PN-1 locus and flanking sequences (−1214 to −596). The PCR products were sequenced.
Results: We found that efficacy of PCR amplification of *PN-1* in tumour DNA samples depended on the presence of PCR enhancers 2-pirrolidone and/or dimethylsulfoxide (DMSO) in the reaction mixture. Three classes of DNA templates were isolated from different tumours. The first group marked P+D+ included 60 tumour DNA samples in which the *PN-1* was amplified only in the presence both 2-pyrrolidone and DMSO. The second group (P-D+) included 27 samples in which this locus was not amplified in the presence of 2-pyrrolidone, but the PCR product was generated when DMSO instead of 2-pyrrolidone was added. Neither 2-pyrrolidone nor DMSO could stimulate PCR amplification of the *PN-1* in the 14 DNA samples (P−D−) belonging to the third group. We supposed that DNA methylation might influence the efficacy of the *PN-1* PCR amplification. To prove our hypothesis methylation status of *PN-2* region was examined. In five tumour DNA belonging to P+D+ group and in five DNA samples from normal endometrium no methylation of *PTEN* promoter cytosine residues was detected. The five tumour DNA of P−D+ group revealed 51−79% of methylated CpG dinucleotides. In this case the non-CpG methylation was also observed. In DNA sample belonging to the P−D− group the fraction of CpG methylation in *PN-2* was 67.4%. In all cases the methylated cytosines were localized in the range of −1036 to −618. In addition to CpG the methylation at CpA, CpC and CpT sites was observed.
Conclusion: We revealed aberrant *PTEN* promoter methylation (epimutations) in endometrial and ovarian tumours and showed that methylation affects PCR amplification of *PTEN* promoter region.

736 The expression of WWOX tumour suppressor gene in colorectal cancer

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Background: *WWOX* gene is located in chromosome region 16q23.3–24.1 (common fragile site FRA16D), an area which is affected by frequent allelic losses in breast cancer. The evidence for tumour suppressor activity was first demonstrated in several cancer cell lines, however numerous studies showed either loss or reduction of the *WWOX* expression in a variety of tumours, including breast and lung cancer. The most common way of affecting *WWOX* function in cancer cells occurs through hemizygous deletions, while point mutations are rare. A number of studies showed that in some cancer types hypermethylation of *WWOX* promoter could be of some importance. Recently, a set of complex heterozygous deletions manifesting as homozygous loss was found at FRA16D in the HCT116 colon cancer cell line. Although this resulted in removing exons 6 to 8 of one *WWOX* transcript, it did not prevent the transcription of wild-type *WWOX* from 3rd allele. In order to clarify the role of *WWOX* gene in colorectal cancer (CRC) tissues, we analysed 99 tumour samples and 4 CRC cell lines: HT-29, HCT116, SW480 and SW620.
Material and Methods: The study was approved by the local Ethics Committee. Informed consent was obtained from all patients. Tissue samples were stored at −80°C until RNA extraction. All qPCR reactions were performed in duplicate, with EvaGreen dye and Corbett Research RG-3000 platform. LOH status was determined by three STS markers: D16S3096, D16S504, D16S518. Methylation status of two *WWOX* promoter regions was performed by *MethylScreen* method utilising qPCR assay on templates generated by combined restriction digestions.
Results: Relative *WWOX* expression in CRC tissues ranged from 0 to 123.18 (median 7.66). In the studied population we did not find any significant hemizygosity suggesting LOH at the studied loci. Also, there was no significant methylation of two examined regions. We found significant difference of DFS in patients with relatively high and low *WWOX* expression ($HR = 0.39$; $p = 0.0452$), but in multivariate analysis it was not an independent prognostic factor. *WWOX* expression correlated with expression of BCL2($r = 0.3996$; $p = 0.0001$), BAX ($r = -0.2671$; $p = 0.0082$) and CCNE1 ($r = -0.3579$; $p = 0.0005$).
Conclusions: Our data suggest that, unlike other tumours, *WWOX* expression in CRC is affected by different mechanisms than deletion or methylation. *WWOX* expression in CRC tumours correlated with expression of genes responsible for cell cycle regulation.
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737 The role of WWOX tumour suppressor gene in colorectal cancerogenesis − a microarray study on HT29 colon cancer cell line

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Background: Colorectal cancer is one of the leading cause of cancer-related deaths in both men and women in western countries. Nowadays, there are three recognized distinct molecular pathways of colon cell cancer transformation. The most common way is an acquisition of chromosomal instability (CIN). Microsatelite instability phenotype(MSI) and CpG Island Methylator Phenotype(CIMP) constitute for the other two pathways. Each of this cancerogenesis phenotype is characterized by molecular profile of genomic, transcryptomic and proteomic alterations.
WWOX is a tumour suppressor gene that spans the common fragile site FRA16D. It has been proven that *WWOX* participates in controlling expression of genes which are responsible for tissue morphogenesis and cell differentiation. Its altered expression has been demonstrated in many tumour types. Moreover, reduction of *WWOX* expression correlates with more aggressive disease stage and higher mortality rate (breast, gastric, lung cancer).
Materials and Methods: Experiments were performed on HT29 colon cancer cell line transfected with *WWOX* cDNA.
Using real-time RT PCR we estimated relevant expression level of 8 cancer marker genes(apoptosis, proliferation, adhesion and cell cycle regulation genes).
We employed whole genome, oligonucleotide microarrays(Human OneArray™;Phalanx Biotech) to assess the influence of *WWOX* on gene expression profiles. Moreover, we performed biological test of anchorage independent growth.
Results: Analysis of microarrays evaluated over 300 differentialy expressed genes in result of increased *WWOX* expression ($p < 0.05$). Our study demonstrated that *WWOX* inhibits expression of genes that are involved in cell cycle progression, WNT and Cadherin signaling pathways and cytoskeletal regulation by Rho GTPase. Genes related to apoptosis and FAS signaling pathway are upregulated. Microarray results are consistent with real time RT-PCR and will be confirmed with Western-Blott and RT-PCR for chosen genes.
Moreover, there was complete inhibition of cell growth in soft agar in cell culture with higher expression of *WWOX* gene.
Conclusions: Microarray gene expression study confirmed the role of *WWOX* in regulation of important pathways in cancerogenesis. As we assumed it has major impact on apoptosis, cell cycle regulation and WNT pathway inhibition in HT29 colon cancer cells.

738 WWOX tumour suppressor gene is affected in glioblastoma multiforme

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Background: Glioblastoma multiforme (GBM) is the most common type of primary brain tumour in adults. This neoplasm is highly lethal with an average survival about 1 year. *WWOX*, a tumour suppressor gene located in a common fragile site FRA16D, is involved in carcinogenesis and cancer progression in many different cancers. Reduced *WWOX* expression is associated with more aggressive phenotype and poor patient outcome in several cancers. Our aim was to investigate *WWOX* expression alternations and its correlations with proliferation, apoptosis and signal trafficking in GBM. We evaluated methylation level of *WWOX* promoter and percentage of loss of heterozygosity (LOH) in *WWOX* genomic region. We also analysed the correlation between mRNA level of *WWOX* and other cancer related genes such as *Ki67*, *Bcl2*, *Bax*, *EGFR*, *ErbB4* (splice variants: *JM-a* and *JM-b*).
Material and Methods: Using real-time RT-PCR we analysed expression levels of 7 genes in 59 cases of GBM. LOH was assessed in 63 patients by high resolution melting. Allelic losses were analyzed for three microsatellite markers: D16S504, D16S518, D16S3096. Methylation detection was performed for two regions of *WWOX* promoter with high contents of CpG. The examination was conducted by MethylScreen method in 67 patients.
Results: We observed a relatively high percentage of LOH for two out of three analysed microsatellites: 38.5% (D16S3096) and 54.5% (D16S504). Concurrent analysis of *WWOX* expression level in reference to promoter methylation and microsatellite markers state revealed a difference in *WWOX* expression in homo and heterozygotes. The highest expression was exhibited by unmethylated, heterozygous samples while the lowest by methylated, homozygous. Loss of heterozygosity lowered expression level in unmethylated samples (with exception of D15S504). Promoter methylation considerably

reduces *WWOX* expression both in hetero and homozygous cases. There was a positive correlation between expression level of *WWOX* and marker of proliferation *Ki67* ($R_s = 0.5440$; $p < 0.0001$), antiapoptotic gene *Bcl2* ($R_s = 0.7092$; $p < 0.0001$) and *JM-a* isoform of *ErbB4* mRNA level ($R_s = 0.7102$; $p < 0.0001$).
Conclusions: Our results suggest that loss of heterozygosity (relatively frequent in GBM) along with promoter methylation may decrease the *WWOX* tumour suppressor expression. We also confirmed that *WWOX* is correlated with *ErbB4* signalling pathway as well as with proliferation and apoptosis in glioblastoma multiforme.

739 Altered expression of miR-205 affects cell proliferation in human cervical cancer

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Background: Cervical cancer is the second most common malignancy of women worldwide. Recently, aberrant miRNA expression has been demonstrated in human cervical cancer, however the functional role of miRNAs in cervical cancer remains unclear. We previously observed a significant over-expression of *miR-205* in human cervical cancer tissues as compared to their matched normal cervical tissues in a sequencing-based miRNA profiling analysis. In this study, we further explore the role of *miR-205* in cervical cancer.
Materials and Methods: We evaluated the expression of *miR-205* in 29 matched pairs of human cervical cancer and normal tissues, as well as eight cervical cancer cell lines using quantitative PCR analysis. To investigate the functional consequences of altered *miR-205* expression in cervical cancer cells, we determined the effect of *miR-205* on cell proliferation in cervical cancer cell lines using a *miR-205* specific mimic or inhibitor. Cell proliferation was measured by WST-1 (water-soluble tetrazolium salt) assay.
Results: We observed significant over-expression of *miR-205* in cervical cancer samples ($p = 0.0052$), as compared to their matched normal counterparts. Over-expressing of *miR-205* in HeLa and SW756 cells, the two cell lines with no detectable or low level of endogenous *miR-205* expression, resulted in significant increase in cell proliferation in both cell lines as compared to the negative control (treated with a non-targeting sequence pre-miR molecule). As a complement to the over-expression experiments, we suppressed endogenous *miR-205* expression in CaSki cells using a miRNA inhibitor, which cell line has a high-level of endogenous *miR-205* expression; which resulted in inhibition of cell proliferaion.
Conclusions: Over-expression of *miR-205* is frequently observed in human cervical cancer, and its dysregulation can alter cell proliferation in cervical cancer cell lines. Our findings provide new insight into the role of *miR-205* in cervical cancer, which may have potential value for clinical diagnosis and therapeutics.

740 Role of the tumour suppressor FOXO3a and its regulation by β-catenin in the pathogenesis of non-Hodgkin's Lymphomas

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Background: Non-Hodgkin's Lymphomas (NHL) are a heterogeneous group of aggressive haematological malignancies with high incidence. This group of tumours is characterized by molecular alterations in cellular processes such as cell cycle regulation and apoptosis in which FOXO3a is involved. The aims of this study are: 1) to evaluate the role of FOXO3a and its regulation by β-catenin in the pathogenesis of NHL; 2) to study its role as a functional target of therapeutic agents.
Materials and Methods: A panel of NHL cell lines was used. Cells were treated with different inhibitors of the PI3K/AKT/FOXO3a pathway (LY294002, Rapamycin, Triciribine and Psammaplysene) and the Wnt/APC/β-catenin pathway (Quercetin) for further analysis. Conventional drugs such as Doxorubicin were also used. Cell viability (Cell Titer-Glo Luminiscent assay kit) and cell cycle analysis (FACS) were performed. Expression and cellular localization of different proteins were studied by means of Western Blot, quantitative PCR and immunocytochemistry.
Results: The role of FOXO3a in a panel of non-Hodgkin's lymphomas has been evaluated. Cells lacking FOXO3a expression are resistant to inhibition of the PI3K/AKT pathway. On the contrary, cells expressing FOXO3a are capable of inducing cell death and apoptosis after PI3K/AKT inhibition, suggesting that FOXO3a inactivation has a role in lymphomagenesis. Moreover, β-catenin, a well known oncogene, is constitutively activated in these cells and interacts with FOXO3a.
Conclusions: The reactivation of FOXO3a and the suppression of β-catenin activity represent a potential therapy for NHL that could enhance the effects of conventional drugs.

741 microRNA-34 expression and the status of TP53 in the primary ovarian cancer

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Background: Changes in TP53 and TP53-dependent pathways are critically important for the pathogenesis of ovarian cancer and seem to determine treatment response. TP53 is known to directly transactivate a set of microRNAs (miRNAs), including miRNA-34 family. The role of TP53-regulated miRNAs in ovarian cancer biology has not been characterised. We aimed to reveal the impact of *TP53* status on the expression of a family of miRNA-34 in ovarian cancer tumours.
Material and Methods: Post-surgical tumour samples from ovarian cancer patients were characterised histopathologically and with regard to the *TP53* status, including differentiation between missense and nonsense mutation as well as to TP53 protein accumulation. Samples were divided into the following groups: low grade carcinomas with the wild-type *TP53* ($n = 7$), high grade carcinomas with wild-type *TP53* ($n = 13$) and carcinomas with the determined types of *TP53* alterations ($n = 23$). Samples containing a minimum of 75% of cancer cells were subjected to miRNA-34a/b/c expression analyses, with the use of TaqMan MicroRNA Assays. Small nuclear RNA, RNU6B (Applied Biosystems) was used to normalise data.
Results: In all series of the samples, a considerable number of ovarian cancer samples showed elevated levels of expression of miRNA-34 family members. Samples with *TP53* mutations presented higher levels of microRNA-34b expression than those with wild-type *TP53*.
Conclusions: (1) Elevated levels of miRNA-34 family members in some ovarian cancer tumours suggest that these TP53-regulated microRNAs may present other roles than tumour suppressors, contradictory to some other cancers, where miRNA-34, has been reported to be downregulated. The miRNA-34 functions apparently depend on the molecular context, tissue type and/or clinical characteristics of a disease. (2) An increase in the miRNA-34b expression in ovarian cancer cells with mutated *TP53* suggests that there are TP53-independent mechanisms of miR-34 regulation.

742 MicroRNAs in the differential diagnosis of aggressive B-cell lymphomas

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Background: Fast and reliable differentiation of Burkitt's lymphoma (BL) *vs* diffuse large B-cell lymphoma (DLBCL) is of major importance for therapeutic decisions, as BL and DLBCL patients need to be treated differently, and in BL, once the diagnosis is established, treatment should be initiated promptly. However, a clear-cut diagnosis between the two malignancies is difficult as some aggressive B-cell lymphomas do not meet the criteria of BL or DLBCL. We aimed to investigate miR-155, miR-21 and miR-26a, as potential biomarkers for the differential diagnosis of BL *vs* DLBCL.
Material and Methods: Fresh ($n = 44$), fine-needle tumour biopsies and formalin fixed paraffin-embedded (FFPE) tumour samples ($n = 5$) from patients with adult BL, DLBCL and intermediate BL/DLBCL were examined. Samples were characterised according to the recent WHO recommendations (2008). miRNAs were measured using TaqMan microRNA assays (Applied Biosystems), with RNU6B as a reference gene. Normal peripheral blood mononuclear cells were used as a calibrator sample. Ten patients' sera were assessed for miR-155 and miR-26a. Expression of the miR-155 precursor, *BIC*, was estimated in 22 samples.
Results: In adult DLBCL miR-155 was significantly higher than in BL and intermediate BL/DLBCL, where it was either absent or very low. In BL and BL/DLBCL also *BIC* expression was very low. So far, we showed no differences in miR-26a expression between DLBCL and BL and a bit higher miR-21 expression level in DLBCL than in BL. FFPE samples were shown to be acceptable as a material to assess microRNA expression.
Conclusions: (1) The expression level of miR-155 differentiates BL from DLBCL, but not BL from intermediate BL/DLBCL. miR-155 assessment may be an additional method of choice for routine differential diagnosis of BL *vs* DLBCL. (2) Low levels of miR-155 expression in BL seem to relate to low/absent transcription of *BIC*, the miR-155 precursor, and not to the alterations in the miRNA-155 biogenesis. (3) The value of miR-21 as a biomarker deserves further studies; miR-26a seems to have no diagnostic value. (4) Supplementing immunophenotype and cytogenetic data in aggressive B-cell lymphomas by microRNA measurements may provide new insights in the pathogenesis of these diseases.