5020 POSTER

Genetic polymorphism of TNF-α and IL-10 in Russian CIN and cervical cancer patients

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Background: An effective host immune response may be an important determinant for the persistence and progression of HPV induced cervical cancer. It was suggested that the cytokine response to HPV infection may potentially affect the disease process. Single nucleotide polymorphisms (SNP) in the human TNF-α and IL-10 genes have been associated with different cytokine production and susceptibility to a number of diseases. The aim of the study was to investigate the possible association of the functionally active polymorphisms in TNF-alpha (−308 G → A) and IL-10 (−1082 G → A; −592 C → A) genes with the development of CIN and cervical cancer in Russian patients.
Methods: Genomic DNA was prepared from the paraffin-embedded tissues from 119 CC patients and 44 patients with CIN I-III. The control DNA was extracted from peripheral blood from 147 females without any cancer, autoimmune or infectious diseases. Polymorphisms of TNF-α and IL-10 were studied using the allele-specific PCR and PCR-RFLP respectively. Fisher exact test was used for the data statistical analysis.
Results: Weobserved the decrease of the (−308GG) TNF-α low-secretor genotype frequency in CIN ($p = 0.022$) but not in CC ($p = 0.23$) patients versus control. The significant increase of frequency of high-producing allele −308 A of TNF-α was found in CIN I-II patients ($p = 0.011$) suggesting that TNF-α activity may be important for CIN regression perhaps due to apoptosis. CC patients demonstrated the significant increase of the low-secretor allele −1082 A of IL-10 ($p = 0.03$) and genotype AA ($p = 0.04$) compared to control women. There were no significant differences in the IL-10–592 alleles distribution between the studied groups.
Conclusions: These data suggest that the genetically detemined ability to produce the different levels of TNF-α and IL-10 cytokines may be associated with cervical carcinogenesis.

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Interim analysis of a prospective study comparing radioresponse of uterine cervical squamous cell carcinoma during external beam radiotherapy and intracavitary radiotherapy

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Background: In definitive treatment for locally advanced cervical cancer, a combination of external beam radiotherapy (EBRT) and intracavitary radiotherapy (ICRT) remains a mainstay, and radioresponse of a tumor is one of major prognostic factors of local disease control. We commenced a prospective study that determines tumor response to each type of radiotherapy individually in terms of tumor regression rate (RR), based on the assumption that ICRT has a stronger treatment impact than EBRT. ICRT delivers much higher doses than EBRT does specifically to the part of the tumor closest to the source. We performed an interim analysis whether concrete conclusions are to be obtained from the study in progress.
Materials and Methods: Subjects were 15 cervical squamous cell carcinoma patients participated in that study between January and December 2006. FIGO stages were IB1 (n = 1), IIB (n = 1), IIIB (n = 12), and IVA (n = 1). Patients were treated according to the radiotherapy protocol of pelvic EBRT (45.0 Gy/5 weeks), followed by high-dose-rate ICRT (6.0 Gy/insertion at point A, 3−5 weekly insertions) and boost EBRT to parametrial induration or lymphadenopathy. Eleven patients were treated by concurrent chemoradiotherapy with cisplatin administered weekly at 35 mg/m^2, while remaining four patients were treated by radiotherapy alone. RR was defined as the slope (day^{-1}) of the tumor-volume shrinkage curve fit to an exponential regression equation. Tumor volume was estimated using MR images obtained before treatment, after pelvic EBRT but before ICRT, and immediately after three ICRT insertions. RRs were compared according to the radiotherapy type and to the tumor geometry type (exophytic, endophytic, or intermediate) adjunctively.
Results: RR was 0.003–0.050 day^{-1} (median, 0.017 day^{-1}) during EBRT and 0.001–0.097 day^{-1} (median, 0.020 day^{-1}) during ICRT, with no significant difference ($p = 0.364$) or correlation ($p = 0.472$) between them. Specifically, RR was higher during ICRT than EBRT for six tumors, similar ($R^2 = 0.99$–1.00, $p = 0.006$–0.055) for six and lower for three. RR tended to be highest for exophytic tumors (n = 3), followed by intermediate (n = 7) and endophytic tumors (n = 5).
Conclusions: RR did not differ significantly between the treatments due probably to weak statistical power at present. The analysis suggested that biological tumor clearance mechanisms must be taken into account in the analysis of physical treatment impact in terms of RR.

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Abnormal splicing of mRNA of Ly6G6D gene may be involved in cervical cancer progression

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Cervical intraepithelial lesions (CIN) and cervical cancer (CC) arrise due to the infection with high-risk human papilloma viruses (HPV). Genetic instability induced by HPV E6 and E7 oncogenes was described for different chromosomes, especcially for the region of major histocompatibility complex (MHC). The microsatellite marker D6S273 (6p21.3) was shown one of the most frequently deleted in CIN and cervical cancer (CC) DNA samples (Mazurenko et al., 2003, 2006). D6S273 is located in the last intron of Ly6G6D gene, spanning about 13 kb within MHC III class region. We have studied Ly6G6D gene by in silico analysis of NCBI database and with RT-PCR analysis of Ly6G6D exon-intron structure. Accoding to our and published data (Ribas et al., 1999, Mallya et al., 2002) the Ly6G6D gene may coded three transcripts: MEGT1, G6F and G6D. Important that all three mRNA share the same reading frame. We suggested that the MEGT1 mRNA involve the exons 1−4 and 8−9 of the Ly6G6D gene; the G6F mRNA – the exons 1−6 and the G6D mRNA – the exons 7−9 of the Ly6G6D gene and possibly transcripted under alternative promoter in intron 6. The MEGT1 protein is 381aa long and contains the immunoglobulin homology region (21–117aa). The MEGT1 shares 266aa with G6F protein that consists of 296aa. The G6D protein consists of 132aa and belongs to the Ly-6 superfamily of leukocyte antigenes that are attached to cell membrane by a GPI-anchor (Ribas, 1999).
Alternative splicing of mRNA G6D (A, B, C) was described for different tumor cell lines (Mallya et al., 2002). However only A splice form coded the G6D protein while B and C splice forms were abnormal. We found the low expression of G6D mRNA (neither G6F nor MEGT1) in normal cervical epithelia, tumor cell lines (HeLa, SiHa) and primary CC. Abnormal G6D splice forms (B and C) were observed in 60% of tested CC. There were no genomic mutations of G6D in CC cell lines that revealed the abnormal B splicing. We found the novel aberrant splice form D in RNA from human myeloblastic cell line KG1 and RNA blood sample from healthy donor but there were no mutations in G6D genomic region in KG1 cell line. We failed to found D splice form of G6D in tested CC samples and tumor cell lines. Thus we suggested that two mechanisms of alteration of Ly6G6D gene expression might be involved in cervical cancer progression: loss of heterozygosity and abnormal splicing.

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5023 POSTER

A genetic three-dimensional model of epithelial ovarian cancer

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Malignant tumours derived from the ovarian surface epithelium (OSE) are the leading cause of death from gynaecological disease in Western societies. Around 70−75% of cases are diagnosed when the disease is at an advanced stage, and so the average survival after diagnosis is only 5 years. Investigating the biology of premalignant and early stages of epithelial ovarian cancer (EOC) may give insight into new