study: 46%). There was an important decrease in the total costs related to TM, mainly due to a decrease in the costs of inappropriate requests.
Conclusions: The present study shows that informative and "self-audit" activities can have a positive impact in the clinical practice, with a decreased in costs.

1042 POSTER

Human homeobox gene (HOX) A10 is overexpressed in human ovarian clear cell adenocarcinoma and correlates with poor survival

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Human homeobox gene (HOX) A10 is a homeobox allotype gene of the HOXA family in the HOX family. HOXA10 may play an important role in cancer development. However, the role of HOXA10 in the carcinogenesis of ovarian clear cell adenocarcinoma (OCCA) has not been established. We have evaluated the prognostic significance of HOXA10 expression for human OCCA and the effects of HOXA10 on proliferation, motility, and invasion of OCCA cells. We found that HOXA10 was not expressed in normal ovarian epithelium, ovarian endometrial cysts, and ovarian serous carcinomas, but 20 of 29 (68.9%) OCCAs were positive for the expression of HOXA10. HOXA10 expression was negatively correlated to the 5-year survival of OCCA patients ($R = -0.442$, $P = 0.043$). When a HOXA10 expression vector was stably transfected into a human OCCA cell line, ES-2, the proliferation rate of ES-2-HOXA10 was much higher than the vector control, the motility of ES-2-HOXA10 cells was significantly increased compared to the control ($P < 0.05$), and the invasion of ES-2-HOXA10 cells was also much higher than the vector control ($P < 0.01$ at 5 hrs and 12 hrs after scratching). In conclusion, HOXA10 was overexpressed in OCCA and was correlated with poor survival. HOXA10 promotes proliferation, migration, and invasion of OCCA cells. HOXA10 could be a promising prognostic marker for OCCA.

1043 POSTER

BRCA1/2 mutation spectra in Serbia: preliminary results

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Background: Breast cancer is the most common female cancer worldwide, as well as in Serbia. The incidence of breast cancer increases in Serbia – it can be described with about 4000 newly diagnosed cases per year. Among them, up to 10% are present with a striking family history, suggestive of Mendelian inheritance, mostly associated with loss-of-function germline mutations in BRCA1/2 genes. BRCA1/2 mutation significantly elevates lifetime risk for the development of breast cancer (about 5 to 8 fold), as well as for ovarian cancer (10 to 20 fold) underling the importance of genetic testing in potential BRCA mutation carriers. This study was performed with the aim to estimate frequency and spectra of BRCA1/2 mutations in Serbian population.
Material and Methods: Complete or partial analysis of BRCA1/2 coding regions has been performed for 87 probands from 73 families. DNA was isolated by phenol/chloroform extraction from peripheral blood samples of the members of the high risk families. Whole gene screen was performed – coding regions of BRCA1 and BRCA2 genes were amplified by PCR, purified, labeled with fluorescent 3′-dye labeled ddNTPs and precipitated by EDTA/ethanol. These samples were bidirectionally sequenced on automatic ABI PRISM 310 genetic analyzer.
Results: 5 known (185delAG, C61G, 2138delA, 3447del4, 5382insC), as well as one novel BRCA1 deleterious mutations (4765del20) were found. 5382insC has been detected in 4 independent families. Novel BRCA2 deleterious mutation (4366insTT) has also been shown in 2 probands from the same family. The mutation frequency was 12.6%. Besides deleterious mutations, two probably damaging unclassified variant of BRCA1 gene (M1652I and R841W), as well as polymorphic variants of BRCA1 (n = 19 including intronic variants). Two BRCA2 unclassified mutations (S599F and IVS14+6 G>A) n = 16) and 21 polymorphisms, including intronic, were detected.
Conclusions: Slavic mutation 5382insC, found in 4 independent families, is probably founder mutation in Serbia. So far, we did not characterize any other mutation as founder for one population. Some of detected polymorphic variants can moderately modify cancer risk in BRCA mutation carriers and their possible impact has yet to be investigated. The presence of more than one polymorphism in several probands without deleterious mutations raises the question of their overall cumulative influence on breast cancer risk.

1044 POSTER

Comparative proteomics of the radioresistant phenotype in head and neck cancer: Gp96 as a novel prediction marker and radio-sensitizing target for radiotherapy

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Background: Radiotherapy is an integral part of the treatment modality for head and neck cancer (HNC). However, cancers can develop radioresistance (RR), leading to recurrence. In this study, we identified genes that may be involved in RR in HNC.
Materials and Methods: The radioresistant sublines from two HNC cell lines were established. Proteomic method were applied to identify the differential proteins between parental and subline cells. Molecular and cellular based studied were used to conform the role of Gp96 on radioresistance.
Results: A total of 64 proteins were identified as candidate RR genes, and those were subjected to analyzing functional network regulatory pathways. Three most significant of which were cellular response to stimulus ($P = 5.67E-26$), regulation of cell apoptosis ($P = 5.36E-22$) and glycolysis ($P = 1.14E-21$). RT-PCR analysis revealed 6 genes that were consistently differentially expressed in both RR sublines, with Gp96, Grp78, HSP60, Rab40B and GDF-15 being up-regulated and annexin V being down-regulated. Gp96 was further investigated for its functions in response to radiation. Gp96-siRNA transfectants displayed a radiation-induced growth delay, reduction in colonogenic survival, increased cellular ROS level, and increased proportion of the cells in G2/M phase. Xenograft mice administered Gp96-siRNA showed significantly enhanced growth suppression compared with radiation treatment alone ($P = 0.009$).
Conclusion: We have identified 64 proteins and verified 6 genes that are potentially involved in the RR phenotype. We further demonstrated that Gp96 knockdown enhances radiosensitivity, which may lead to a better prognosis of HNC treatment.

1045 POSTER

Human epidermal growth factor receptor 2 (HER2) testing in operable breast cancer: comparison of immunohistochemistry (IHC), fluorescent in situ hybridization (FISH), chromogenic in situ hybridization (CISH), and quantitative real-time polymerase chain reaction (qRT-PCR)

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Background: HER2 protein is overexpressed in approximately 15−30% of breast cancers. Amplification is the primary mechanism of HER2 overexpression. As it is not only a predictive factor but also a prognostic factor, HER2 testing should be routinely performed in patients with a new diagnosis of invasive breast cancer. However, approximately 20% of current HER2 testing may be inaccurate and the best method to assess HER2 status, in regards both to the type of assay used and the optimal method to perform each assay, remains controversial. So, we decided to compare IHC, FISH, CISH and qRT-PCR assays.
Material and Methods: This prospective study included 54 patients with a diagnosis of operable breast cancer whose fresh tumor tissues were obtained between 2005 and 2007. IHC, FISH and CISH analyses were performed on paraffin-embedded samples. Frozen tumor specimens were used for qRT-PCR assay. A positive HER2 result was IHC staining of 3+ (uniform, intense membrane staining of >30% of invasive tumor cells), a FISH result of more than 6.0 HER2 gene copies per nucleus or a FISH ratio (HER2 gene signals to chromosome 17 signals) of more than 2.2. For CISH assay, high HER2 amplification was defined as >10 dots or large clusters of the HER2 gene present per nucleus in >50% tumor cells. According to qRT-PCR method, final results were expressed as a ratio of HER2 gene expression value in the tumor sample normalized with

reference gene expression value and HER2 gene expression value in the normal breast tissue. A ratio $\geqslant$2.6 was regarded as being positive for HER2 amplification. Pearson and partial correlation analyses were done and $p < 0.05$ was considered statistically significant.

Results: Comparison of the assays was detailed in Table 1. One case of chromosome 17 monosomy and 2 cases of chromosome 17 polysomy were found. IHC had significant correlation with only FISH ($P = 0.001$); other amplification methods revealed significant correlation with each other (qRT-PCR & CISH, $P = 0.000$; qRT-PCR & FISH, $P = 0.035$; FISH & CISH, $P = 0.012$).

Conclusions: FISH seems to be best standard assay for determination of HER2 status but other amplification methods must be prospectively tested in large tumor samples and clinical trials. Clinical impact of polysomy 17 must also be evaluated.

Table 1: Comparison of assays for the assessment of HER2 protein expression and gene amplification.

IHC N = 54		FISH N = 54		CISH N = 54		qRT-PCR N = 54	
Score	No. of pts	Amplified	Not Amplified	Amplified	Not Amplified	Amplified	Not Amplified
0	25	0	25	4	21	2	23
1(+)	10	1	9	4	6	4	6
2(+)	8	0	8	2	6	2	6
3(+)	11	7	4	4	7	3	8

N: No. of pts

1046 POSTER

Does lactate have an impact on enzyme activity?

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Background: We still do not fully understand all roles of lactate in tumor growth. At present it is postulated that lactate serves as a whole-organism metabolic signal. Following this hypothesis we can suggest that lactate like a hormone can modulate enzymes activities. Adenosine deaminase (ADA) is a key enzyme of purine nucleotides metabolism. Increased lactate concentration and ADA's activity in tumor tissues is a characteristic feature for neoplasm.

The aim of the current investigation was to determine if lactate has an impact on activity of ADA in erythrocytes.

Materials and Methods: The different amounts of Lithium lactate have been added to red blood cells suspended in buffer-solution (pH 7.4). The range of lactate concentration was 7.5 to 30 mM whilst pH was supported on constant level. An incubation period with lactate was 10 minutes. Then erythrocytes have been hemolysed and activity of ADA was determined spectrophotometrically.

Results: We found that the dependence of activity of ADA on lactate concentration has extremum-like shape. The 3-times growth of ADA activity was observed upon concentration of lactate from 7.5 mM to 20 mM. Further elevation of lactate concentration led to decreasing ADA activity to initial level (obtained in control tube without lactate).

Table. Activity of ADA in erythrocytes after lactate addition

	Lactate, mM						
	0, control	7.5	10	15	20	25	30
ADA*, nmol/min?mg	6.2±0.7	8.2±0.3	9.7±0.4	11.8±0.6	20.8±1.9	13.2±0.3	9.0±0.2

* $p < 0.005$

Conclusion: The lactate impact is not a simple effect. Our results suggest that lactate can definitely modulate ADA activity and we can reject effects concern with genes expression due to erythrocytes metabolism features. Such complicated impact can be described by two different lactate influence (activation and inhibition) with different dose-sensitivity to lactate.

1047 POSTER

Extracellular matrix of glioblastoma inhibits polarization and transmigration of T cells: a role of tenascin-c in immune suppression

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Background: A tumour-bearing host can raise a tumor-specific T cell response. However, dense accumulations of T cells are often found in peritumoral areas, which could reduce the efficiency of contact-dependent lysis of tumour cells. The mechanisms contributing this phenomenon remain unclear. In this study we evaluated the effect of extracellular matrix (ECM) of tumor cells on T cell migration.

Methods: Transwell system was used to measure the transmigration of Jurkat, Molt-4, and primary T cells through cell monolayers and ECM of MCF-7, U-87MG, U-118MG, U-373MG, and HepG2 cells. Morphological changes and protein kinase expressions of T cells on cell monolayers and ECM of the tumour cells were detected by time-lapse microscopy and western blotting, respectively. Location of the protein kinase in migrating T cells was identified by confocal microscopy. Components of tumor ECM were verified by RT-PCR. The individual component was downregulated by shRNA.

Results: The transmigration rate of T cells measured for U-87MG, U-118MG and U-373MG (glioma cells) was consistently low as compared with that for HepG2 or MCF-7 cells. Jurkat T cells showed reduced ameba-like shape formation and delayed ERK activation when they were in contact with monolayers or ECM of glioma cells as compared with those in contact with HepG2 and MCF-7 cells. Glioma cells, but not MCF-7 and HepG2 cells, expressed tenascin-C. Knocking down the tenascin-C gene converted glioma cells to a transmigration-permissive phenotype for Jurkat T cells regarding ERK activation, transmigration, ameba-like shape formation, and phospho-ERK locating at the leading edge of migrating cells. Exogenous tenascin-C protein reduced the amoeba-like shape formation and transmigration of Jurkat T cells through MCF-7 and HepG2 cell monolayers. In addition, high level of tenascin-C was visualized immunohistochemically in human glioma tumor tissues. $CD3^+$ T cells were detected in the boundary tumor area stained strong positive for tenascin-C.

Conclusions: ECM produced by tumor cells could directly regulate T cell migration. Glioma cells actively paralyze T cell migration by the expression of tenascin-C, representing a novel immune suppressive mechanism achieved through tumour ECM.

1048 POSTER

Requirement of fully activated dendritic cells for elicitation of potent anti-tumour immune responses in cancer patients with impaired immunity

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A number of clinical trials of cancer immunotherapy have been done so far. None of them has reported satisfactory outcomes. It is mainly because anti-tumor immune responses are hardly activated in cancer patients whose immunity is generally impaired. In patients with metastatic cancer, immunosuppressive factors such as transforming growth factor (TGF)-beta, vascular endothelial growth factor (VEGF) and interleukin (IL)-10 produced by tumor cells or tumor-stromal cells abrogate the maturation of dendritic cells (DC) which have a crucial role in eliciting potent anti-tumor cellular immune responses. In this study, we established a strategy for inducing fully activated mature DCs *ex vivo*. Immature DCs prepared from peripheral blood monocytes by a conventional culture supplemented with granulocyte macrophage-colony stimulating factor (GM-CSF) and interleukin (IL)-4, were maturated by further 48 h-culture in the presence of anti-CD40 antibody and penicillin-killed streptococcus pyogenes (OK432). The induced DCs showed higher levels of CD80, DC86 and HLA-DR expression when compared with those in mature DCs which were prepared by a conventional strategy using tumor necrosis factor (TNF)-alpha or lipopolysaccharide (LPS). Furthermore, the fully activated DCs continued to produce a large amount of T-helper 1 type cytokines, i.e., interferon (IFN)-gamma and IL-12 for at least 3 days. In contrast, the conventional mature DCs demonstrated the predominant production of T-helper 2 type cytokines and production of low levels of T-helper 1 type cytokines. The cytokine production was expired within 3 days. Cytotoxic T lymphocytes (CTL) that were induced from peripheral blood lymphocytes of patients with metastatic breast cancer *in vitro* using fully activated mature DCs as a stimulator showed a strong antigen-specific killing activity of target cells, whereas CTLs induced using conventional mature DCs did not kill the target. Therefore, we expect that the fully activated DCs are applicable to DC-based vaccine for cancer patients with impaired anti-tumor immunity.