Oral presentations (Tue, 25 Sep, 09.30−10.30)
Gynaecological cancer (2)

5006 ORAL

Pathway analysis of gene signatures associated with platinum-based chemotherapy resistance in ovarian cancer: the big picture

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Background: Ovarian cancer is the leading cause of death from gynecological cancers in the Western world. Despite improved surgery and advances in chemotherapy the overall 5-year survival is only 30%, which is for a significant part due to platinum-based chemotherapy resistance.
We aim to gain more insight in platinum-based chemotherapy resistance mechanisms in ovarian cancer. We have therefore performed a pathway meta-analysis on seven published gene-sets associated with platinum resistance in ovarian cancer, including a study by us [1−7].
Materials, Methods and Results: A Gene Ontology analysis was done to determine which functional processes were common in the seven gene-sets. The six processes selected were cell growth and-or maintenance (84 genes), transcription (53 genes), protein metabolism (53 genes), signal transduction (45 genes), organismal physiological process (35 genes) and response to external stimulus (31 genes). With the genes belonging to these processes (i.e. focus genes), networks were generated using Ingenuity Pathway Analysis (IPA). The genes linked to most of the focus genes were labeled as key genes. Remarkably, tumor necrosis factor (TNF) was a key gene for each process, P53 for five processes and transforming growth factor beta (TGFB) for four of the processes. The same analysis with four mock data sets resulted in less keygenes and networks per process and these keygenes were found less often for the different processes indicating the validity of this approach.
Another pathway analysis was done for a subset of eight genes that showed a remarkably similar expression profile (the so-called 'extracellular matrix gene cluster') [1]. IPA generated one network with transforming growth factor beta (TGFB) as the key gene and IPA indicated that TGFB increases the expression of four of the eight focus genes.
Conclusions: TNF as well as TGFB are involved in the inflammatory response and these analyses suggest an increased presence of activated inflammatory cells and fibroblasts in the tumor microenvironment of platinum resistant ovarian carcinomas. The role of inflammation in platinum resistance and of conditions induced by inflammation such as a mutagenic environment or different ECM constitution, are subjects of future investigations.

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5007 ORAL

Correlation of the AKT/mTOR/p70S6K1 pathway and phosphorylated vascular endothelial growth factor receptor 2 in human epithelial ovarian cancer

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Background: The Akt/mTOR/p70S6K1 (Amp) pathway is activated in 55−68% of epithelial ovarian cancers. pS6 and p4EBP1 are downstream targets and presumed to be representative for the activation of Amp. pS6 has been shown to be a reliable biomarker for mTOR inhibition while p4EBP1 has been shown to be associated with tumor grade and reduced survival in ovarian cancer. VEGF-A and angiogenesis are important targets because bevacizumab as a single agent induces encouraging responses. pVEGFR2 has been shown to be a biomarker for bevacizumab treatment in inflammatory breast cancer patients. Our primary objective was to evaluate the correlation between the Amp pathway and angiogenesis. Our second objective was to evaluate whether metastatic lesions show the same expression as the primary tumor because relapses usually involve these lesions.
Material and Methods: Epithelial ovarian cancer FFPE material from 1999–2004 was collected in a tissue micro array. Immunohistochemistry was performed using pS6, p4EBP1 and pVEGFR2. An H-score was used to quantify the staining.
Results: Patients (n = 89) were FIGO stage I in 21%, stage II in 4%, stage III in 62% and 11% stage IV. 65 patients had available tissue material of both primary tumor as well as other (multiple) abdominal metastatic lesions. Considering both primary as well as metastatic lesions together, pVEGFR2 was correlated with p4EBP1 (Spearman = 0.174; $p = 0.008$). More profound correlation was found between pS6 and pVEGFR2. ($r = 0.333$; $p < 0.0001$). The correlation of pS6 and pVEGFR2 was present in tissue of primary tumors ($r = 0.279$; $p = 0.002$) but was more pronounced in tissue of metastatic lesions. ($r = 0.444$; $p < 0.0001$).
Conclusions: Although bevacizumab seems to be very active, it is at present associated with unacceptable treatment induced toxicities. Inhibiting the Amp pathway with mTOR inhibitors could potentially also influence VEGF-A mediated mechanisms in ovarian cancer with the potential of more manageable side effects. This study provides evidence that there is a relationship between the Amp pathway and pVEGFR2 in ovarian cancer. Since the correlation of activated VEGFR2 and pS6 was found on tumor cells, this suggests that VEGF-A might be a key-stimulating growth factor to the tumor cells itself by influencing downstream cell signaling proteins. The anti-tumoral activity of bevacizumab could be explained by reducing an important tumor cell growth factor besides anti-angiogenesis. Further research is necessary and ongoing.

5008 ORAL

miRNA signatures in recurrent ovarian cancer

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Background: Conventional chemotherapy in ovarian cancer is still unsatisfactory as it ignores aspects of tumor biology during recurrence. A new class of RNA regulatory genes known as microRNAs (miRNAs) are thought to confer a novel layer of gene regulation in cancer cells. We aimed to determine whether a differential miRNA gene expression pattern exists between primary and recurrent ovarian cancers.
Materials and Methods: miRNA was isolated from 3 advanced primary and 3 recurrent serous papillary ovarian adenocarcinomas using the Ambion mirVana® miRNA isolation kit. miRNA expression levels were examined using the Applied Biosystems TaqMan® MicroRNA Assays Human Panel-Early Access Kit consisting of 180 miRNAs. miR16 and let-7a were used as endogenous controls. Quantification of primary samples was carried out relative to recurrent using the Delta Delta Ct method.Target prediction was carried out using the miRGen webserver.
Results: Differential expression patterns were identified between primary and recurrent tumours. We observed expression of miRNAs previously reported in other human cancers such as mir-155, miR-21, miR-221 and miR-222. 60 miRNAs were greater than 2-fold dysregulated between primary and recurrent specimens. 12 miRNAs were not detectable in the ovarian samples. miR-9 and miR-147 were the most differentially dysregulated genes and are predicted to target genes previously identified in our transcriptome studies. miR-147 appears to be specific for recurrence as it was detected only in recurrent specimens.
Conclusions: We report a distinct miRNA signature between primary and recurrent ovarian cancers. Some of the miRNAs identified are predicted to target dysregulated genes identified in our transcriptomic analysis of the same specimens. Three selected miRNA targets are currently being validated in an independent set of 40 primary and recurrent ovarian tumours using archival tissue. These miRNAs might represent attractive biomarkers or therapeutic targets in recurrent ovarian cancer.

5009 ORAL

Significant antitumour activity of the novel epothilone ZK-EPO against in vitro and in vivo models of ovarian cancer

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Background: The high mortality in ovarian cancer (OC) underlines a need for more effective therapeutic options for the treatment of this disease. Epothilones are a new class of microtubule-stabilising agents that may have potential to replace taxanes in OC, and the novel epothilone ZK-EPO has shown promising activity against a range of human tumour models.

Materials and Methods: ZK-EPO was compared with paclitaxel and the epothilones ixabepilone and epothilone B for its ability to inhibit proliferation in a range of human tumour cell lines. Activity against OC in vivo was assessed in tumour xenografts in SCID mice.
Results: ZK-EPO demonstrated sub-nanomolar IC_{50} values for established OC cell lines, including the A2780 and multidrug-resistant A2780/Adr OC cells, unlike ixabepilone, epothilone B and paclitaxel. In vivo, ZK-EPO showed significant dose-dependent inhibition of OVCAR-3 and OVCAR-8 tumour growth compared with paclitaxel and cisplatin. In tumour cell cultures newly isolated from OC patients, ZK-EPO displayed a high level of activity against all 27 isolates tested, and was significantly more active than paclitaxel and docetaxel, and the epothilones ixabepilone, epothilone B and KOS-862. This was clearly evident after only 1 h exposure, when the mean IC_{50} across the 27 isolates was 4 nM (epothilone B), >50 nM (docetaxel), >60 nM (paclitaxel), >90 nM (ixabepilone) and >100 nM (Kos-862). After 3 days, ZK-EPO showed sub-nanomolar IC_{50} values for all isolates tested, compared with higher nanomolar IC_{50} values for the other agents. These effects were seen irrespective of the parent tumours' clinical response to platinum-containing therapy. Xenograft models from a number of primary OC cell lines were established in SCID mice, and their sensitivities to treatment correlated with those of in vitro models.
Conclusion: ZK-EPO is highly active against all OC tumour model systems examined in vitro and in vivo. Importantly, all newly isolated patient-derived OC cell lines tested have been sensitive to ZK-EPO, which has demonstrated significantly higher antiproliferative activity than comparator compounds such as ixabepilone, Kos-862 and paclitaxel, even after short-term exposure. ZK-EPO is now in Phase II clinical trial in patients with OC.

5010 ORAL

Phosphorylated 4E binding protein 1 (p4EBP1) correlates with pathologic grade and prognosis in cervical cancer treated with surgery and radiation therapy

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Purpose: To assess the prognostic value of phospho-4E binding protein 1 (p4EBP1) in cervical cancer patients treated with surgery and radiotherapy. p4EBP1 is a signaling molecule downstream of mTOR and ERK pathways that may integrate membrane-generated signals and promote cellular proliferation.
Methods: Upon revision of medical records, 66 women who underwent surgery and adjuvant radiotherapy at our institution between 1996 and 2004 for early stage cervical cancer were identified. 13 patients received concomitant chemotherapy. Tumor tissue blocks were cut and immunohistochemically stained for expression of p4EBP1. The extent and intensity of staining were measured and an immunohistochemical store determined. Survival curves were generated and the outcome compared by the log-rank method.
Results: 66 patients were evaluated. Median follow-up was 24 months and median age was 58 years. Histologic type was squamous cell, adenocarcinoma, adenosquamous and other in 33, 26, 3 and 4, respectively. FIGO stage was IA, IB and II in 1, 46 and 19 patients, respectively. High-level expression of p4EBP1 was identified in 53% of samples. Freedom from local recurrence was significantly poorer in tumors with high-level expression of p4EBP1 ($p = 0.034$). No impact of p4EBP1 on metastatic disease was observed. High-level expression of p4EBP1 was significantly associated with cancer-specific survival ($p = 0.037$). Interestingly, higher levels of p4EBP1 were observed in poorly differenciated tumors ($p = 0.044$).
Conclusion: In this study, expression of p4EBP1 was significantly associated with high-grade tumors and poor prognosis in cervical cancer patients treated with surgery and radiation therapy. Further evaluation of this factor may help understand the oncogenic role of p4EBP1 in cervical cancer.

5011 ORAL

COX-2 polymorphism and susceptibility to gynaecological malignancies: −765C allele confers increased risk for ovarian cancer

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Background: Invasive cervical cancer (ICC) and Ovarian cancer (OC) are the most frequent malignancies among women worldwide (almost 14% of all newly diagnosed cases). Although the etiology underlying OC in not fully understood, unlike ICC, in both neoplasias chronic inflammation plays an important role in the onset of the disease. Cyclooxygenase-2 (COX-2) is highly inducible by growth factors, tumor promoters and has an important role in the inflammatory process, as well as in key steps of tumor development. Several polymorphisms in COX2 have been identified, although only a few appear to influence the susceptibility to cancer development. The 765G>C COX2 polymorphism, in the Sp1 binding site of the gene's promoter region, has been associated with the development of several diseases. The aim of our study was to assess the influence of this polymorphism in the development of OC and ICC.
Materials and Methods: This cross-sectional study involved 727 women, 150 of which had ovarian adenocarcinoma and 351 cervical lesions (60 squamous intraepithelial lesions and 291 invasive cervical cancer). The remaining 226 women had no evidence of malignant disease (control group). The 765G>C COX2 polymorphism genotypes were determined by PCR-RFLP.
Results: We found no statistically significant differences in the distribution of the 765G>C COX2 polymorphism genotypes between ICC cases and controls ($p = 0.879$). The frequency of the −765GG, GC and CC genotypes were, respectively, 64%, 31% and 5% in controls and 49%, 47% and 4% in women with ovarian cancer. We observed that women with GC and CC genotypes had a nearly two-fold increased risk for development of OC ($p = 0.004$; OR = 1.8; 95% CI: 1.211−2.787). This susceptibility was even higher, nearly 3-fold, when considering women younger than or with 53 years ($p < 0.0001$; 95% CI = 1.623−4.838).
Conclusion: The −765C allele seems to increase the susceptibility to develop OC, especially in women younger than or with 53 years. The different influence that this polymorphism seems to have on the onset of OC and ICC could be explained by the distinct etiologies of both cancers. The role of −765GC COX2 polymorphism in the susceptibility to ovarian cancer could be due to an enhanced expression of COX2 by the −765C allele that will promote an increased inhibition of apoptosis, enhanced tumor proliferation, angiogenesis and metastasis.

Poster presentations (Wed, 26 Sep, 14:00−17:00)
Gynaecological cancer

5012 POSTER

Durable clinical responses with autologous dendritic cells pulsed with MUC1: a phase II trial in ovarian carcinoma patients

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Background: The Mucin 1 (MUC1) glycoprotein is highly expressed by ovarian carcinoma and is thus a potential antigen for an immunotherapy approach. We report a phase II trial in patients (pts) with ovarian carcinoma treated with dendritic cells (DC) pulsed with mannan-MUC1 fusion protein (DC-MFP), following successful phase I results in similarly treated patients [1].
Methods: Eligibility criteria were: incurable disease; age >18 yrs; PS 0−2; no autoimmune disorders; rising CA125 levels (>25% in 1 mth, confirmed). The primary endpoint was CA125 response: major response >50% reduction, minor >25% (each confirmed at 4 wk) or stabilisation (>3 mths). PBMC were collected by leukapheresis, cultured with IL-4 and GM-CSF to generate DC, and pulsed with MFP on day 5. DC were reinjected on day 6 as i.d. injections to 8 body sites (each 5×10^6), given 4-weekly $\times$ 3, then 10-weekly to 12 months. Excess cells were cryopreserved for subsequent injections.
Results: 28 pts were recruited, with all evaluable for toxicity and 21 for efficacy (received at least 3 vaccinations). Characteristics were: serous histology 24 (86%) pts; 88% of tumours were MUC1+ on IHC; median age 58 yrs (34−78); PS 0−1 27 (96%) pts; prior systemic therapy (all pts platinum-treated) 1 line 5 pts, 2 lines 4 pts, 3 lines 10 pts, 4 or greater lines 9 pts. Leukapheresis was generally required only 6-monthly. Following ex vivo culture, the proportion of CD86+ cells ranged from 40−85%. There was no grade 3 or 4 therapy-related toxicity. Of 21 pts, 4 (19%) showed CA125 response or stabilisation. 2 pts had major response: 1 pt with 4 previous lines of systemic therapy (received DC-MFP 12+ mths) and 1 pt treated second line (duration 14 mths). One pt had stable disease of 7 mths duration which included 10 wks classified as minor response and one pt, treated fifth-line, had stable disease for 5 mths. An additional pt, treated fourth-line, had >25% CA125 reduction which was not confirmed by repeat