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CHK1 frameshift mutations in genetically unstable colorectal and endometrial cancers

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Introduction: The protein encoded by the CHK1 gene plays an important role in the G2 checkpoint in mammalian cells. In its coding region it presents a poly (A) 9 tract, a potential site of mutations in tumors with microsatellite instability (MSI). We analysed the presence of frameshift mutations in the CHK1 gene in MSI+ colorectal and endometrial cancer samples. Other genes reported altered in these tumours (BAX, TGFβRII, IGFIIR) were also analysed.

Methods: MSI+ colorectal and endometrial cancer cases were selected from two series of 100 and 50 cases, in whom MSI had been assessed at 10 and 14 loci, respectively. MSI was determined according to the Bethesda criteria in colon cancers, and according to the presence of no instability, low instability (MSI-L, MSI at 1 or 2 loci) and high instability (MSI-H, MSI at more than 2 loci). in endometrial cancers. Three MSI+ endometrial, 2 MSI+ colorectal cancer and one MSI− cell line were also analysed. DNA fragments containing repetitive sequences within CHK1, BAX, TGFβRII and IGFIIR genes were analysed by PCR.

Results: 13 colorectal and 9 endometrial MSI+ cancer samples were studied. CHK1 frameshfit mutations were found in HEC59 cell line, in 1/10 colon and 2/7 endometrial cancers showing MSI. IGFIIR and BAX were mutated in 3/6 cases, TGFβRII in 4/5 among the colorectal MSI-H cases. For the endometrial cancer, IGFIIR mutations were detected in 1/7 MSI-H cases, BAX in 3/7, TGFβRII in none. None of the MSI-L or MSS cancers had alterations in the coding regions analysed.

Conclusions: Our data suggest that the alteration in CHK1 gene could represent an alternative way of cancer cells to escape from cell cycle control.

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Dukes'C colon cancer after adjuvant treatment: Can KRAS and PT 53 mutations serve as prognostic indicators?

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Introduction: Mutations in KRAS and TP53 genes are common in colorectal cancer and affect the biological behavior. Current prognostic variables are not able to identify those patients who might have a benificial effect of adjuvant treatment. Therefore, we analyzed the prognostic value of KRAS and TP53 mutations in patients with Dukes'C colon cancer treated in a randomized trial of adjuvant 5-FU/Levamisole ± Leucovorin.

Methods: Genotyping of mutation hotspots of KRAS gene and evolutionarily conserved regions of the TP53 gene was performed in 55 patients. The median follow-up of the patients still alive is 47 months (range 32–66 months). Twenty-eight patients were treated with 5FU/Levamisole and 27 patients received Leucovorin/5FU/Levamisole for a period of one year.

Results: In the hotspot regions of KRAS, point mutations were found in 15 of the 55 carcinomas (27%). These included 11 codon 12 mutations and four codon 13 mutations. No mutations were found at codon 61. Probably causative mutations in the evolutionarily conserved regions (exon 5–8) of the TP53 gene, were found in 24 of 55 biopsies (44%). In this study, in which all patients received postoperative chemotherapy, TP53 mutated and KRAS mutated tumors did not significantly influence cancer specific survival compared to non-mutated tumors (Log-Rank test p = 0.72 and p = 0.77 respectively).

Conclusions: Current results indicate that 5-year cancer-specific survival after adjuvant chemotherapy with 5FU/Levamisole ± Leucovorin is not different for tumors with or without KRAS or TP53 mutations.

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No evidence for a link between p53 polymorphism at codon 72 and head and neck carcinomas. No correlation with the papillomavirus status, p53 gene mutation status nor with clinical characteristics

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Purpose: p53 polymorphism at codon 72 generates either a proline (Pro) or an arginine (Arg) form of the wild type p53 protein. Controversial data have been reported on the role of this polymorphism in the development of human cancers, including papillomavirus (HPV)-positive cervical carcinomas for which the Arg p53 genotype was considered a risk factor. The aim of this study was to determine whether the distribution of Pro or Arg alleles could play a role in the development of head and neck carcinomas, some of which harbour HPV DNA sequences.

Methods: Sixty-four matched germline-tumour DNA specimens were analysed for p53 polymorphism at codon 72 using BstU1 restriction. Germline DNA from 84 healthy blood donors of the same ethnic origin were used as controls.

Results: The distribution of germline genotypes in patients was not significantly different from that found in healthy blood donors and was not found to be related to the HPV DNA status, the p53 gene mutation status, the tumor site nor the clinical stage.

Conclusion: p53 polymorphism at codon 72 does not seem to play any role in the development of head and neck carcinomas.

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Methylation status of CpG island of adenomatous polyposis coli (APC) gene and its relationship to gene expression in colon cancer cell lines

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Purpose: The human APC gene has been shown to be mutated in the germline of patients with familial adenomatous polyposis. Somatic mutations of the APC gene have also been found at very early stages of tumorgenesis in sporadic colorectal cancer. In this study, we examined the relationship between the methylation status of the CpG island in the APC gene promoter and its expression.

Materials and Methods: Genomic DNA extracted from six colon cancer cell lines (DLD-1, SW480, Colo320, HT29, WiDr and Colo201) was modified with sodium bisulfite and subjected to nested PCR to amplify the APC promoter region. Northern blot analysis was done using full-length APC cDNA as a probe. Immunostaining was carried out with antibodies Ab3 and Ab4 against the APC gene product, where Ab3 recognizes the N-terminal portion and Ab4 the non-truncated Cterminal portion of the APC protein. Southern blot analysis was conducted to exclude major genetical alteration of the APC gene.

Results and Discussion: Cell lines with methylcytosine around the transcription start site (DLD-1, SW480 and HT29) expressed fewer APC transcripts than those without methylation. Consistent with the Northern blot analysis, DLD-1, SW480 and HT29 showed little or no immunostaining with Ab3. In all cell lines except HT29, immunostaining with Ab4 was negative. Our study suggested that methytaion of the CpG island around the transcription start site may down-regulate APC gene expression, regardless of the presence or absence of mutation.

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Chromosomal regions involved in the pathogenesis of osteosarcomas

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The comparative genomic hybridization (CGH) technique was used to identify common chromosomal imbalances in osteosarcomas (OS), frequently displaying complex karyotypic changes. We analyzed 15 primary tumors, 5 corresponding cell lines, and 1 recurrent tumor from 16 patients. The CGH-results were verified by classical cytogenetics and/or FISH, using probes specific for the centromere of chromosome 8, 8q21.3 and 8q24.1 (MYCC). In 15/16 cases chromosomal imbalances were found. Gains of chromosomal material were more frequent than losses. The most com-

mon overrepresentations were seen at 8q (11 cases), 4q (9 cases), 7q (8 cases:), 5p (7 cases), and 1p (8 cases). The smallest regions of overlap were narrowed down to 8q23 (10 cases), 4q12–13 (8 cases), 5p13–14 (7 cases), 7q31–32 (7 cases), 8q21 (7 cases), and 4q28–31 (5 cases). This data demonstrates that a number of chromosomal regions and even two distinct loci on 4q and 8q are involved in the pathogenesis of OS. More than 40% of the OS samples displayed low to moderate amplification of the MYCC oncogene, verified by FISH. Interestingly, amplification of the MYYC oncogene had no adverse prognostic impact in the OS cases studied.

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EWS-FLI1 gene rearrangement and CD99 positivity identify a breast tumor as a PNET

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Rearrangements of the *EWS* gene with ETS transcription factor genes as a result of chromosomal translocation and high expression levels of $CD99^{MIC2}$ characterize the Ewing family of tumors which usually affects bone and soft tissue in children and young adults. We report on a case of a $CD99^{MIC2}$ positive small round cell tumor m the breast of a sixty year old woman in which by cytogenetic analysis a t(11;22)(q24;q12) chromosomal aberration was identified. Reverse transcriptase polymerase chain reaction (RT-PCR) followed by sequence analysis revealed expression of a chimeric transcript in which *EWS* Eggs exon 10 was fused to *FLI1* exon 6. The specific gene rearrangement of *EWS* intron 10 was confirmed on Southern blots of genomic DNA. This case further contributes to the growing list of unusual neoplasms in adults that carry genotypic and phenotypic traits of the Ewing family of tumors. After mastectomy, adjuvant chemotherapy was performed with VACA protocol consisting of vincristine, adriamycin, cyclophosphamide and acfmomycin D with G-CSF support and radiotherapy with 50 Gy was given to the thorax wall. The patient has been tumor-free for 1 year now.

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Radiation-induced chromosome aberrations in two cell types of healthy donors and breast cancer patients

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Purpose: In recent studies different DNA-repair deficiencies were identified in lymphocytes of certain cancer patients. Defects in DNA-dsb-repair processes can be detected by analyzing chromosome aberrations after in vitro irradiation. We compared radiation-induced aberrations in lymphocytes and fibroblasts of healthy donors and breast cancer patients.

Material and Methods: Plateau-phase skin fibroblasts and blood G0 lymphocytes obtained from healthy donors or from 5 breast cancer patients were irradiated in vitro with a test dose of 3 Gy of 200 kV X-rays. The genomic yields of dicentrics, acentrics and the partial yields of reciprocal translocations (FISH-method, #4, #7, #9) were scored in 1st and 2nd post-irradiation metaphases.

Results: With respect to reciprocal translocations we found no differences neither between the two cell types, nor between the groups of healthy donors and breast cancer patients. Dicentric chromosomes were slightly increased in fibroblasts from the cancer patient group. Acentric fragments associated with chromosome deletions were significantly increased in both cell types of the cancer patient group.

Conclusion: In agreement with the reported increased levels of chromatid breaks or micronuclei in lymphocytes of certain cancer patients we observed increased levels of deletions in five breast cancer patients. In these patients, increased levels were also measured in skin fibroblasts. These findings indicate that at least two cell types from breast cancer patients display an increased level of unrepaired DNA-dsb's.

737 POSTER

Susceptibility to breast and ovarian cancer: The role of glutathione S-transferase polymorphism

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Purpose: Polymorphism[Γ]s in many xenobiotic metabolizing enzymes occur leading to variation in the level of enzyme expression in vivo. We hypothesize that women who carry deletions (null genotype) in glutathione-s-transferase genes, GSTM1 and GSTT1, may be more susceptible to the effects of environmental carcinogens than women who carry wild type (wt) alleles.

Methods: We studied this genotypes in a total of 105 cases, 85 cases with breast cancer (BC) and 20 cases of women with ovarian cancer (OC), using a differential polymerase chain reaction to simultaneously characterize inactivating mutations responsible for the null alleles of GSTM1 and GSTT1. We also studied 123 healthy controls (HC).

Results: 43 of 85 (50.6%) of BC were GSTM1 null, frequency not significantly different than HC (58%). 24 of 83 (28.9%) of BC were GSTT1 null and not significantly different than the frequency in HC (32.7%). When stratified by age at diagnosis we found a frequency of 46.6% in women diagnosed with BC after age 40. However, in women diagnosed with breast cancer before the age of 40, a tendency for a higher frequency of GSTM1 null genotypes (8 of 10 or 80%) was found and the trend for the differences between the two age groups was significant ($p = 0.048$, Fisher exact test). No association was found for the GSTT1 null genotype. In OC cases, GSTM1 null genotypes were found in 45.0% and GSTT1 null genotypes in 50.0%. Comparison of frequency distributions did not show significant differences from the HC.

Conclusion: Our results may suggest that the alteration in the metabolic pathways of xenobiotics may be associated with an earlier onset of breast cancer. The lack of the glutathione S-transferase M1 (GSTM1 null alele) did not appear to influence susceptibility to BC or OC. Further studies are necessary to understand if there are some differences in the tumor behavior or in the response to chemotherapeutic agents.

738 POSTER

Cytogenetic characteristics of 81 cases of human thyroid tumours

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Thyroid tumors, especialy benign ones like coloido-nodular goiters and follicular adenomas have been rarely analysed genetically. The results of cytogenetic analysis 41 coloido-nodular goiters, 33 follicular adenomas and 7 cancers (2 follicular, 2 papillary and 3 anaplastic) are presented. The method of short-term in vitro tissue culture were used. The chromosomes were G-band stained, In 7 colloido-nodular goiters single clonal structural chromosomal rearrangements were found. In 13 out of 33 follicular adenomas more complex rearrangements were detected – 13 numerical or structural In benign tumor thyroid tumours characteristics chromosal aberrations were not found. All thyroid cancer displayed the presence of sturtural aberration. The number of more complex rearrangements were higher in anaplastic cancers than in follicular and papillary ones. Structural chromosomal abberations of long arms of chromosome 4 were detected in 2 anaplastic and in 1 follicular cancer.

739 POSTER

Familial neoplastic clustering in 81 gastric cancer patients

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Gastric cancer (GC) is rarely due to a genetic syndrome (FGP, HNPCC), but many studies revealed that 15% of GC patients has a positive familial history for GC.

Methods: We asked 81 consecutive italian GC pts (52 male, 29 female; mean age 57) their family history and identified the cases of cancer family syndromes (CFS): FGP; HNPCC; other unspecified. Among the relatives of pts without CFS we assessed the total number of subjects affected by cancer at any site and analized the differences of the number of neoplasms occurred in the families after stratifying the sample by age (pts < 50 vs pts