of the known inhibitor cerulenin caused 40% inhibition. PA treatment led to increased expression of phosphorylated-eIF2α after treatment for 6 hours with 2.5 and 5.0 μg/mL. Constitutive activation of STAT3 in U266 multiple myeloma cells was not affected by PA and activation of ERK1/2 in RPMI-8226 multiple myeloma cells was only partially inhibited.
Conclusion: The results suggest that the anti-proliferative and pro-apoptotic effects of PA are not primarily mediated through inhibition of signalling from growth factor receptors but may be the consequence of ER-stress possibly related to disturbed lipid metabolism.

871 Deficiency of the WWOX Fragile Gene Impairs DNA Damage Response

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Common chromosomal fragile sites are preferential targets of replication stress in preneoplastic lesions, resulting in deletions involving archetypal fragile genes encoded at these conserved chromosome regions, such as *FHIT* and *WWOX*. The *WWOX* (WW domain-containing oxidoreductase) gene encompasses the second most active chromosomal fragile site, FRA16D; a region involved in loss of heterozygosity and homozygous deletions in cancers and cancer-derived cell lines, in chromosome translocations in multiple myeloma, and its promoter region is frequently hypermethylated in cancers. Indeed, Wwox expression is reduced or absent in most common human cancers and its restoration in Wwox-negative cells suppresses tumourigenicity both *in vitro* and *in vivo*. Targeted ablation of the *Wwox* gene in mice demonstrated *bona fide* tumour suppressor function. Recently, it has been suggested that damage to fragile sites, with lost function of genes located at these sites, is coincident with activation of DNA damage response (DDR) checkpoint proteins suggesting that fragile sites might function as DNA damage warning sensors. Nevertheless, role of the *WWOX* fragile gene and the mechanism it might play in DDR are largely elusive. Here, we demonstrate that *Wwox*-deficient murine fibroblasts (MEF) display increased number of total chromosomal breakage as compared to wild type counterparts following treatment with aphidicolin, a mild DNA replication inhibitor. Overexpression of Wwox in *Wwox*-deficient MEF rescued this phenotype. Moreover, our findings show that this genomic instability in murine fibroblast is associated with delayed gH2AX foci formation. Furthermore, manipulation of Wwox expression in human cancer cell lines is associated with altered DDR checkpoint activation and DNA repair. Our data suggest that loss of the *WWOX* fragile gene product impairs DDR thus contributing to genomic instability. These findings present a fresh prospective on the role of Wwox as a tumour suppressor, which is inactivated early in pre-neoplastic cells, and how its loss may provide a selective advantage for clonal expansion of neoplastic cells.

872 Promoter hypermethylation in Bulgarian patients with glial and laryngeal cancer

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Background: Promoter hypermethylation is one of the major mechanisms in the transcriptional inactivation of certain carcinoma-associated genes. O6-methylguanine-DNA methyltransferase (MGMT) repairs the cytotoxic and mutagenic O6-alkylguanine produced by alkylating agents such as chemotherapeutic agents and mutagens. *hMGMT* expression is inversely linked to hypermethylation of the CpG island in the promoter region. Methylation in the promoter region of the DNA mismatch repair gene *hMLH1* is responsible for its inactivation and is associated with increased mutations in simple repeats in genomic DNA and microsatellite instability. The methylation analysis of these DNA repair genes may provide important information about laryngeal and glial carcinogenesis.
Materials and Methods: Genomic DNA was extracted from 50 tumour tissue samples (30 glial and 20 primary laryngeal tumours) and bisulfite conversion was performed. All samples were analyzed for promoter hypermethylation of *MGMT* gene by using a methylation-specific polymerase chain reaction (MSP) assay. The other DNA repair gene *hMLH1* was analyzed by MSP in 20 primary laryngeal carcinomas.
Results: MSP analysis demonstrated hypermethylation of *hMGMT* gene in 9 patients (30%) with glioma and 6 patients (30%) with laryngeal cancer. Promoter hypermethylation of *hMLH1* was observed in 11 (55%) of the cases with laryngeal cancer, whereas promoter hypermethylation of both *hMLH1* and *hMGMT* was found only in 3 cases (15%). The epigenetic inactivation of *hMLH1* and *hMGMT* in Bulgarian patients was detected in similar frequencies to relative studies of both cancers.
Conclusions: Our results indicate that methylation modifications in *hMLH1* and *hMGMT* genes are implicated in a significant proportion of cases with glial and laryngeal cancer.

873 Association study of polymorphic variants in chromosome locus 8q24 linked with prostate cancer in Bulgarian patients

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Background: In developed countries, prostate cancer (PC) is the most common noncutaneous malignancy in men. The molecular pathology of PC is not clear yet. Twin studies and epidemiologic observations have suggested a substantial genetic contribution to the disease risk. Linkage, admixture mapping and genome-wide studies have identified variants with moderate effects on PC risk at multiple loci in 8q24. Three distinct regions within this hot spot locus in the genome have been associated with PC risk. The locus itself is a 1.2-Mb region devoided of genes, delimited by the genes *FAM84B* and *MYC*. It is not yet known how 8q24 variants influence PC development.
Material and Methods: We have performed a case control study of the polymorphic variants rs1447295, rs16901979, and rs10505477 on locus 8q24 for association with PC. One hundred and ten PC samples and 195 controls were genotyped by using TaqMan® method.
Results: The three polymorphic variants did not show association with increased PC risk after comparison of all samples and controls. Significant association was found for rs6983267 and rs10505477 when we compared genotype and allele frequencies of patients with Gleason score above seven with the controls samples. The A/A genotype of rs10505477 (OR = 3.29, 95% CI=1.38–7.83, p = 0.007) and G/G genotype of rs6983267 (OR = 3.04, 95% CI=1.28–7.24, p = 0.011) showed association with PC in patients with Gleason score above 7. The results for the A allele (OR = 2.06, 95% CI=1.10–3.89, p = 0.016) and the G allele (OR = 1.94, 95% CI=1.03–3.65, p = 0.027) of the same variants are analogous and show statistical significance.
Conclusions: Although rs1447295 is not associated with the total PC risk or with grade and stage of the carcinoma, rs6983267 and rs10505477 demonstrated association with PC in Bulgarian patients with high Gleason score. These two polymorphisms lead to three fold increased risk for development of aggressive form of the disease.

874 Large genomic aberrations in MSH2 and MLH1 genes in Bulgarian colorectal cancer patients

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Background: Hereditary nonpolyposis colorectal cancer is caused by inactivating mutations in the genes of the DNA mismatch repair (MMR) system. Previous studies have shown that large-fragment aberrations in MMR genes are responsible for a considerable proportion of hereditary colorectal cancer (CRC) in different populations.
Material and Methods: In the present study we performed ligation-dependent probe amplification analysis (MLPA) of large genomic *MLH1*/*MSH2* alterations in 38 Bulgarian patients with CRC, in which neither epigenetic changes nor mutations were found by traditional screening methods.
Results: The frequency of the large genomic *MLH1*/*MSH2* alterations was 13.2%, which was in consistency with previous studies in other populations. One deletion was found in *MLH1* (2.6%): **del MLH1 ex 7** in a patient from family with Lynch syndrome. The observed genomic alterations in *MSH2* were four (10.5%). Two patients from HNPCC families possessed **dup MSH2 ex 9** and **del MSH2 ex 4**, respectively. The **del MSH2 ex 1** and **del MSH2 ex 3** were found in two patients with sporadic CRC and early onset, correspondingly. All cases with deletions/duplications correlated with high microsatellite instability.
Discussion: Our results indicate that genomic large-fragment deletions and duplications in *MLH1* and *MSH2* genes play a role in the pathogenesis of Bulgarian patients with both familial and sporadic CRC, as reported in other populations.