level of fibrinogen, D-dimer or fibrin, antithrombin III and prothrombin time (hypercoagulation group). Second group (n = 28) had normal values of coagulation (control group). All pts had received at least 2 cycles low-dose immunotherapy (IL-2, 1 MIU, i.v, 3 tiw + IFN, 5 MU, s.c, 3 tiw – 3 weeks on, 3 weeks off). Tumor response was assessed radiographically every 2 cycles using RECIST criteria. Median overall survival (OS) was estimated according to Kaplan-Meier method.
Results: Hypercoagulation was present at study entry in 38.8% of MRCC pts. 71.4 and 75% of pts were male, median age at on-study was 62 and 60.1 years in hypercoagulation and control group, respectively. 46.4% of pts had poor prognosis by MSKCC score in both groups (13 = 13 pts), and 53.6% of pts had good or intermediate prognosis. 25 (89.3%) pts of control group and 26 (92.9%) pts of hypercoagulation group had clear-cell histology. Pts with normal coagulation and treated with IL-2+IFN had a statistically longer survival and higher response rate than those who had abnormal coagulation (Table). Pts with hypercoagulation had predisposition to disease progression after 2 cycles of immunotherapy.

	Hypercoagulation group	Control group
CR	–	1 (3.6%)
PR	1 (3.6%(	5 (17.9%)
OR	3.6%	21.4%
SD	11 (39.3%)	14 (50.0%)
PD	16 (57.1%)	8 (28.6%)
Median OS*, months	7.1	14.5
95% CI	6.0−8.2	10.4−18.6

*Cancer-related survival; logrank p < 0.001.

Conclusions: These early results demonstrate that abnormal coagulation can be an independent prognostic factor for survival and efficacy to therapy in pts with MRCC.

543 POSTER

Soluble E-selectin levels and CEA expressing blood-borne cells in colorectal cancer patients. A causal relationship?

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Purpose: The recognition of E-selectin by colorectal cancer (CRC) cells is an essential step for adhesion to activated endothelium and metastatization. Increased expression of E-selectin has been found in small vessels surrounding lesions in CRC and elevated levels of soluble (s) E-selectin have been found in metastatic compared with non-metastatic CRC patients. One of the newer areas being explored in the management of CRC is the use of reverse transcription-PCR (RT-PCR) to analyze the blood of cancer patients for the detection of mRNA expressed in tumor cells. Thus, this study was aimed to verify whether CEA mRNA levels in blood-borne cells correlate with cytokines and adhesion molecules involved in the haematogenous spread of CRC cells.
Methods: CEA mRNA (by RT-PCR), proinflammatory cytokines (IL-6, IL-1beta, TNF-alpha) and sE-selectin levels (all by R&D immunoassays) were analyzed in blood samples obtained from 64 CRC patients, treated at "Tor Vergata" Clinical Center, 40 patients with benign CR diseases and 59 control subjects. Patients were histologically diagnosed with primary [Dukes' Stage A (n = 4), Stage B (n = 27), Stage C (n = 17) and Stage D (n = 2, with a single resectable liver metastasis)] or relapsing (metastasis to the liver: n = 8, peritoneum: n = 2, lung: n = 2 and multiple metastasis: n = 2) CRC. The study was performed under the appropriate institutional ethics approvals, and informed consent was obtained from each patient.
Results: Median sE-selectin levels were higher in patients with CRC (44 ng/ml) compared to controls (34 ng/ml) or patients with benign CR diseases (31 ng/ml, H = 18.5, p = 0.0001). Increased levels of sE-selectin were significantly associated with CEA mRNA positivity by RT-PCR (p
Conclusions: The findings obtained suggest that circulating cancer cells, or their released products, might be responsible, through cytokine release, for the elevation of circulating adhesion molecules in CRC patients.

544 POSTER

Searching for susceptibility alleles: emphasis on bilateral breast cancer

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Background: Polygenic inheritance plays an essential role in cancer susceptibility, however identification of at-risk alleles is compromised by poor reproducibility of case-control studies. We and others suggested earlier that the use of subjects with "extreme" characteristics of cancer risk, e.g. patients with multiple tumors, may provide highly demonstrative results of molecular epidemiological analysis.
Materials and Methods: Literature has been searched for case-control gene-association studies which analyzed both bilateral and unilateral breast cancer (BC) cases. 8 relevant reports have been identified, including 6 papers involving our contribution and 2 articles published by independent groups. The results of these investigations were compared against reference studies (i.e. meta- or pooled analyses) for each at-risk allele.
Results: Good concordance has been observed between the data obtained on limited number of bilateral BC and the larger data sets involving unilateral BC cases: all at-risk alleles (e.g., BRCA1 5382insC, CHEK2 1100delC, NBS1 657del5, ATM Ser49Cys) demonstrated some degree of overrepresentation both in women with a single tumor and in those with multiple cancers, while the "negative" studies (e.g., those for p53Arg72Pro, CYP17 −34 T/C polymorphisms) failed to reveal an effect in either of the patient groups. Most importantly, in all instances where a gene-disease interaction has been firmly established, the odds ratio observed for bilateral BC patients evidently exceeded the one calculated for unilateral series. Furthermore, the results of the analysis of bilateral BC corresponded well with the published reference studies.
Conclusions: For truly at-risk alleles, comparison of bilateral BC against controls always provides higher odds ratio estimates than the traditional analysis of non-selected BC cases; therefore, use of bilateral BC relaxes the requirements for the study size. Emphasis on bilateral form of breast cancer may significantly facilitate the search for genetic determinants of BC predisposition.

545 POSTER

Possible participation of fragile sites in her2/neu gene amplification on 17q12−21 chromosome in breast cancer

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Background: Overexpression of HER2/neu protein which plays a significant role in breast cancer development and progression was strongly associated with amplification of its coding gene her2/neu. A number of genes located around her2/neu were shown to be co-amplified with it in breast cancer. However initial events and mechanism of amplification in this locus is not clear until now. The aim of this study was to investigate 17q12-q21 chromosome region for potential fragile sites that could play a major role in amplification of her2/neu in a group of patients with breast cancer.
Materials and Methods: We examined genomic DNA from fresh frozen breast cancer tumor samples of 130 patients for amplification of HER2/neu by Real Time PCR with TaqMan technology. The sequence of the region around her2/neu was investigated by TwistFlex software to reveal loci with high level of flexibility.
Results: 35 out of 130 cases (27%) had increased her2/neu gene dosage. Among these 35 cases we analyzed amplification level of genes located in 17q12−21 chromosome region around HER2/neu: LASP1, MLN64, PPARBP, CASC3, TOP2A. Gene dosage of genes located in HER2/neu-TOP2A region was higher as compared with normal tissue in 14 out of 35 cases. PARBP-HER2-GSDML region had high level of amplification in 21 out of 35 cases.
Recent publications described involvement of fragile sites in various chromosomal rearrangements. One of the basic features of fragile sites is sequence flexibility. We analyzed the region around her2/neu gene for the presence of flexible sequences. Two loci with high flexibility were detected. First locus located within intron sequence of ZNFN1A3 gene, which situated 36 kb telomeric to her2/neu, the second locus was found within FBXO40 gene, which located on 720 kb centromeric to her2/neu gene. Interestingly, both genes were described as tumor suppressor genes in different tumor types.
Conclusion: We proposed that these two sites with high level of flexibility might play a critical role in amplification of this region consistent with the