transported methotrexate into the cells when it was phosphorylated. This protein was unphosphorylated in the resistant cells. The protein was purified and digested with trypsin. Two peptides were sequenced. Using TBLASTIN, this protein was identified to be a heat shock cognate protein (HSC). The HSC protein is coded by the HSCa8 gene. A methotrexate binding assay was developed and results using this assay indicated that the HSC protein was indeed a methotrexate- binding protein. The protein from the cloned gene from L1210 cells for HSCa8 was expressed in E. coli using a polyhistidine-tag purification system. The protein showed methotrexate binding characteristics. The protein was found not to be phosphorylated in cisplatin-selected ovarian cancer cell line. In view of these findings, we propose that heat shock proteins may play important role(s) in tumors' sensitivity to methotrexate and to other drugs and as such may have important and clinically relevant implications in cancer treatment.

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ANTIDEPRESSANT DRUGS MODIFY THE CYTOTOXIC EFFECT OF TEMOZOLOMIDE ON HUMAN GLIOBLASTOMA CELLS: *IN VITRO* STUDIES

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Introduction: Chemoresistance of glioblastoma cells and side effects of standard treatment result in the need for the use of adjuvant drugs and therapies. However, literature data demonstrate that adjuvant drugs can affect efficacy of chemotherapy, which was confirmed in breast and colon cancer patients. Antidepressant drugs constitute a significant group of commonly-used adjuvant drugs due to their analgesic, antidepressant, anxiolytic and hypnotic properties. However, there are no detailed data on whether and how these drugs influence glioblastoma basic metabolic processes and efficacy of therapy with temozolomide, the first choice drug in patients diagnosed with glioblastoma multiforme (1, 2). *Materials and Methods:* Previous studies have shown that hypoxia of inner compartments of the tumor is a dynamic process (the level of pO_2 inside the tumor ranges from 0% to 5%) (3, 4). In order to reproduce *in vivo* conditions, for the first time we conducted studies in 6 experimental oxygen models. The experiments were conducted on the T98G cell line of glioblastoma multiforme and we investigated the effect of temozolomide (1000 μM), antidepressant drugs (imipramine, fluoxetine, tranylcypromine; concentrations of 1, 10,100 μM) and the interaction of temozolomide with antidepressant drugs on: cell viability, cell division and early and late cell apoptosis of glioblastoma cells. *Results:* Our study showed that glioblastoma cells cultured in conditions of chronic hypoxia were almost completely resistant to the effect of the temozolomide and antidepressant drugs. Moreover, we observed that the higher oxygen availability to glioblastoma cells was the greater statistical significance of imipramine and tranylcypromine reducing effect on temozolomide cytotoxicity was obtained. Fluoxetine did not influence the action of the chemotherapeutic drug. *Discussion:* Through these studies, we hope that oncologists and researchers will focus on the role of antidepressant drugs in patients treated with temozolomide. Knowledge of the interaction between these drugs may contribute to enhancement of anticancer effect.

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EFFECTS OF HESPERIDIN ON NON-SMALL CELL LUNG CANCER CELLS

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Background: Hesperidin, a glycoside flavonoid that is found in citrus fruits, and the mechanisms of hesperidin-induced apoptosis are not well understood. In this study, we aimed to investigate the cytotoxic and apoptotic aspect of hesperidin induction in lung cancer. *Materials and Methods:* The relation of hesperidin and non-small cell lung cancer (NSCLC) was determined by WST-1, LDH cytotoxicity, cell death detection and AnnexinV-FITC assays in a time- and dose-dependent manner. Then, changes in whole genome gene expression levels were examined using Illumina Human HT-12v4 beadchip microarrays. *Results:* The findings showed an increasing apoptotic cell population by hesperidin. Fibroblast

growth factor (FGF) signal transduction and the NF-kB pathway were the most statistically significant pathways in NCI-H358 and A549 cells in GO analysis. *Conclusion:* Our results show that hesperidin has a positive impact by modulating the immune response through a role played on the apoptotic process. After confirming its effects *in vivo*, hesperidin could be proposed as a novel anticancer agent against NSCLC.

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ADAPTIVE MECHANISMS TO ANTI-VASCULAR THERAPY

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Glioblastomas (GBMs) are highly vascular tumours and anti-vascular therapy is regarded as a promising yet controversial treatment. In theory, VEGF inhibition by using the monoclonal antibody, bevacizumab, should cause reduced tumour growth with a prolongation of patient survival. Current clinical evidence shows a dramatic reduction in tumour contrast enhancement leading to reduced vascular leakage and blood vessel normalization. This leads to an increased progression free survival but there is little evidence for increased overall survival. Thus, the tumours develop mechanisms of therapy resistance. To address mechanisms of resistance, we assessed the tumour response to bevacizumab at the phenotypic, physiological, and molecular level in clinically relevant intracranial human GBM xenograft models. We show by magnetic resonance imaging (MRI) that treatment caused a strong decrease in contrast enhancement but with only marginal effects on tumour growth. Histological observations revealed a strong reduction of large blood vessels indicating blood vessel normalization. Yet, dynamic contrast-enhanced MRI revealed a significant reduction in intra-tumoural blood flow indicating that the treatment did not lead to increased perfusion of the tumours. Interestingly we observed a significant increase in tumour cell invasion in the treated tumours.

At the molecular level bevacizumab treatment led to an increase in HIF1α with a subsequent increase in lactate and alanine metabolites indicating that the treatment induced vascular remodelling leading to a more hypoxic tumour microenvironment. This indicates that bevacizumab causes a metabolomic change in the tumours toward glycolysis.

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TUMOR SUPPRESSOR ACTIVITY OF PROTEIN KINASE C A IN EPITHELIAL CANCERS

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Protein kinase C α (PKCα), a member of the conventional (classical) class of PKC isozymes, has been linked to both negative and positive regulation of cell proliferation, survival, and motility in normal cells, and downstream effects of PKCα can be tumor promoting or tumor suppressive depending on cell type and context. Extensive evidence from our laboratory and other supports a tumor suppressor role for PKCα signaling in the intestine/colon, endometrium, lung, and prostate, among others. Our studies have shown that expression of the enzyme is broadly lost by transcriptional repression in intestinal and endometrial neoplasia, and PKCα deficiency is associated with more aggressive/invasive tumors and reduced survival. These effects likely reflect the ability of PKCα to induce cell cycle withdrawal in non-transformed epithelial cells and to potently suppress anchorage-independent growth in epithelial cancer cells. PKCα acts on multiple signaling pathways to elicit these tumor suppressive effects. The enzyme inhibits PI3K-AKT signaling at multiple levels to restrain protein synthesis and cell growth. PKCα signaling also promotes sustained activation of the ERK/MAPK pathway to elicit cell cycle withdrawal. Mechanistic analysis points to inhibition of mitogenic molecules such as cyclin D1 and inhibitor of DNA binding 1 (Id1) and induction of cell cycle inhibitory proteins such as the cyclin-dependent kinase inhibitor $p21^{Waf1/Cip1}$ in mediating the tumor suppressive effects of PKCα. In contrast to PKCα, other PKC isozymes (*e.g.*, PKCε, PKCι) are tumor promoting in epithelial tissues and the interplay between their opposing activities dictates cellular responses. Based on these findings, we are also exploring the potential of these signaling pathways as prognostic indicators and therapeutic targets in epithelial cancers.

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NEW ASPECTS CONCERNING THE RADIATION OF BREAST CANCER

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Background/Aims: Radiotherapy (RT) improves overall survival (OS) of breast cancer patients after breast conserving surgery and after mastectomy in patients with involved lymph nodes (LN). The contribution of RT to the regional LN to this survival benefit was poorly understood. Recently, the results of three large randomized trials addressing this question have become available. *Patients and Methods:* The published abstracts (full publication pending)