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

ICI treatment. E2 treatment caused down-regulation of ERα after 1h, but no significant change in Jab1 expression. Previous data showed that the subcellular distribution of Jab1 can be regulated. Thus, we also investigated if E2- or anti-E2 treatment could affect the cellular distribution of Jab1, using confocal microscopy and immunofluorescence. Neither E2 nor anti-E2 treatment resulted in a major shift of Jab1 between the nucleus and the cytoplasm. Interaction of ERα and Jab1 under E2- and anti-E2 treatment was investigated using coimmunoprecipitation (Co-IP). A small amount of ERα was Co-IPed with Jab1, which was enhanced by 4-HT treatment. Pre-treatment of MCF7 cells with curcumin increased the portion of Co-IPed ERα. In addition, using siRNA to knock-down Jab1 expression, a significant down-regulation of ERα was observed (P = 0.049). To conclude, a strong correlation between Jab1 and ERα expression occurs in breast tumors in vivo. In ERα+ MCF7 breast cancer cells, there is no short-term regulation of Jab1 expression by E2 and/or anti-E2 treatment. However, Jab1 and ERα may interact directly or within a complex, and this may be influenced by ligand. As well transient knock-down of Jab1 using RNAi resulted in a small but significant decrease in ERα expression suggesting that longer term knockdown of Jab1 may decrease ERα steady-state levels further. Jab1 expression is generally over-expressed in breast cancer compared to normal breast tissue and therefore may have a role in upregulating ERα expression which occurs during breast tumorigenesis.

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The identification of new tumour suppressor micrornas epigenetically silenced in drug resistant cancer cells

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Background: MicroRNAs (miRNAs) are a recently discovered class of non-coding short length RNAs (21-24 nucleotides in length) that play a fundamental role in gene regulation. These molecules down-regulate the expression of their target genes by base pairing to 3' UTR of the target messenger RNAs (mRNAs). These small RNAs are involved in the control of several biological processes, from cell differentiation to cell proliferation, thereby playing an important role in cancer. It was previously demonstrated that miRNA-127 (miR-127), is embedded in a CpG island and is highly induced from its own promoter after treatment with the demethylating agent 5-aza-2'-deoxycytidine (AZA) and the chromatin-modifying drug 4-phenylbutyric acid (PBA). In addition, it is usually expressed as part of a miRNA cluster in normal cells but not in prostate, bladder, and colon cancer cells, suggesting that it is subject to epigenetic silencing.

Materials and methods: Real Time PCR was used in order to evaluate the expression of miRNAs and their corresponding host genes in cancer cell lines. We analysed promoter sequences in cell lines by bisulfite-sequencing and methylation-specific polymerase chain reaction (MSP) to assess methylation status.

Results: Transcriptional silencing in cancer by CpG island methylation of genes that contain miRNAs can down-regulate the expression of the miRNAs as well while up-regulating mRNA expression, classifying both as tumour suppressors. We have mapped several miRNAs inside the introns of putative tumour suppressor genes and have observed down-regulation in cancer cell lines compared to the non invasive counterpart, and in cancer cell lines compared to the drug resistant counterpart, once again classifying both as tumour suppressors. Furthermore, this down-regulation appears to be due at least in part, to CpG island methylation.

Conclusions: This work permitted us to identify new miRNAs and new genes that are silenced in cancer due to an epigenetic event.

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The role of the estrogen-responsive B box protein (EBBP) in cancer cell cycle progression

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We have previously identified EBBP as a transcription factor involved in the retinoid anti-cancer pathway in Neuroblastoma. Currently, we aim to characterise EBBP's mechanism and role as a novel regulator of cancer in cell cycle progression. EBBP is a member of the evolutionarily conserved RBCC/TRIM (RING finger, B-box, coiled-coil/tripartite motif) group of proteins, which have diverse functions including; apoptosis, proliferation, differentiation, and transcriptional regulation.

In the absence of retinoid, EBBP overexpression induced growth inhibition and apoptosis in both retinoid-sensitive and -resistant cancer cells. Growth arrest correlated with reduced Cyclin D1 expression and phosphorylation of Rb. Furthermore, EBBP induced growth arrest in 7 human cancer cells in the absence of retinoid, but not in 4 normal cell lines. Retinoid treated neuroblastoma cells (retinoid-sensitive) displayed increased EBBP in nuclear aggregates. Contrastingly, retinoid-resistant breast cancer cells treated with retinoid displayed peri-nuclear EBBP aggregations. We have identified E2F transcription factor 1 (E2F-1), and Vimentin, both as EBBP-binding proteins by mass spectrometry and co-immunoprecipitation. EBBP also modulated E2F-1 and Vimentin protein expression in neuroblastoma cells as demonstrated by EBBP transfection and siRNA knock-down experiments.

Like other TRIM family members, EBBP may act as a corepressor in protein-protein complexes, or depending on the cell context it may act as a coactivator. Recently, we demonstrated that down-regulation EBBP expression with specific EBBP siRNA also reduced cell proliferation, induced apoptosis, and blocked phosphorylation of pRb, in retinoid-sensitive cancer cells, but not in retinoid-resistant cancer cells. To determine whether EBBP overexpression influences tumour-forming ability and sensitivity to retinoid treatment in vivo, we established breast cancer (MDA-MB-231) and neuroblastoma (BE(2)-C) stable cell lines overexpressing EBBP. As anticipated, exogenous EBBP induced growth inhibition and increased retinoid sensitivity in these stable clones.

Thus, EBBP has both retinoid-dependent and -independent functions, which may relate to cell cycle regulation and cell structure. These properties make EBBP an exciting new therapeutic target for anticancer compounds that are designed to target cancer cells while having reduced side effects on normal cells.

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Hh-Gli signaling in tumors; Hh-Gli activation and effects on cell cycle progression

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Ovarian dermoid cysts (DC) or benign cystic teratomas are benign tumors descending from germinal cells composed of elements descending from all three of the germinal layers.

We present an investigation of Hh-Gli signaling pathway in ovarian dermoids. Previously, we have shown that methylation of Ptch promoter may contribute to pathway malfunctioning.

We developed several different clone lines derived from primary cell cultures of ovarian dermoid tissue. RNA was isolated and Real-Time PCR analysis was performed. Real-Time PCR demonstrated expression of the Hh-Gli pathway genes. This expression, although present in all clone lines, differs among them, confirming the heterogeneity of this tumor type.

Some of the clone lines were additionally analyzed by immunofluorescent staining. Our results show difference in localization of some of Hh-Gli pathway proteins among the clone lines, and some of them show reactivity to cyclopamine treatment, on both mRNA and protein level. I.e. we have seen difference in localization of Ptch and Smo during cell cycle.

For cell cycle analysis cells were treated with cyclopamine, tomatidine or Shh protein, stained with propidium iodide and analyzed by flow cytometry. In this way we have also demonstrated effect of cyclopamine treatment on cell cycle progression of these clone lines. Taken together, this data suggests Hh-Gli pathway involvement in tumorigenesis and cell cycle progression of ovarian dermoids.

Since similar results were previously shown on ovarian carcinoma, we suggest Hh-Gli pathway aberration is an early event in transformation of ovarian cells in their progression towards malignancy.

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The Na+/H+ exchanger regulation factor (NHERF1) is a component of epidermal growth factor receptor (EGFR) signalling complex and regulates EGFR degradation

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Background: NHERF1 (Na+/H+ exchanger regulating factor 1) is a PDZ domain-containing protein that recruits membrane receptors and transporters and cytoplasmic signalling proteins into functional complexes. Recent evidence obtained from our laboratory and from other groups shows that NHERF1 is an important player in cancer progression. Interestingly, NHERF1 was shown to associate with proteins involved in cancer progression. Some of these are tumor and metastasis suppressors, such as PTEN (phosphatase and tensin homologue deleted on chromosome 10). Other NHERF1 associated proteins are oncogenic, such as EGFR.