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

M. Zelazowski[1], E. Pluciennik[1], M. Nowakowska[1], K. Seta[1], K. Kosla[1], G. Pasz-Walczak[2], P. Potemski[3], R. Kordek[2], A.K. Bednarek[1]. *[1]Medical University in Lodz, Molecular Carcinogenesis, Lodz, Poland, [2]Medical University in Lodz, Department of Pathology, Lodz, Poland, [3]Medical University in Lodz, Department of Chemotherapy, Lodz, Poland*

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

M. Nowakowska[1], K. Seta[1], U. Lewandowska[2], E. Pluciennik[1], M. Zelazowski[1], K. Kosla[1], A.K. Bednarek[1]. *[1]Medical University in Lodz, Department of Molecular Cancerogenesis, Lodz, Poland, [2]Medical University in Lodz, Department of Medical Enzymology, Lodz, Poland*

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

K. Kosla[1], E. Pluciennik[1], K. Seta[1], M. Nowakowska[1], M. Zelazowski[1], A. Kurzyk[1], A. Jesionek-Kupnicka[2], R. Kordek[2], P. Liberski[3], A.K. Bednarek[1]. *[1]Medical University of Lodz, Department of Molecular Cancerogenesis, Lodz, Poland, [2]Medical University of Lodz, Department of Pathology, Lodz, Poland, [3]Medical University of Lodz, Department of Molecular Pathology and Neuropathology, Lodz, Poland*

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