Cancer genetics

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Detection of p53 mutations via Chemical Cleavage of Mismatches: fundamental aspects of the methodology and further perspectives

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Breast Cancer is one of the most common cancers among women. Efforts to decrease breast cancer mortality are focused on improved treatment, early diagnosis and monitoring. Point mutations represent a new peculiar characteristic of tumour cell, and recently identification of mutant DNA has been applied for early diagnosis. The presence of p53 point mutations in breast cancer can reach 30-50%, but they are localised randomly in more than 50 positions. 90% of p53 mutations disordering gene function fall within a sequence encompasses exon 5 through 9. The most sensitive and efficient modern methodology used for random mutation detection is Chemical Cleavage of Mismatches. Moreover this method permits to identify the mutation. It is based on recognising mismatches in linear heteroduplexes consisting of wild and mutant strands. Unfortunately CCM applied to p53 analysis was characterised by non-specific background that prompted an investigation of possible reasons for non-specific DNA cleavage. In solutions of different PCR products both after purification and after heteroduplex formation reaction we have revealed branched DNA structures, in particular Holliday junctions, formed by homologous duplexes. Now we have elucidated the mechanism of this novel type of DNA-DNA interaction that enable us to elaborate different approaches for CCM optimisation. Using optimised solid phase silica beads CCM in tumour samples of 89 breast cancer patients we revealed 48 positive signals. Eight of which were confirmed by direct sequencing and others are under SSCP analysis followed by sequencing of mutant band. Further improvement of CCM is planed to be achieved via combination of this method with Ligation-mediated PCR that will enable us to avoid the necessity of denaturing electrophoresis usage and corresponding fluorescent or radioactive labelling of DNA probes.

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Differential diagnosis in adipose tissue tumors: complex rearrangement involving chromosome 1 and 8 found in a retroperitoneal lipoma

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Superficial lipomas are very common benign adipose tissue tumors. In contrast, lipomas occurring in deep location, such as retroperitoneal lipomas, are extremely rare and have to be carefully distinguished from well-differentiated liposarcomas for appropriate treatment and follow-up of the patient. We report here the first cytogenetic analysis of a retroperitoneal lipoma in an adult patient, showing a complex rearrangement interpreted as a t(1;8)(q32;q22-23) followed by a pericentric inversion of the der(8). There was no detectable rearrangement of chromosomes 12, and in particular no 12q14-15 amplification. Because rearrangements of 8q11-13 region, involving the PLAG1 gene, have been described in another kind of benign adipose tumor, lipoblastomas, we used florescence in situ hybridation (FISH) analysis to determine that in the present case, the chromosomal breakpoint on 8q was located between the ETO (8q22) and COX6C (8q22-23) genes, at a considerable distance from PLAG1. Karyotype analysis of additional cases of retroperitoneal lipomas will be required to assess the significance of the chromosome 1 and 8 rearrangements in the continuous effort for a better classification of adipose tissue tumors. Of great importance is the determination of such genetic markers as additional tools for the differential diagnosis between benign and malignant forms of adipose tumors and for the avoidance of erroneous diagnoses.

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Differential expressed genes in the cancerous tissues of head and neck cancer of Taiwan

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Purpose: Head and neck cancer (HNC) including cancers of pharynx, larynx and oral cavity (ICD 140-149,except 147), is the fifth leading cancer of male in Taiwan. However, the knowledge about the carcinogenesis of HNC, the genetic alterations, and the factors leading to cellular abnormality is very limited. In order to further understand the genes that predisposing HNC, we used differential display methods to globally search potential mutated genes in the cancer.

Methods: We utilized mRNA differential display method to identify transcripts differentially expressed in the HNC cancers as compared to those derived from non-tumor tissue, by using the HT11X and AP1-8 primers. Twenty cDNA fragments, which showed discrepant expression patterns between HNC tumor and non-tumor sample pairs, were selected, subjected to sequencing analysis, and blasted their sequences through GenBank databases for identify search. To further confirm the differential expression of these genes, total mRNA were separately extracted from 47 pairs of HNC tissues to assay relative amounts of these genes mRNA by using real-time RT-PCR method.

Results: we have found six novel genes which are drg1/RTP/Rit42, cdc2, ches1, NF1-alpha, HMG-CoA reductase and alpha -NAC that were overexpressed in the tumor tissues. After confirmation analysis by real-time RT-PCR methods, we found the fractions of over-expression in tumor tissues are 62%in Drg1 gene, 47%in alpha-NAC gene, 75% in CDC2 gene, 43%in CHES1 gene, 60%in EF1-alpha gene and 60% in HMG-CoA reductase, respectively.

Conclusion: These genes were first reported overexpressed in HNC. The clinical relevance and the functions of these genes in HNC will be further investigated.

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Loss of heterozygosity on chromosomes 3p, 18q21.3 and 11p15.5 as a poor prognostic factor in stage II and III (FIGO) cervical cancer treated by radiotherapy

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Purpose: Loss of heterozygosity (LOH) has been shown to be an important prognostic factor in a variety of malignant naoplasmas. Cervical cancer develops as result of multiple genetic alterations. However, the effect of such alterations on the recurrence of cervical cancer after radiotherapy remains unknown.

Methods: Studies were performed on tissue specimens and venous blood from 20 patients with cervical cancer (squamos cell carcinoma) in stage II and III (FIGO) treated by radiotherapy. DNA was isolated using organic or Chelex-100 extraction. Additional microcolumn purification was performed. The fluorescent multiplex polimerase chain reaction (PCR) was used to amplify 10 microsatellite loci induced in commercially available human identification kits (D3S1358, vWA, D16S539, D2S1338, D8S1179, D21S11, D18S51, D19S433, TH01, FGA). Microsatellite marker BAT 26 was amplified in the separate PCR reactions.

Results: LOH in BAT analysis was present in 75% cervical cancers. More then 65% of the cases showed LOH in one or more of other examined loci. The most common sites of LOH were 3p, 18q21.3, and 11p15.5. LOH was found on 3p in 41%, on 18q21.3 in 28% and on 11p15,5 in 20% of examined cases. In all cases with poor prognostic histological factor (G3) LOH was present in all three loci.

Conclusion: Cervical cancer tissues manifested LOH on chromosomes 3p, 18q21.3 and 11p15.5. Patients with LOH have worse prognosis for survival and relapse-free survival time compared to patients without LOH. Association of high stage of histological malignancy (G3) and LOH seems to be a connected with poor prognosis.