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.