetaxel, lowered the metastatic potential, and induced the production of cytokines known to stimulate the immune response against cancer. The treatment was effective for prostate cancer cells independently on their androgen sensitiveness.

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SiRNA-mediated Apollon gene silencing induces apoptosis in breast cancer cells via p53 stabilization and caspase-3 activation

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Aims: Apollon is a member of the Inhibitor of Apoptosis Proteins family that contains baculoviral-IAP-repeat and ubiquitin-conjugating enzyme domains. It inhibits apoptosis by promoting ubiquitination and proteasomal degradation of Smac/DIABLO and by interfering with caspase activity. In the present study, we proposed to analyze cellular and molecular effects consequent to Apollon gene knockdown in breast cancer cells using small interfering RNA (siRNA).
Materials and Methods: Three human breast cancer cell lines differing in p53 gene status were used in the study: ZR75.1 and MCF-7 cells (wild-type p53), and MDA-MB-231 (mutant p53). The knockdown phenotype arising after transfection of cells with 10 nM of Apollon siRNA was evaluated at different time points. The effects of the treatment on Apollon expression were determined by RT-PCR and Western blotting. Cell death was evaluated by fluorescence microscopy analysis of cells stained with propidium iodide and by monitoring the catalytic activity of caspase-9 and caspase-3. The expression pattern of mitochondrial apoptosis-related proteins was analyzed by Western blotting, and the release of cytochrome c from mitochondria was assessed by ELISA.
Results: In ZR75.1 cells, silencing of the Apollon gene resulted in a significant and time-dependent decline in cell proliferation and an increase in the rate of spontaneous apoptosis, which was associated with p53 stabilization and activation of the mitochondrial-dependent apoptotic pathway (up-regulation of Bax and Bad, marked release of cytochrome c, and activation of caspase-9 and caspase-3). Conversely, only a modest reduction in cell survival was observed in MDA-MB-231. Pre-incubation of ZR75.1 cells with p53-specific siRNA resulted in a partial rescue of the cell growth inhibition induced by Apollon knockdown. Furthermore, the activation of caspase-3 seemed to be essential for the induction of apoptosis mediated by Apollon silencing, since Apollon siRNA had no effect on the viability of caspase-3-deficient MCF-7 cells or ZR75.1 cells transfected with caspase-3-specific siRNA.
Conclusions: Our results indicate that p53 stabilization and caspase-3 activation concur to determine the apoptotic response induced by Apollon siRNA and suggest that targeting Apollon may have therapeutic potential for the treatment of breast cancer.

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Inhibition of autophagy significantly enhances the anticancer activity of SAHA by promoting ubiquitin-conjugated protein accumulation and oxidative stress in colon cancer cells

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Background: Autophagy is a degradation process responsible for the turnover of organelles and long-lived proteins by targeting them to lysosomes. The scope of the role that autophagy plays in cancer remains highly controversial as its induction has been proposed to both mediate cell death and promote cell survival in the face of metabolic stress. The histone deacetylase (HDAC) inhibitor, SAHA has been reported to induce autophagy, which may contribute to its anticancer activity. However, we have demonstrated that inhibitors of autophagy stimulate ubiquitin-conjugated protein accumulation, increase oxidative stress, and induce synergistic levels of apoptosis when administered in combination with SAHA.
Materials and Methods: The effects of autophagic inhibition on the anticancer activity of SAHA were investigated in the colon cancer cell lines, HCT8 and HT29. Autophagy was inhibited using Atg7 siRNA and the pharmacological inhibitors, chloroquine (CQ) and 3-methyladenine (3-MA). Apoptosis was measured by propidium iodide and active caspase-3 staining followed by flow cytometry. Oxidative stress was evaluated by using the dyes hydroethidine and DCF-DA and subsequent flow cytometric analysis. Ubiquitin-conjugated protein accumulation was measured by immunocytochemistry. The combination of CQ and SAHA was further investigated in vivo using HCT8 and HT29 xenograft models of colon cancer.
Results: Inhibition of autophagy resulted in the accumulation of ubiquitin-conjugated proteins and potently enhanced SAHA-induced apoptosis in the HCT8 and HT29 colon cancer cells. While SAHA disrupted bortezomib-induced aggresome formation, it enhanced ubiquitin-conjugated protein accumulation stimulated by CQ. The CQ+SAHA combination also produced a strong increase in the levels of the superoxide anion, which was determined to be critical for apoptosis induction. Furthermore, the CQ+SAHA combination significantly reduced tumor burden in both HCT8 and HT29 xenograft models.
Conclusions: Inhibition of autophagy by CQ in combination with SAHA stimulates ubiquitin-conjugated protein accumulation, oxidative stress, and apoptosis in the HCT8 and HT29 colon cancer models. This combination warrants further investigation for the treatment of colon cancer and other malignancies. A Phase 1 clinical trial evaluating the efficacy of the CQ/SAHA combination for the treatment of advanced solid malignancies is planned.

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Apoptosis and oxidative stress induced by 2-chloroadenosine in PC3 cell line

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Background: 2-Chloroadenosine (2-CADO) is an adenosine analog capable of inducing apoptosis in several cell lines by acting either via adenosine receptors or via uptake through nucleoside transporters that is followed by metabolic transformations leading to nucleotide analogs.
Material and Methods: Androgen-independent and -sensitive (PC3 and LNCaP) prostate cancer cells cells were used in the study. RT-PCR analysis was performed to determine the expression of nucleoside transporters and adenosine receptor subtypes. Proliferation, cell cycle progression and apoptosis were analyzed by MTT assay and cytofluorimetric analysis. DNA damage was assessed by COMET assay. Deoxy-and ribonucleoside triphosphate pools were determined by HPLC. The molecular mechanism was examined by assessing the involvement of DNA synthesizing enzymes in the cellular response. Reactive oxygen species (ROS) production and glutathione (GSH) concentration were analysed by spectrofluorimetric and spectrophotometric assay, respectively. Nuclear factor erythroid 2-related factor (Nrf2) nuclear translocation was assessed by Western blotting.
Results: 2-CADO treatment dramatically reduced the number of prostate cancer cells and permanently blocked cell-cycle progression in the S-phase. The block of prostate cancer cell proliferation is mediated by 2-CADO uptake through equilibrative nucleoside transporter, followed by sequential phosphorylations to 2-Cl-ATP that irreversibly inhibits several key-enzymes for DNA biosynthesis. 2-CADO treatment also induced ROS accumulation, GSH depletion and Nrf2 nuclear translocation.
Conclusions: 2-CADO caused oxidative stress and arrested DNA synthesis by irreversibly inhibiting purine/pyrimidine ribo-and 2-deoxyribonucleotides salvage enzymes.

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Histone deacetylase inhibitors restore caspase-8 expression and overcome TRAIL resistance in cancers with epigenetic silencing of caspase-8

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Resistance to apoptosis is a hallmark of human cancers and can be caused by epigenetic silencing of caspase-8, a key component of the death receptor pathway. Loss of caspase-8 correlates with poor prognosis in medulloblastoma, the most common primary malignant brain tumor in childhood. In search for novel strategies to restore defective