2. PDI and TF are co-located (shown by co-immunoprecipitaion)
3. TF is located on microparticles
4. No truncated TF can be found
5. TF in malignant effusions is coagulatory active (shortening time)

We found a loose inverse correlation between tissue factor activity and PDI levels.

Conclusions: These insight and the development of new, more specific coagulation inhibitors such as FXa-inhibitors will help to treat hypercoagulabilitiy with all negative consequences for cancer patients.

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1127 POSTER

Expression of HER-2 and Its Relation With Pathological and Clinical Features in Differentiated Thyroid Cancers

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Background: Human epidermal growth factor receptor2 (HER-2) is a well recognized prognostic and predictive factor in breast cancer. Its overexpression in other human cancers may have prognostic significance. The role of HER-2 in thyroid cancer is controversial. The aim of this study is to evaluate HER-2 expression in a large retrospective series of non-metastatic differentiated thyroid cancers (DTC) and to compare it with other clinical and pathological features of the patients.
Methods: We have studied 69 patients with DTC; 58 papillary and 11 follicular carcinomas. HER-2 was detected by immunohistochemistry (IHC) test on sections from formalin-fixed, paraffin-embedded tumour tissues. Dako test was used and results were scaled by Hercept test criteria. Tumours with HER-2 +2 were retested with chromogenic in situ hybridization (CISH) test. All clinical and pathological data was summarized from the hospital files of the patients.
Results: HER-2 overexpression was found in 4 (6.9%) of 58 patients with papillary carcinoma. There was no HER-2 overexpression in 11 cases of follicular carcinoma. No association of HER-2 expression was found with tumour size, pathological grade, age, gender and cervical lymph node metastases.
Conclusion: There were no HER-2 positive cases of follicular carcinoma. The incidence of HER-2 overexpression in papillary carcinoma is very low. HER-2 cannot be used routinely as a prognostic or predictive factor in DTC. The expression of other epidermal growth factor receptors in DTC merits further future studies.

1128 POSTER

Mutations in CHEK2 and TP53 Genes in High-Risk Hereditary Breast and Ovarian Cancer Patients in the Czech Republic

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Background: Germ-line mutations in *BRCA1* and *BRCA2* genes account for only 40% of inherited breast or ovarian cancer cases. Thus, mutations in additional susceptibility genes also influence the risk of cancer. In this study we focused on the role of mutations in *CHEK2* and *TP53* genes in families at high risk of breast and ovarian cancer.
Material and Methods: Mutation analysis was performed in a series of 626 unrelated patients previously tested negative for *BRCA1/2* mutations. The complete coding region of *TP53* gene was analyzed by sequencing of cDNA; multiplex ligation-dependent probe amplification (MLPA) was used for the detection of the two most frequent Czech alterations in the *CHEK2* gene: c.1100delC and genomic deletion of 5395 bp comprising exons 8−9 that is probably of Slavic origin. All identified gene alterations were confirmed and characterized by direct DNA sequencing.
Results: In our cohort, 10 (1.6%) patients carried pathogenic mutations in *CHEK2* (5 carriers for each tested mutations) and 4 (0.6%) patients carried mutation in *TP53.* The two *TP53* mutations (c.818G>A and c.815T>G) have been repeatedly identified in sporadic breast tumours and seem to be pathogenic. The clinical importance of the third sequence variant (c.760A>G) which was found in two patients is not known. One of these mutations was detected in a woman with a familial breast cancer that also carried large deletion in the *CHEK2* gene. The 2 pathogenic *TP53* mutations were identified among hereditary cancer cases (2/296; 0.7%), whereas the majority of CHEK2 mutations was found in non-familial cancer cases (7/330, 2.1%).
Conclusions: Pathogenic mutations in *CHEK2* and *TP53* genes were much less frequent than mutations described in major predisposition genes *BRCA1/2*. However, our results indicate that testing for locally prevalent recurrent mutations in *CHEK2* gene may be of an important clinical relevance in our population. On the other hand, families with mutation in *TP53* gene were rare and the role of this gene in breast tumorigenesis is limited. Two pathogenic mutations were detected in cases of breast cancer prior to age 28 years. Analysis of *TP53* may be restricted to cases of early onset breast cancer.
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1129 POSTER

DNA Repair Enzyme, O6-methylguanine DNa Methyltransferase, Modulates Therapeutic Efficacy of Platinum Drugs With Radiation and Its Clinical Significance

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Background: In this study, we were aiming to evaluate the role of DNA repair enzyme, O^6-methylguanine–DNA methyltransferase (MGMT) in regulating the therapeutic efficacy of platinum drugs and radiation, and also investigate its clinical significance.
Materials and Methods: Tetracycline-regulated Tet-On system and RNA interference method were used to investigate the correlations between MGMT expression and platinums/radiation-induced DNA damage and cytotoxicity in cultured cells. Furthermore, 83 NPC patients received cisplatin (CDDP)-based concurrent chemoradiotherapy (CCRT) were analyzed the relationship of MGMT expression and survival.
Results: CHO-derived Tet-On-inducible cells (S12+) showed MGMT overexpression and statistically significant more resistance to CDDP, carboplatin and oxaliplatin than parental CHO cells. Knockdown of MGMT expression with small interfering RNA in HONE-1 cells conferred increased sensitivity to those platinum drugs as compared with scrambled control. Further study showed that the amount of CDDP-DNA adduct and double strand DNA breaks after CDDP exposure were significantly lower in MGMT-proficient cells than that of MGMT-deficient cells in both Tet-On and RNAi system. Host reactivation assay revealed that protection of CDDP-induced DNA damage and cell death by MGMT is through enhanced global DNA repair capacity. Otherwise, Resistance to X-ray irradiation was observed in MGMT-proficient cells, and *vice versa* in MGMT-deficient cells. The result from clinical specimens revealed that the NPC patients, who received CDDP-based CCRT, with lower level of MGMT expression had a better disease-free survival (DSS) ($P = 0.015$) and local recurrent-free survival (LRFS) ($p < 0.05$) than patients with high expression of MGMT. Multivariate analyses indicated that high expression of MGMT is an independent predictor for poor survival, with a risk ratio of 2.14 for DSS (95% CI=1.14–4.02), and 3.62 for LRFS (95% CI=1.33–9.88).
Conclusion: Our results suggested that MGMT plays an important role in determining the therapeutic efficacy of platinum drugs and radiation, and may have a relevance to clinical use of CCRT.

1130 POSTER

Spectrum of Mutations in BRCA1 and BRCA2 Genes in Families at High Risk of Breast and Ovarian Cancer in the Czech Republic

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Introduction: *BRCA1* and *BRCA2* are major genes related to hereditary breast and ovarian cancer. The purpose of our study was to estimate the incidence and spectrum of inherited mutations in these genes in a large series of Czech patients.
Materials and Methods: We evaluated DNA and RNA samples from 820 high-risk breast or ovarian cancer patients for germline mutations in *BRCA1* and *BRCA2* genes. A complete sequence analysis of *BRCA1* and *BRCA2* coding sequence was performed by protein truncation test (PTT) and direct DNA sequencing of PCR products. A total of 640 patients tested negative for point mutations and small deletions or insertions were screened for large genomic deletions and rearrangements (LGRs) at *BRCA1/2* loci by multiplex ligation-dependent probe amplification (MLPA), long range PCR and genomic sequencing. The chromosome 17-specific aCGH was used to locate deletion breakpoints in regions flanking the *BRCA1* gene.
Results: Of the 820 analyzed individuals, PTT and sequencing identified 132 (16.1%) and 48 (5.8%) mutations in *BRCA1* and *BRCA2* genes,

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.
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1131 POSTER

PALB2 Mutations in Familial Breast Cancer in the Czech Republic

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Introduction: *PALB2* (for *P*artner *A*nd *L*ocalizer of *B*RCA2) 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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1132 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: 15 mm 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, VNM (positive when expression in $\geqslant$40% of tumour cells), SNL (positive when + in $\geqslant$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 $\geqslant$ 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.

1133 POSTER

Proteasome System in Regulation of Insuline-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 transcripton 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 NFkappa-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 insuline-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.