two cases, 0.5%, 1.2%). All specimens of dysplasia high, dysplasia high with area atypia and carcinoma in citu were positive for Bcl-2 and Ki-67 expression (individual variation Bcl-2, 2.6–16.4%; Ki-67, 5.8–13.4%). The high level of Bcl-2 (>10% cells staining) was observed in 50% cases and high proliferation in 30% cases.
Conclusion: Areas of cells with expression of p53, Bcl-2, Ki-67 are an indication of the transformation phenotype in tumor distant mucosa and represent high risk of development of second tumors after treatment. Diagnosis and prognosis for treatment of OSCC should not only be focused on the tumor but also on alterations in tumor-distant oral mucosa.

Prevention of miscellaneous cancers

P35
Changes in the system of proteolysis at the growth and metastasis of Lewis lung carcinoma upon development of cisplatin-resistance

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Drug resistance is critical in treating malignant tumors. At present, drug resistance, together with metastasis are considered as different manifestations of tumour progression. The interrelation of these processes with the system of proteolysis, which plays an important role in tumour progression, remains still weakly studied. The informative parameter revealing the abnormality in the proteolytic system is the proteinase-antiproteinase balance.

The aim of the work is to study the dynamics of the change of total proteolytic activity (TPA), the level of main proteinase inhibitor (α1-proteinase inhibitor, alpha1PI) in the blood plasma of C57B1/6 mice upon the growth and metastasis of Lewis lung carcinoma (LLC) with different resistance to the anticancer drug cisplatin.
Materials and Methods: The development of the cisplatin resistance was achieved by sequential intramuscular transplantations of carcinoma cells from cisplatin-treated animals. Three variants of drug resistant LLC (LLCR9, LLC19, and LLCR27 obtained in result of 9-, 19-, and 27-courses of cisplatin therapy, respectively) as well as the reference (sensitive) variant (LLC/S) have been used in our work. The studied indexes were determined on the day 10th, 15th, 20th, 25th, 28th after tumor transplantation. The intact animal blood plasma has been used as reference.
Results: A considerable change of the growth kinetics of LLC has been observed as a result of the decrease of carcinoma drug sensitivity. The growth rates of LLCR19 and LLCR27 tumours have increased considerably. Such modifications of the kinetic parameters of tumour have been preceded by the changes of TPA in the latent period of carcinoma growth (up to 10 days). The increase of TPA during this period correlates with the tumour growth rate. A considerable increase of alpha1PI (>60%) in the exponential phase of tumour growth (LLCR19 and LLCR27) leads to the subsequent growth deceleration. The value of TPA/alpha1PI ratio has shown that the development cisplatin-resistance of LLC is accompanied by the imbalance between proteolytic and antiproteolytic activities shifted to the activation of proteinases in blood plasma and deficiency of alpha1PI despite of the elevation of its level in blood plasma. A decrease of the cisplatin-sensitivity of LLC has been shown to proceed together with the considerable increase of the metastasis process.
Conclusion: The decrease of the cisplatin-sensitivity of LLC has been experimentally shown to be accompanied by the increase of tumour growth rate and metastatic activity and the imbalance between proteolytic and antiproteolytic activities. One may assume that the shift of the proteinase-antiproteinase balance in the blood plasma can be used for the prognosis of metastasis and for the search of the ways to prevent the metastasis through the influence on the proteolytic system.

P36
Multiple anticancer targets of chemopreventive curcumin in squamous cell lung carcinoma in vitro

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Introduction: Throughout the world, lung cancer is infamous for high mortality. Curcumin, a chemopreventive has chemotherapeutic potential but its mechanisms are still being elucidated. In this study, newer genes targeted by Curcumin were investigated to identify new targets for chemoprevention/therapy of squamous cell lung carcinoma (SCC) in vitro.
Methods: Lung squamous cell carcinoma cells (H520) were cultured in vitro. Apoptosis was detected in these cells after exposure to Curcumin (25μM) for 24 hours by morphological examination, MTT assay, flowcytometry and TUNEL assay. Microarray analysis of gene expression profiles on curcumin treatment was done. Real time quantitative RT-PCR and western blotting followed the microarray study.
Results: Curcumin (25μM for 24 hours) produced 29.8$\pm$2.1% cytotoxicity (MTT assay). Apoptosis was corroborated by flowcytometry (23.7$\pm$1.4%) and TUNEL (21.6$\pm$1.8%). Using microarray analysis, 34 genes were seen to be upregulated and 31 genes downregulated after curcumin treatment. Among several apoptosis related genes that were upregulated, Growth arrest and DNA damage gene, GADD45a and Peroxiredoxin-I were upregulated more than 2-fold. Real time quantitative RT-PCR and western blotting validated the results.
Conclusions: This study helps to identify novel putative intervention sites as chemopreventive and chemotherapeutic targets for curcumin in squamous cell lung carcinoma (SCC) in vitro and can contribute to better understanding of lung carcinogenesis and anticancer therapy.

P37
Prostate-specific antigen gene polymorphism and prostate cancer risk

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Prostate-specific antigen (PSA, kallikrein-related peptidase 3) is an androgen-regulated serine protease that is part of the kallikrein superfamily, produced predominantly by the prostate and primarily by secretory luminal epithelial cells. The action of androgens is regulated by androgen receptor (AR). After binding to androgen, the AR recognizes and binds androgen response elements (AREs) in the promoter regions of androgen regulated genes, such as the PSA gene. A single-nucleotide polymorphism in the ARE-I region at position –158 relative to the transcription

start site of the PSA gene was identified. It has been hypothesized that the AR binds the two PSA alleles (A and G) with differing affinity and hence may differently influence prostate cancer risk. We have investigated the potential functional significance of this polymorphism and its association with prostate cancer susceptibility in 145 men diagnosed with prostate cancer and 219 healthy men. PSA polymorphism was determined by the PCR-restriction fragment length polymorphism analysis using DNA from peripheral blood samples. We did not find a significant association between PSA polymorphism at position –158 and prostate cancer risk. The OR, calculated relative to subjects with the A/A genotype, was for the A/G genotype 0.95 (95% CI 0.56–1.6), and for the G/G genotype 0.62 (95% CI 0.34–1.12), respectively. No significant associations were found between the PSA polymorphism and the serum PSA level ($P = 0.49$). In conclusion, the PSA-158 ARE-I genetic polymorphism may not be associated with the risk of prostate cancer development and its disease progression.

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Study of drug resistance in patients with acute leukaemia: determination of mRNA ABC-transporters and apoptotic proteins

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Cellular drug resistance is an important determinant of the response to chemotherapy, and its precise measurement may have clinical relevance. Various cellular mechanisms can give rise to multidrug resistance (MDR). Expression of ATP binding cassette (ABC) transporters and apoptotic proteins is the best-studied mechanism of chemoresistance. In presented study, expression of several ABC efflux pumps (P-gp, MRP, BCRP) and apoptotic proteins (p53, bax, bcl-2 and bcl-x) have been studied by RT-PCR in leukaemic cells of patients with acute leukaemia. In addition, our study focuses on determination levels of mRNA apoptotic proteins among leukaemic cells and normal lymphocytes from healthy donors. We have demonstrated that acute leukaemia, both myeloblastic (AML) and lymphoblastic (ALL), is associated with significantly elevated levels of p53 and bax mRNA in leukaemic cells. With respect to ALL, significantly elevated levels of bcl-xl mRNA could explain for relative resistance of ALL cells to p53-dependent apoptosis. P-gp exhibited strong variation in transcription level among different leukaemia patients; however, it was significantly higher in relapsed than in de novo patients. The expression of MRP was more consistent and no significant differences between de novo and relapsed patients were observed. The expression level of BRCP was very low, however, significantly higher in relapsed than in de novo patients. Supported by grant aAV/1106/2004 from Ministry of Education of Slovak Republic.

Late abstracts

P39

Chemoprevention of kava and its potential active components against lung tumorigenesis in A/J mouse induced by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and benzo(a)pyrene

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Lung cancer has been the leading cause of death among all the malignancies for decades with relatively small improvement in its treatment. Preventing the development of lung cancer, therefore, would be an alternative strategy to help fight this disease. As smoking is the major cause of lung cancer, smokers and ex-smokers would be the population to benefit the most from an effective lung chemopreventive agent.

Based on epidemiological information, kava – a traditional beverage in the South Pacific Island region, is potentially chemopreventive against lung tumorigenesis. Its chemopreventive effect, however, has never been evaluated. In addition, the potential hepatotoxicity of kava presents a major barrier to its chemopreventive application. In this study, we evaluated whether oral kava could prevent NNK- and B[a]P-induced lung tumorigenesis in A/J mouse and whether such kava treatment would induce hepatotoxicity.

Lung tumorigenesis was induced in A/J mouse with weekly gavage administration of 2 μmol NNK and 2 μmol B[a]P for eight consecutive weeks. To evaluate the potential chemopreventive activity, kava was administered orally as a supplement to standard diet at the dose of 10 mg/g diet. Such diet was administered to A/J mice (20 mice per group) through three courses – either administered only during the carcinogen-treatment (eight weeks); or administration started after the last carcinogen treatment (22 weeks); or administered throughout the whole experimental period (30 weeks). It was found that the 30-week kava treatment had statistically significant lower lung tumor multiplicities (i.e., mean number of tumors/mouse) than NNK + B[a]P-treated mice [12.8 tumors/mouse in the NNK + B[a]P group versus 5.65 tumors/mouse in the NNK + B[a]P + Kava group; difference, 7.15 tumors/mouse; 95% CI, 3.9 to 10.6 tumors/mouse; $P < 0.0001$], corresponding to a 55.9% of tumor reduction. The 8-week treatment concurrent with carcinogen treatment also significantly reduced lung tumor multiplicities [12.8 tumors/mouse in the NNK + B[a]P group versus 6.79 tumors/mouse in the NNK + B[a]P + Kava group; difference, 6.01 tumors/mouse; 95% CI, 2.4 to 9.7 tumors/mouse; $P = 0.0018$], corresponding to a 47.1% of tumor reduction. More excitingly, post-carcinogen treatment reduced the tumor multiplicity significantly as well [12.8 tumors/mouse in the NNK + B[a]P group versus 6.59 tumors/mouse in the NNK + B[a]P + Kava group; difference, 6.21 tumors/mouse; 95% CI, 2.6 to 9.9 tumors/mouse; $P = 0.0014$], corresponding to a 48.7% of tumor reduction. Mechanistically, kava inhibited proliferation and enhanced apoptosis of lung cancer cells as demonstrated by a reduction of proliferating cell nuclear antigen (PCNA), an increase of caspase-3 and cleavage of Poly (ADP-ribose) polymerase (PARP). Kava treatment inhibited the activation of nuclear factor-κB (NF-κB), a potential up-stream pathway of kava chemoprevention. Under these treatments, kava induced no detectable hepatotoxicity as characterized by the following parameters – body weight, liver weight, ALT, AST, GGT enzymatic activities, and liver pathology. In this