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.