

respectively. A total of 20 different mutations, 15 previously reported and 5 novel, were detected in *BRCA1*. In *BRCA2*, we identified 37 different mutations, 31 previously reported and 6 novel. The four most common recurrent mutations in *BRCA1* (c.300T>G, c.1806C>T, c.3819_3823del5, and c.5385dupC) accounted for 64.4% (85/132) of alterations in this gene and the c.5385dupC was detected in 40.9% (54/132) of mutation positive women. The four most frequent mutations (c.1642C>T, c.5910C>G, c.5991dupT and c.9631delC) accounted for 20.8% (10/48) of alterations in *BRCA2*. MLPA analysis revealed 9 different LGRs in *BRCA1* gene in 17 (2.7%, 17/640) probands. In total, large deletions accounted for 11.4% (17/149) of all identified *BRCA1* mutations. Five LGRs were novel, four LGRs were previously reported. No LGRs were found in the *BRCA2* gene. **Conclusions:** Mutation analysis demonstrated the high frequency of recurrent *BRCA1* mutations in the Czech population. Our analyses confirm that screening for LGRs in *BRCA1* should include high-risk breast and ovarian cancer patients. On the contrary, our analyses do not support the need to screen for LGRs in the *BRCA2* gene. Supported by grants from IGA MZCR, No. NS10304-3/2009 and No. NS10566-3/2009, and from the Ministry of Education No. MSM0021620808.

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POSTER

PALB2 Mutations in Familial Breast Cancer in the Czech Republic

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Introduction: *PALB2* (for Partner And Localizer of *BRCA2*) has been discovered to belong to an increasing number of moderate-risk breast cancer susceptibility genes. The *PALB2* gene product functions as a tumour suppressor and interacts with both *BRCA1* and *BRCA2* proteins during DNA double-strand break repair. Biallelic mutations in *PALB2* cause Fanconi anemia subtype FA-N, whereas heterozygous mutations predispose to breast cancer. The *PALB2* mutation frequency was estimated to be 1–3.4% in different studies. The aim of our study was to determine the contribution of *PALB2* gene to the development of hereditary breast cancer in the Czech Republic.

Material and Methods: We performed a mutation analysis of the *PALB2* gene in a cohort of 190 patients selected on the basis of family history of breast cancer and negative for *BRCA1/2* mutations and in a group of 1227 control samples. The complete coding region was divided into four overlapping fragments, amplified from cDNA and sequenced on an ABI 3130 genetic analyzer. Mutations were confirmed in a corresponding DNA sample.

Results: We identified 4 truncating *PALB2* alterations in 6 independent breast cancer patients (6/190; 3.1%). Two of them were described previously (c.509_510delGA; c.172_175delTTGT – three patients) and two were novel; nonsense mutation c.73A/T, p.K25X and frameshift mutation c.1227_1231delTGTTA, p.409YfsX0. One truncating mutation (c.509_510delGA) was found in one control sample (1/1227; 0.08%).

Conclusion: We found relatively high frequency of *PALB2* mutations comparing to the most of other studies. Our results confirm that truncating mutations in the *PALB2* gene contribute to the development of breast cancer at least in patients with strong family history. Thus, screening for the *PALB2* mutations can be recommended to these patients. However, more data about the gene's mutations penetrance are needed to fully understand their clinical implications.

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POSTER

Epithelial-Mesenchymal Transition in Cancer of Unknown Primary

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Introduction: Epithelial to Mesenchymal Transition (EMT) refers to the phenotypic changes that occur in an epithelial cell that render it loose from epithelial junctions, motile and invasive. EMT has been associated with advanced stage, invasion, metastasis and poor outcome in patients with several solid tumours, but not in Cancer of Unknown Primary (CUP).

Methods: 15mm tissue cores from 100 CUP tumours were loaded in tissue microarrays in duplicate and studied for immunohistochemical expression of E-Cadherin (ECADH- Zymed, dilution 1:30), N-Cadherin (NCADH-Zymed, dilution 1:30), Vimentin (VMN-Zymed, dilution 1:30), Snail (SNL-Abcam, dilution 1:10). EMT phenotype was defined as loss of ECADH, expression of any of NCADH, VMN with concomitant expression of SNL, as assessed by percentage of staining tumour cells. Complete clinicopathologic and management data were electronically recorded for analysis.

Results: The study population consisted of 100 patients with CUP (47 males, 53 females), of a median age of 65 and fit performance status (PS 0–1 in 75%). Histological diagnosis was adenocarcinoma in 60%, squamous ca in 22% and undifferentiated carcinoma in 17% of cases, with high grade seen in 50% of CUP. The clinicopathologic subgroups were visceral 30%, axillary nodal 8%, peritoneal carcinomatosis 22%, nodal disease 40%. Therapy consisted of palliative chemotherapy (platinum-based combination regimens in 55%). The median progression-free survival and overall survival (OS) were 7 and 12 months respectively. We performed distributional studies of IHC markers by examining frequency histograms and chose natural cut-offs for expression of ECADH (negative when expression in <60% of tumour cells), NCADH, VMN (positive when expression in ≥40% of tumour cells), SNL (positive when + in ≥80% of tumour cells). EMT phenotype was seen in 8 cases (8.1%) and was strongly associated with poor OS (EMT- median OS 13 months vs EMT+ median OS 8 months, logrank p=0.023). Presence of EMT phenotype correlated significantly with male gender, high grade and presence of visceral metastases (p < 0.05). Other factors prognostic for poor survival were male gender, PS ≥ 2, non-platinum therapy (p < 0.05).

Conclusions: EMT is infrequently seen in a heterogeneous population of CUP tumours, however it carries significant adverse impact on the outcome of these patients, probably through early systemic dissemination in visceral sites and anaplasia.

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POSTER

Proteasome System in Regulation of Insulin-like Growth Factors and NF-kappaB in Endometrial Cancer

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Background and aims: The aim of the study was to study the role of proteasomes in regulation of insulin-like growth factors (IGF), insulin-like growth factor binding proteins (IGFBP-3 and IGFBP-4), their protease PAPP-A and NF-kappaB in endometrial cancer.

Methods: A total of 50 endometrial cancer patients with I-II Stage were enrolled. The total proteasome activity and the 26S and 20S proteasome activities in tumours were determined by fluorogenic substrate hydrolysis. The concentrations of IGF-I, IGF-II, IGFBP-3, -4, PAPP-A, NF-kappaB (p50) and (p65) in tumours were determined using ELISA kits (R&D Systems, DSL, Caymanchem, USA).

Results: The increased proteasome activity results in reduction in expression of transcription factors. We found negative correlation between 26S proteasome activity and NF-kappaB expression (p50). The decreased NF-kappaB expression leads to decrease in expression of intracellular pool of growth factors and PAPP-A. The positive correlations between the NF-kappaB (p50) and NF-kappa-B (p65) expressions and IGF-I expression ($r_1 = 0.52$; $r_2 = 0.40$) and between the NF-kappaB (p65) and PAPP-A expressions ($r = 0.72$) were found in endometrial tumours. Negative correlations between IGF-I expression and the 26S and 20S proteasome activities were also revealed in endometrial cancer samples. These correlations are likely explained by proteasome degradation of insulin-like growth factor 1 receptor.

Conclusion: The detected correlations between 26S proteasome activity and NF-kappaB expression (p50); between NF-kappaB (p50) and NF-kappaB (p65) expressions and IGF-I expression; between NF-kappaB (p65) and PAPP-A expressions as well as between IGF-I expression and 26S and 20S proteasome activities indicate the delicate proteasome regulation of both the NF-kappaB expression and the components of insulin-like growth factor system.