Transfection with pDsRed2-C1-DF3 and pDsRed2-C1 vectors was evaluated in breast cancer hormone-dependent adenocarcinoma MCF-7 and carcinoma T47D, hormone-independent adenocarcinoma HBL-100 and carcinoma MDA-MB-435S, ovarian carcinoma Sk-Ov-3, mouse fibroblasts OMEGA-E, green monkey renal epithelium Cos-1 cell cultures. Specific expression of reporting FP was observed in transfected MCF-7, T47D, Sk-Ov-3 and HBL-100 cell lines 36-48 hours after transfection. There was no detectable FP expression in non-specific cells OMEGA-E, Cos-1 and MDA-MB-435S. Transfection efficiency of pDsRed2-C1-DF3 was 20-40% depending on cell culture without great difference with control vector but level of DsRed2 expression from DF3 is only 40-50% of that CMV promoter delivers. Flow cytometry and confocal microscopy analysis showed high presentation of MUC1 receptor in hormone-dependent MCF-7 and T47D, lower in HBL-100, Sk-Ov-3 and MDA-MB-435S and absence of expression in negative control cell lines.

Conclusions: Clinic evidences of MUC1 hyperexpression in 95-98% of breast cancer cases, especially in 30% of ER- and 65% of HER2-neo-negative primary tumors, made this antigen one of the most important diagnostic markers in genotyping and proteomics assays. The enhancement of MUC1 promoter's expression activity is a prospective target for development of selective metastatic breast cancer therapy.

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Orchestrating role of bisindolylmaleimide IX in integration of extrinsic and intrinsic apoptosis in COLO 205 cells

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Introduction: Resistance to apoptosis is the strategy used by cancer cells to avoid elimination. We have previously shown that in COLO 205 cells the blockage of TNF-alpha-dependent extrinsic apoptosis results from cFLIP overexpression. Thus, our efforts are focused on the restoration of cell harmony by the use of metabolic inhibitors, such as bisindolylmaleimide-IX (Bis-IX), which is believed to return the balance in apoptosis. Methods: The experimental model was human colon adenocarcinoma COLO 205 cell line. Cell survival was evaluated by MTT assay. The apoptosis induction was visualized by Hoechst/propidium iodide staining. Immunoprecipitation and Western-blot techniques were used to show the expression of proteins and their respective cellular interactions. Additionally, Scan^R Screening System allowed monitoring of the expression of proteins, engaged in apoptosis machinery. Results: The application of Bis-IX sensitized COLO 205 cells to TNF-alpha-mediated apoptosis. The susceptibility of human COLO 205 cells to apoptogenic stimuli resulted from time-dependent reduction in cFLIPL and TRADD protein levels. At the same time, the level of FADD protein was up-regulated. Additionally, the presence of Bis-IX caused caspase-8-independent cytochrome c release and caspase-9 cleavage. In turn, the treatment with bisindolylmaleimide III (Bis-III) did not evoke neither TNF-alpha-dependent nor intrinsic apoptosis. Conclusions: The results of this study indicate that Bis-IX facilitates the death receptor signal mediated by TNF-R1. Moreover, Bis-IX is able to activate mitochondria in caspase-8-independent intrinsic apoptosis. Targeting antiapoptotic protein(s) with TNF-alpha and Bis-IX is a promising tool to activate apoptosis in order to improve efficacy of cancer treatment.

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Molecular markers of human brain tumors and their participation in cellular signaling pathways

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The aim of this investigation is identification characterization of genes with significant changed expression in brain tumors and their possible interaction with signaling pathways. Such knowledge is necessary not only for understanding the tumorigenesis, but also the normal brain functioning.

SAGE, Northern, RT-PCR, Western blot analysis, histochemistry were used to identify 129 genes with 5-fold changes of expression in glioblastomas, the most aggressive form of human brain tumors. This altered pattern of gene expression in tumor cells can be viewed as a molecular marker in the analysis of malignant progression of astrocytic tumors, and as possible clues for the mechanism of disease. Moreover, several of genes overexpressed in glioblastomas produce extracellular and membrane proteins or proteins involved in signaling pathways, thereby providing possible therapeutic targets. Next step includes functional analysis of encoded proteins, their potential partners and participation in cellular signaling pathways. High levels of HC gp-39 gene expression, the product of which reveals a mitogenic effect, similar to the effect of insulin-like growth factor I (IGF-I), correlates with unfavorable course of disease.

Since deregulation of the IGF system/HC-gp39 is a frequent pattern in tumours, IGFs/ IGFBPs/HC-gp39 should be included in the panel of tumour markers used for the diagnosis and serological surveillance in various malignancies.

As a functional antagonist to the potential oncogene HC gp-39, gene TSC-22 has significantly lower expression in astrocytic gliomas. Differential expression of TSC-22 was confirmed by histochemical analysis. TSC-22 may serve as a mediator of TGF-β signals. A substantial decrease of TSC-22 expression on the RNA and protein levels revealed in glial tumors together with known negative role of TSC-22 in the cell proliferation regulation have evidenced about its tumor-suppressed function, it allows to offer TSC-22 as a prognostic factor for gliomas.

Further characterization of these genes will thus allow them to be exploited in molecular classification of glial tumors, diagnosis, prognosis, and anticancer therapy. Novel antisense and iRNA strategies targeting components of cellular signaling pathways may offer additional options for treatment of malignant gliomas.

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Full-length tissue transglutaminase is a resistance factor for cell differentiation in neuroblastoma

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Transcriptional activation of tissue transglutaminase (TG2) is essential for neuroblastoma cell differentiation induced by retinoic acid (RA). We have previously shown that the MYCN oncogene suppresses neuroblastoma cell differentiation through repressing TG2 gene transcription. Due to the alternative splicing of pre-mRNA, there exists at least 4 isoforms of TG2.

In this study, we aim to determine the effects of the full-length isoform of TG2 (TG2-L) on neuritic differentiation and cell viability in neuroblastoma cells. MYCN amplified neuroblastoma BE(2)-C and LAN-1 cells were transiently transfected with scrambled control siRNA, total-TG2 siRNA targeting all isoforms of TG2 or TG2-L siRNA specific for TG2-L, followed by treatment with 1μM of all-trans RA (atRA) or control for 5 days, to determine the effect of TG2-L on cell differentiation and the combinational effect of repression of TG2-L and retinoid. siRNA transfection efficiency was determined by competitive RT-PCR. Cell viability was assessed by trypan blue assay.

Treatment with atRA induced transcriptional activation of TG2-L in both BE(2)-C cells and LAN-1 cells. Repression of TG2-L with siRNA alone induced neuritic differentiation with morphological transition to neuronal type in the cells within 48 hours, and more dramatically 5 days post-transfection. The induction of differentiation was further amplified when cells were transfected with TG2-L siRNA and treated with 1μM atRA, compared with atRA alone or TG2-L siRNA alone ($p < 0.05$). Moreover, combination of TG2-L siRNA and atRA resulted in a dramatic decrease in cell viability 5 days post-treatment in both BE(2)-C and LAN-1 cells.

Taken together, our data suggests that TG2-L is a resistance factor to neuritic differentiation in MYCN-amplified neuroblastoma cells, and that decrease in cell viability after TG2-L repression is secondary to terminal differentiation.

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Jab1 and estrogen receptor alpha (ERa) in human breast cancer

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Jab1 (Jun activation domain-binding protein 1) may have a role in the development and progression of breast cancer. Interestingly in a recent study conducted in our laboratory with 283 ERα-+ve breast tissues, examined by immunohistochemistry, we found a significant positive correlation between Jab1 and ERα expression. This result was unexpected given previous reports in the literature. To investigate the potential mechanisms underlying this relationship, we determined the expression of Jab1 in breast cancer cell lines after estrogen (E2) and anti-E2 treatment by western blot. Exposure of cells to 4-Hydroxy-tamoxifen (4-HT) resulted in a little up-regulation of Jab1 after 24h. As expected we observed an increased expression of ERα protein after 4-HT treatment at > 24 hours, and a strong down-regulation of ERα due to treatment with ICI 182,780 (ICI). However, no significant change in Jab1 expression was found due to