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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 evolution-arily 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 iden-tify 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-