Materials and Methods: In order to identify tumor cells in urine, we loaded tumoral or normal proliferating cells in a urine sample of healthy volunteers. The cells were recovered by centrifugation, fixed and attached to positively charged slides. We performed a double FISH technique using specific ODN probes for each one of the ncmtRNAs; the SncmtRNA was identified with a specific probe labeled with Alexa fluor 488, and the ASncmtRNA with a Texas red labeled probe. The same set of experiments was performed in suspension cells, and analyzed by flow cytometry. Finally, patients' urine samples were analyzed in the same way.
Results: The double FISH approach developed here showed high sensitivity and specificity in the differentiation of tumor from normal cells in a mixture obtained from human fluids, constituting a promising new and highly accurate cancer diagnostic method.
Conclusion: We developed a double FISH approach with specific ODN probes complementary to SncmtRNA and ASncmtRNA which can identify normal or tumoral status in single cells. The same results were corroborated by flow cytometry. This approach, based on visualization of the expression of these new cancer biomarkers can be used as a novel approach for the non invasive diagnostis of bladder and prostate cancer.

PP23

Immunohistochemical marker profile in colorectal cancer: Our experience

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Background: Our aim was to examine whether certain immunohistohemical molecular markers, specifically PCNA, Ki-67 and p53, could be used to predict the tumor response of rectal cancer to determine the overall and disease-free survival rates of patients following adjuvant therapy.
Materials and Methods: In "Sveti Vracevi" Hospital in Bijeljina 301 patients suffering from colon cancer received treatment from 1st January 2000 to 31st December 2008. We analyzed the prognostic value of PCNA, Ki-67, and p53 by immunohistochemistry on formalin-fixed, wax-embedded sections in a series (n = 153) of stage III (Dukes C) colorectal cancers. An immunohistochemical score based on the intensity of immunoreactivity and, where relevant, the proportion of immunoreactive cells was established for each marker. We elected to investigate PCNA, Ki-67 as a marker of cell proliferation indices and p53 oncogenes/tumor suppressor gen because these markers have been demonstrated in a number of studies to have potential value in defining populations of individuals who either may or may not benefit from the use of adjuvant chemotherapy.
Results: Using 9 years of follow-up data, our retrospective analysis demonstrated an association between PCNA intensity (relapse-free survival [RFS]: risk ratio [RR] = 1.47, P = 0.01; overall survival [OS]: RR = 1.49, P = 0.002), Ki-67 (RFS: RR = 0.71, P = 0.05; OS: RR = 0.6, P = 0.05), and p53 (RFS: RR = 1.42, P = 0.01; OS: RR = 1.19, P = 0.0013) for RFS and OS. High PCNA intensity levels and positive p53 staining were associated with a worse outcome. Tumors containing a high percentage of Ki-67-positive cells enjoyed an improved outcome compared with those patients whose tumors contained relatively few positive cells. An interaction with treatment was not identified for any of the markers.
Conclusion: Immunohistochemical analysis is not used in the routine analysis of colon cancer. This retrospective investigation demonstrated that PCNA, and p53 staining each had significant prognostic value for patients colon carcinoma. There was not statistically significant difference in the survival rate of patients with positive immunohistochemical Ki-67 values in relation to the patients with the negative values.

PP84

Na+/H+ exchanger regulatory factor 1 (NHERF1) and angiogenesis in familial breast cancer

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Background: NHERF1 is a scaffolding protein that recruits membrane and cytoplasmic proteins into functional complexes. Our recent evidences demonstrate that in breast cancer NHERF1 overexpression is associated with increased tumor hypoxia and poor prognosis. Hypoxia is implicated in tumor proliferation and angiogenesis that interests neoplastic regions. In fact, the hypoxia-inducible factor-1 (HIF-1α), mediating trascriptional activation of vascular endothelial growth factor (VEGF) gene, is considerated the central initiator of angiogenic activity in tumor. Our aim was to determine NHERF1 expression on a series of familial and sporadic breast cancer patients and examine the relationship with other progression markers (HIF-1α, VEGFR 1 and HER2/neu).
Materials and Methods: NHERF1, VEGFR1, HIF-1α and HER2/neu proteins expression were analysed by immunohistochemistry on a tissue microarray, including 94 familial and 93 sporadic breast tumors. Cytoplasmic, membrane and nuclear NHERF1 reactivity was analysed.
Results: Membrane NHERF1 expression was significantly higher in sporadic than familial patients (p = 0.000). Familial cancers showed high levels not statistically significant of cytoplasmic NHERF1 expression compared with sporadic cancers. In familial breast patients, cytoplasmic NHERF1 overexpression was related with VEGFR1 positivity, in 48.3% of cases (p = 0.009). Furthermore, high levels of nuclear NHERF1 in familial cancers were associated with positive HIF1α tumors (p = 0.003). No significant correlation was found between NHERF1 and HER2/neu. In contrast, 48% of overexpressing HER2/neu sporadic tumors, showed a significant association with high cytoplasmic NHERF1 levels (p = 0.007). Moreover, in these tumors, nuclear NHERF1 protein is significantly correlated with HIF1α expression (p = 0.019). Any NHERF1 significant association between both VEGFR1 and HIF-1α was found.
Conclusion: In familial breast cancer, NHERF1 resulted strongly related with VEGFR1 and HIF-1α proteins with respect to sporadic tumors. In this context, we suggest an emerging role of NHERF1 in angiogenesis.

PP19

Randomised phase III clinical trial of 5 different arms of treatment for patients with cancer-related anorexia/cachexia syndrome (CACS)

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Background: Cancer-related anorexia/cachexia syndrome (CACS) is a multifactorial syndrome characterized by tissue wasting, loss of body weight, particularly of lean body mass (LBM), metabolic alterations, fatigue, reduced performance status, very often accompanied by anorexia.
Materials and Methods: In April 2005 we started a phase III randomised study to establish the most effective and safest treatment of CACS addressing as primary endpoints: LBM, resting energy expenditure (REE), total daily physical activity, serum IL-6, TNF-α, and fatigue evaluated by the Multidimensional Fatigue Symptom Inventory − Short Form (MFSI-SF). The sample size was 475 patients (pts). Eligibility criteria: hystologically confirmed tumors of any site; weight loss $\geqslant$5% in the last 3 months and/or abnormal values of proinflammatory cytokines and oxidative stress parameters predictive of the onset of CACS; life expectancy >4 months. Patients could be treated with either antineoplastic therapy with palliative intent or supportive care. All pts enrolled received as basic oral treatment: polyphenols + alpha lipoic acid + carbocysteine + Vitamins ACE. Pts were then randomised to one of the following 5 arms: (1) Medroxyprogesterone Acetate (MPA)/Megestrol Acetate (MA); (2) Pharmaco-nutritional support containing EPA; (3) L-carnitine; (4) Thalidomide; (5) MPA/MA + Pharmaco-nutritional support + L-carnitine + Thalidomide. Treatment duration was 4 months. Interim analyses were planned after every 100 randomized pts.
Results: At April 2009, 332 pts were randomized and 310 were evaluable: M/F 180/130, mean age 62 yrs (range 30−84), 96% were stage IV. A first interim analysis on all 125 pts enrolled showed a significant worsening of LBM, REE and fatigue in arm 2 (Pharmaco-nutritional support containing EPA) in comparison to the others and it was withdrawn from the study. A second interim analysis after the enrolment of 204 pts showed arm 1 (MPA/MA) significantly less effective than the others for primary efficacy endpoints: it was withdrawn from the study.
Statistical analysis at April 2009 showed a significant increase of LBM (by DEXA) and decrease of REE, IL-6 and fatigue in arm 5. As for safety, the treatment was overall well tolerated and patient compliance was very good.
Conclusion: The results so far seem to suggest that the most effective treatment for cancer pts with CACS should be the combination regimen.

PP106

DNA promoter methylation in breast cancer as possible biomarkers for screening breast cancer and association with molecular breast cancer subtypes

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Background: Aberrant DNA methylation has been found in breast cancers associated with the loss of expression of regulatory genes for growth.
Purpose: To investigate the association between DNA methylation as possible biomarkers for screening breast cancer and association with clinico-pathological and molecular breast cancer subtypes.
Materials and Methods: We quantified methylation levels of genes; APC, RAR-Beta, E-Cadherin, ESR1 and 14-3-3σ gene in 107 women with breast cancer and 108 control subjects. A sensitive PCR quantitative technique was used to analyze the utility of hypermethylation gene promoter regions.