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.