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.

5021 POSTER

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.

5022 POSTER

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

approaches to disease detection at earlier stages. We have established a model of immortalised normal ovarian epithelial (nOSE) cells based on overexpression of the human catalytic sub-unit of telomerase, hTERT. Immortalised nOSE cells have a diploid karyotype and can be passaged extensively without any evidence of neoplastic transformation. However, this is a two-dimensional model of nOSE cell proliferation and may not reflect the biological behaviour of these cells in vivo. To address this, we tested three different platforms for growing nOSE cells as three-dimensional models. Cells were grown on poly-HEMA coated plates, on an AlgiMatrix scaffold, and in rotary cell culture vessels. Cells were analysed for proliferative and apoptotic markers, as well as levels of secretion of hormones, steroids and expression of certain cell surface receptors known to be typical of the normal ovarian surface epithelium.
The primary aim of these studies is to use three-dimensional models of nOSE cells in order to develop a genetic model of neoplastic initiation and development for ovarian cancer. The cMyc, K-ras and B-raf oncogenes have been introduced into nOSE-hTERT cultures by retroviral infection. Mutations in the mitogen-activated protein kinase (MAPK) pathway and cMyc, a downstream effector of this pathway, are common in cancers and are thought to be early events in tumourigenic transformation. Interestingly, although mutations in the MAPK pathway occur in around two thirds of mucinous and low-grade serous ovarian tumours, B-raf mutations appear to be limited to the low-grade serous subtype. Amplification or overexpression of the cMyc gene is also detected in EOC tumour samples at frequencies of around 40%. The cellular and molecular effects of overexpressing these three genes, independently and in combination, are being studied, with the aim of looking for differences in the characteristics of neoplastic transformation in cells expressing mutant K-ras compared to cells expressing mutant B-raf.

5024 POSTER

TP21 genetic polymorphism in cervical cancer and radiotherapy response

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Background: Cervical cancer (CC) is the most common gynecologic malignancy worldwide. In Portugal CC has an age-standardized incidence rate of 17.0 per 100.000 women and is responsible for 4.2% of malignant deaths. Treatment primarily involves surgery and/or radiotherapy. Apoptosis is frequently activated and one of the causes of cell death in CC following radiotherapy. Apoptosis is known to be regulated by a variety of genes. TP21, one of the P53 target genes, is responsible for inactivating G1 cyclin-dependent kinases blocking cell cycle progression through G1 and encodes the p21 protein, also known as the cyclin kinase inhibitor WAFf1/CIP1.
Materials and Methods: The study consisted of 84 patients treated with a combination of external radiotherapy and intracavitary brachytherapy. DNA was extracted from biopsies and corresponding peripheral blood samples from all patients. P21 nt590 polymorphism was analyzed using PCR/RFLP.
Results: In our study we found that overall survival in P21 nt590 polymorphism women carriers was significantly higher (120 months vs. 103 months, P = 0.001). When stratifying the analysis according to FIGO staging it revealed that P21 CT genotype was associated with protection for progression from stage II to stage III (P = 0.045). Moreover, in the group of patients that presented tumor recurrence, all women that were disease-free after treatment were P21 nt590 polymorphism carriers (P = 0.0001).
Conclusions: The TP21 nt590 polymorphism is located at the UTR (untranslated region), which has been shown to be an important region in p21 functions on cellular proliferation, apoptosis, tumor and metastasis supression. Deregulation of genes involved in the activation of the apoptotic process may lead to radioresistance. Our findings suggest that TP21 nt590 polymorphism is related to treatment response of cervical cancer and may play an important role in the regulation of radiosensitivity.

5025 POSTER

Cytological diagnostics of ovarian carcinoma using immunocytochemistry

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Quite frequently the primary diagnosis of ovarian carcinoma is determined using cytological method.
An objective of this work was to study potentialities of cytological diagnostics of ovarian carcinoma using immunocytochemistry.
Cytological material of 189 females who had been treated in Altai Oncological Hospital was investigated using standard punctional method and traditional staining methods were used. Potentialities of Cytocentrifuge “Cytosine IY”, immunochemical and immunocytochemical methods with Streptavidin-biotin system with a set of markers (11 antibodies) were used. Serous adenocarcinoma of ovarian (n = 181) predominated. Endometrioid and mucous carcinoma were noticed in 2.1% (n = 4 and n = 4). Ascitic and pleural effusions with using of immunocytochemistry technique were investigated of 15 (7.9%) patients (n = 12 and n = 3). Patients range in age was from 41−76 years. Stages III and IY of disease were determined. Metastases to lung were deterined from 5 patients, to liver from 3 patients, to peritoneal nodes from 2 patients.
The cells of ovarian carcinoma were immunonegative with monoclonal and immunopositive with polyclonal Carcinoembrion antigen (CEA). In all casesthe cells of ovarian carcinoma expressed Keratins-pan (C MNF 116, C AE1/AE3) and had positive reaction with Epithelial antigen (Ber-EP4).
In three cases of serous carcinoma positive reaction with Calretinin and with Mesothelin were noticed. Endometrioid carcinoma was diagnosed using reaction with Vimentin, which is not typical of serous carcinoma. Reaction with CD-15 was weakly positive only in two cases. Cytoplasmatic immunoreactivity of Epithelial Membrane Antigen (EMA) was noticed in all cases.
The data showed, that cytological diagnostics of ovarian carcinoma with immunocytochemistry helped to verify morphological type of tumour, that influences to treatment and choosing of polychemiotherapy. Serous carcinoma sometimes showed mesothelioma-like reactivity and positive reaction with Calretinin and Mesothelin. Immunopositive reactions with polyclonal CEA, Ber-EP4 are typical and sometimes with CD-15. Immunopositive reaction with Vimenyin is typical for endometrioid carcinoma.

5026 POSTER

Prognostic value of angiogenesis related genes in advanced ovarian cancer (AOC)

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Background: Ovarian cancer is the most lethal gynecologic malignancy. Despite the improved median overall survival in patients with regimens such as paclitaxel (P) and carboplatin (C) combination, relapse still occurs in the majority of those with advanced disease, and only 10 to 30 percent of such patients have long-term survival. Angiogenic process has been underlined in ovarian carcinogenesis and clinical trials are already being designed to evaluate agents that inhibit the VEGFR and other molecules involved in angiogenesis in patients with newly diagnosed disease and in those with relapse. Rational selection therapy in AOC requires improving prognostic profiling. The purpose of this study was to evaluate the role of angiogenesis related genes in ovarian cancer prognosis.
Materials and Methods: 61 patients with III/IV FIGO stage ovarian cancer who underwent surgical cytoreduction and received a C plus P regimen were included. RNAs were collected from formalin-fixed paraffin-embedded AOC samples. Expression levels of 82 angiogenesis related genes were measured using quantitative real time polymerase chain reaction. Statistical analysis was performed using a stepwise method to generate a predictive model of progression free survival.
Results: 47 patients progressed with a median progression free survival (PFS) of 16 months. A predictive model was generated ($p < 0.0001$). According to this prediction, patients were divided in four groups and the Kaplan-Meier plot and log-rank test showed that the groups were significantly different in PFS. The model comprises 40 genes including VEGF receptors, VGEF-D and MMP7, the last ones have been described as prognostic factors in epithelial ovarian cancer.
Conclusions: It is possible to generate a predictive model of PFS for ovarian cancer based on angiogenesis related genes using formalin-fixed paraffin-embedded samples. The present results are consistent with the increasing weight of several angiogenesis genes in prognosis of ovarian cancer. Although these results should be confirmed in larger series of ovarian cancer patients, this model could identify a subgroup of patients with a poor prognosis. Furthermore, it could be used to tailor therapy in those patients.