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(E)-3-Benzyl-6, 8-dihydroxyoct-2-en-4-one, a novel compound isolated from *Streptomyces* sp., induces apoptosis in LNCaP cells through mitochondrial-mediated pathway

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Background: Apoptosis has been characterized as a fundamental cellular event to maintain the physiological balance and homeostasis of the organism. The relationship between dysregulation of apoptosis and cancer formation has been emphasized. Accordingly, many chemotherapeutic agents reportedly exert their antitumor effects by inducing apoptosis in cancer cells. (E)-3-Benzyl-6,8-dihydroxyoct-2-en-4-one, designated as F3-2-5, a novel compound isolated from the culture broth of *Streptomyces* sp. KACC91015, was investigated for its inductive effect on the cell cycle arrest and apoptosis in human prostate carcinoma LNCaP cells.
Materials and Methods: To confirm the effects of F3-2-5 on the induction cell cycle arrest and apoptosis in LNCaP cells obtained from the American Type Culture Collection (Rockville, MD, U.S.A.), MTT assay, flow cytometric analysis, Immuno-blotting, and TUNEL assay, as well as cytochrome c analysis were performed.
Results: F3-2-5 exhibited anti-proliferative effect against LNCaP cells (IC50: 27.5 ?M), and the accumulation of the sub-G1 phase was observed in F3-2-5-treated LNCaP cells in the flow cytometric analysis using propidium iodide (PI) staining. Moreover, the typical apoptotic DNA fragmentation of the LNCaP cells treated with F3-2-5 was confirmed in the TUNEL assay. This apoptotic induction of F3-2-5 in the LNCaP cells was associated with the cytochrome c release from the mitochondria to cytosol, and the activation of procaspase-8, -9 and -3, as well as the specific proteolytic cleavage of poly (ADP-ribose) polymerase (PARP). In addition, F3-2-5 treatment caused the down-regulation of the anti-apoptotic protein, such as Bcl-2 and Bcl-XL, but the proapoptotic Bax protein was not influenced by F3-2-5 treatment.
Conclusions: A novel benzyldihydroxyoctenone derivative (F3-2-5) exhibits an anti-proliferative effect by induction of apoptosis that is associated with increase of Bax/Bcl-2 ratio, cytochrome c translocation, activation of caspase-3, -9, and -8, and PARP degradation in LNCaP cells. Indeed, the pan-caspase inhibitor was found to counteract F3-2-5-induced apoptosis. Thus, our findings suggest that the mitochondrial-mediated, caspase-dependent pathway is one possible mechanism underscoring F3-2-5-induced apoptosis.

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Autophagy and autophagic cell death are next targets for elimination of the resistance to tyrosine kinase inhibitors

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Background: The relationship between autophagy and cell death of various malignant cells has been investigated, nevertheless, there are many unknown points of the mechanisms of autophagy in hematological malignant cells. K562 is chronic myelogeneous leukemia (CML) cell line and has an ability of differentiating both erythroid and megakaryocytic pathways. Autophagic degradation system plays a role in terminal differentiation of erythroid cells. An HDAC inhibitor, SAHA is known to induce autophagy and apoptosis in K562 cells. Here, we investigated some mechanisms of autophagy during megakaryocytic differentiation and imatinib-induced cell death of K562 cells. Furthermore, we studied about the relation of autophagy in the cell death of imatinib-resistant cells.
Materials and Methods: To observe megakaryocytic differentiation and induction of autophagy, we treated K562 cells with TPA for 2−7 days and examined the expression of LC3, which is associated with the completed autophagosomes, by Western blotting and observed the appearance of autophagy by electron microscopy. Then, we performed double staining of anti-CD41 antibody and monodansylcadaverin (MDC) that is thought to accumulate in autophagosomes. The activation of MAPK during TPA treatment was investigated by Western blot analysis, and the appearance of autophagy under the inhibition of RAS/MAPK was accessed. Furthermore, we investigated the appearance of autophagy in dominant negative of MAPK-transfectant. We added imatinib on K562, imatinib resistant cell line, BaF3/T315I and BaF3/E255K cells under inhibition of autophagy by chloroquine diphosophate. Chloroquine blocks the fusion of autophagosome and lysosome, and then inhibits autophagosomal degradation. After two to three days, we counted viable and dead cells by trypan-blue exclusion test, and analysed by TUNEL assay. Then, the expression of LC3 was observed by Western blotting.
Results: Electron microscopy demonstrated TPA-induced autophagy, and the LC3 expression was increased in TPA treatment. Excess of autophagy could induce autophagic cell death in K562 cells. There were few cells that were double-positive of both MDC and CD41. These data suggested that degradation of cytoplasmic component by autophagy is not necessary for megakaryocytic differentiation. Western blot analysis demonstrated the activation of MAPK under the TPA treatment, and autophagy was inhibited in the dominant negative transfectant cells. TPA-induced autophagy was mediated by the Ras/MAPK pathway. The inhibition of autophagy induced acceleration of imatinib-induced cell death. Furthermore, autophagy also occurred in imatinib-resistant cell lines, and inhibition of autophagy induced cell death under the imatinib treatment in even T315I.
Conclusions: Autophagic cell-survival mechanisms might be usually promoted in imatinib-resistant cell lines, resulting the inhibitions of autophagy might disrupt the defense against imatinib and enhance drug-sensitivity.

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Activity of the anti-cancer aptamer AS1411 includes regulation of Bcl-2 family members

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AS1411 is a G-quadruplex forming oligonucleotide aptamer and is currently in phase II clinical trials. AS1411 has been shown to bind nucleolin, which is present at elevated levels in the cytoplasm and on the surface of various cancer cell lines. It therefore has been proposed that AS1411 uses nucleolin to gain access into the cell, where it arrests some cell lines in S phase of the cell cycle and always causes cytostatic followed by cytotoxic effects.
We have previously shown that AS1411 exerts an effect across a wide range of cancer cell lines, with IC_{50}s in the range of 1−10 μM, while non-cancer cell lines or normal primary cells are unaffected by similar concentrations of drug. Further characterisation of a number of paediatric cell lines showed these were also sensitive to AS1411. Furthermore the ALL cell line SUP-B15 demonstrated a shorter time course to cell death.
AS1411 has also been shown to destabilise mRNA encoding the anti-apoptotic regulator Bcl-2 by inhibiting the binding of nucleolin to mRNA, we have now shown here that the pro-apoptotic protein Bax is up-regulated in response to AS1411 in several cell lines.
The data presented here suggest that AS1411 is a novel drug with a broad spectrum of specific anti-cancer activity, and provide further rationale for its ongoing clinical development.

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A novel monosaccharide-based antimetabolite 2,2-difluoro-D-glucose (2-DFG) blocks glycolysis and induces cell death in gliomas

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Glycolysis is the major energy producing pathway for fast growing, glycolytically depended tumors, such as gliomas. Blocking glycolysis is therefore an important therapeutic strategy when used alone or in combination therapy to enhance the effects of chemotherapy in energy starved tumors. In our previous studies, we have examined D-glucose antimetabolites 2-deoxy-D-glucose (2-DG), 2-fluoro-2-deoxy-D-glucose (2-FG) and 2-fluoro-2-deoxy-D-mannose (2-FM) and confirmed their ability to block glycolysis and discovered their ability to induce autophagic cell death in vitro and established that their antitumor activityin vivo in orthotopicglioma model was comparable to that oftemozolomide, the standard of care therapy. In our attempts to discover more selective inhibitor of glycolysis we have synthesized 2-deoxy-2,2-difluoro-D-glucose (2-DFG) and initially assessed its ability to block glycolysis and induce autophagic cell death in comparison with our previously tested analogs. Cell viability assays were carried in U87 cells treated for 72 h with increasing concentrations of the 2-DFG under hypoxic and normoxic conditions. The results showed 2-DFG to have comparable cytotoxicity as that demonstrated by 2-DG, 2-FG and 2-FM in U87 cells treated for 72 h with IC50 values of 2.9 mM in cells treated under normoxic conditions (21% O2) and 1.2 mM, when cells were under hypoxic conditions (0.1% O2). To test the ability of the 2-DFG to induce autophagic cell death, we monitored the increase in acidic vesicular organelles (AVO) using acridine orange staining in U87 cells treated with increasing concentrations of 2-DFG for 72 hours under hypoxia and normoxia. The results showed concentration

dependent increase in acridine orange staining indicating the increase in autophagic cell death. The confirmation of autophagy (Type II programmed cell death) in U87 cells following 5 mM 2-DFG treatment was demonstrated using transmission electron microscopy (TEM) showing the presence of multilamellar structures, otherwise called autophagosomes. Our studies show that 2-DFG is an equally potent inhibitor of cell proliferation and a potent inducer of autophagic cell death in gliomas as are 2-DG, 2-FG, and 2-FM. Therefore, targeting the energetic metabolism of cancer cells and the autophagic survival response using inhibitors of glycolysis is a promising therapeutic approach to the treatment of cancers that are dependent on glycolysis for survival.

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Preliminary evidences for recruitment of innate responses to rectal cancer cell death elicited by neo-adjuvant radio-chemo therapy

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Colorectal cancer is the fourth cancer in the world with 1.023.000 new cases and 529.000 death per year, 27% are rectal and 73% colon cancer. 30% of rectal cancer patients with a T3N+M0 tumor stage (locally extended tumor with a lymph node involvement, but without metastasis) responds to the neo-adjuvant therapy, which causes necrosis and inflammation in situ. We cannot predict which patients will response. The aim of this study is to verify: (i) whether the pattern of innate response to synchronized death of the tumor cells elicited by the neo-adjuvant radio-chemotherapy is heterogeneous among patients and whether (ii) this information can be used to identify which patients will benefit from the treatment. We focused our attention on macrophages, which represent specialized sensors of injury in the midst of living tissues; in particular we assessed the expression of Heme Oxygenase (HO-1), CD68, CD163, CD206, Tie2, RAGE. Moreover, we assessed inflammatory molecules and soluble pattern recognition receptors. We are also verifying polymorphism of Tlr4 gene. We are analyzing at diagnosis, before the second cycle of chemo-radio therapy and after surgical resection the monocyte phenotype in the peripheral blood by flow cytometry, the infiltrating tumor macrophages by immunoistochemistry and immunofluorescence and the levels of inflammation molecules by ELISA assays.
The results so far obtained confirm a substantial involvement of macrophages and of innate molecules in coping with the neoplasm and with the effects of the therapy.

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Influence of 2-methoxyestradiol on cell numbers, metabolic activity, morphology, cell cycle progression and gene expression in a breast adenocarcinoma cell line

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It has been reported that the natural metabolite of estradiol (E2), namely 2-methoxyestradiol (2ME) exerts both antitumorigenic and antiangiogenic effects *in vitro* and *in vivo*. However, several questions regarding the action mechanism of 2ME remain to be answered. The aim of this study was to determine the influence of 2ME on cell numbers, metabolic activity, morphology, cell cycle progression and gene expression in a breast adenocarcinoma (MCF-7) cell line.
Influence on cell growth and metabolic activity was investigated spectrophotometrically. Effects of 2ME on morphology were determined by means of light- and fluorescent microscopy and transmission electron microscopy (TEM). Flow cytometry was conducted to assess cell cycle progression and the Annexin V kit to determine possible occurrence of apoptosis. Microarray slides containing 20,173 known human 60-mer oligonucleotide probes were subsequently employed to study the influence of 2ME on gene expression profiles in MCF-7 cells.
2ME (10^{-6} M) caused 50% decrease in cell number and metabolic activity after 24 hours of exposure. Light- and fluorescent microscopy revealed hallmarks of apoptosis including abnormal metaphase cells, membrane blebbing and apoptotic bodies. Annexin V indicated 3.8% cells to be in early apoptosis compared to 0.5% of the control cells after 24 hours exposure to 10^{-6} M 2ME. TEM revealed increased apoptotic bodies and large intracellular vacuoles in the 2ME-exposed MCF-7 cells suggesting the induction of apoptosis and autophagy. Fluorescent microscopy showed increased acidic lysosomes and cells with compromised membranes. Bioinformatics analysis conducted on microarray data identified 681 differentially expressed genes (B-value >2.5) when compared to vehicle-treated control cells including CALM2, BAK1 and AKT1S1. These genes are involved in the regulation of apoptosis, autophagy and the G_2/M-phase transition.
The above-mentioned study is currently also being conducted to assemble the possible mechanism of action of 2ME in a non-tumorigenic breast epithelial cell line (MCF-12A). These results will thus indicate differences in signal transduction *in vitro* exerted by 2ME in cancer and normal cells respectively. Research concerning unravelling the exact mechanism of action of 2ME will enable scientists to focus on affected cellular mechanisms, as well as the identification of possible new targets for therapeutic intervention.

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Platinum (IV) complex LA-12 induces cell cycle arrest and phase specific apoptosis in colon carcinoma cells HCT116

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Background: Platinum antitumor drugs are employed in treatment of various tumor types. They create DNA adducts generating DNA damage signals resulting in cellular stress response, which forces the cell to slow down or arrest the cell cycle to allow time for DNA repair or undergo cell death. LA-12 is a novel platinum (IV) complex expressing cytotoxic effects in many cancer cell lines. It has been shown to overcome intrinsic and acquired resistance to cisplatin and oxaliplatin in ovarian and colon cancer cell lines.
Material and Methods: Colon carcinoma cells HCT116 were continually incubated with LA-12. DNA content, marker of DNA double strand breaks (gamma-H2A.X), mitotic (phospho-histone H3) and apoptotic markers and protein expression were analyzed by flow cytometry in individual cells. Cell cycle phase specific apoptosis was assessed by bivariate analysis of DNA content and presence of caspase-cleaved cytokeratin 18 (M30 antibody), as an early apoptotic marker.
Results: LA-12 induced histone H2A.X phosphorylation in S-phase cells and accumulation of the cells in G2 phase of the cell cycle, whereas the proportion of mitotic cells declined. In contrast, cells expressing cyclin B1 and active form of CDK1 were still present, even after 48 hours of incubation with LA-12, when apoptosis was massively induced. Bivariate analysis of caspases-cleaved cytokeratin 18 fragments and DNA content revealed increase in early apoptotic cells with G1 DNA content.
Conclusions: On the basis of our results, we hypothesize that in HCT116 cells, LA-12 activates DNA damage signaling by creating double strand breaks in S-phase cells by perturbation of DNA replication. This early event is followed by accumulation of the cells in G2 phase. However, certain part of the cells is somehow able to overcome this arrest and proceed to mitosis and G1 phase, where apoptotic cascade is activated and cells eventually die.
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Expression of the genes involved in apoptosis, proliferation and endoplasmic reticulum stress in ionomycin/PMA treated Jurkat cells

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The principal objective of our study was to test the regulation of gene expression of SCF/c-kit tyrosine kinase signaling pathway and the genes involved in apoptosis, proliferation and endoplasmic reticulum stress in ionomycin/PMA treated Jurkat cells.