Conclusions: In this prospective study, low expression of BRCA1 and probably high expression of ERCC1 appeared to predict better prognosis in advanced relapsed OC. Unlike prior retrospective findings in T treated sarcoma, low BRCA1 and high ERCC1 levels did not predict longer PFS and OS in this trial. These results warrant further study to identify molecular biomarkers of outcomes with T therapy.

8006 ORAL

Improved outcome after first line chemotherapy in BRCA1- and BRCA2-associated ovarian cancer compared with sporadic ovarian cancer patients

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Background: Data of *in vitro* and small retrospective studies suggest that ovarian cancer (cells) without functional BRCA1 of BRCA2 proteins are more sensitive to DNA-damaging chemotherapy, such as platinum-containing regimens. In some clinical studies a better survival has been observed for BRCA1/2-associated versus sporadic ovarian cancer patients. Data, however, are not consistent; moreover, separate data for BRCA1- and BRCA2-associated ovarian cancer are lacking. Therefore, we compared response to, progression free (PFS) and overall survival (OS) after first line chemotherapy between BRCA1-associated, BRCA2-associated, and sporadic ovarian cancer patients, respectively.
Patients and Methods: From the database of the family cancer clinic, we selected 86 BRCA1 and 12 BRCA2 mutation carriers diagnosed with ovarian cancer between 1980 and 2008, and having been treated with chemotherapy. Patients were matched in a 1:2 ratio for year of birth and diagnosis (within 5-years periods) with 194 sporadic ovarian cancer patients. A Chi-square test was used to test for differences in type of responses, and Kaplan-Meier survival analysis to calculate PFS and OS.
Results: Mean age at diagnosis was 52 years. Stage III/IV was observed in 73%, and was not significantly different between the groups. First line chemotherapy mainly consisted of platinum-based regimens (96%). A complete (CR) or partial response (PR) was significantly more often observed in both BRCA1- and BRCA2-associated than in sporadic ovarian cancer patients (88% vs 100% vs 65%, respectively). The median PFS was significantly longer in BRCA1- (2.3 years, $p = 0.008$) and BRCA2-associated patients (2.9 years, $p = 0.03$) than in the sporadic group (1.4 years). Also, the 2- and 5-years PFS rates were significantly higher in the BRCA1- and BRCA2-associated groups than in the sporadic group, being 55% vs 58% vs 34%, and 33% vs 50% and 18%, respectively. The median OS was 6.2 yrs vs >10 yrs vs 3 yrs in the BRCA1, BRCA2 and sporadic groups, respectively ($p = 0.004$; and 0.009). In multivariate analysis, corrected for FIGO-stage and differentiation grade, the longer PFS and OS in the mutation carrier cohorts remained significant.
Conclusion: The response to, as well as the outcome after chemotherapy is significantly better in BRCA-associated than in sporadic ovarian cancer. Further, BRCA1- as well as BRCA2-associated ovarian cancer are different entities, with a trend for BRCA2 being most sensitive to chemotherapy.

8007 ORAL

Enhanced expression of Annexin A4 in clear cell carcinoma of the ovary and its association with chemoresistance to carboplatin

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Background: Clear cell carcinoma (CCC) of the ovary is known to be resistant to the platinum-based chemotherapy compared to serous or endometrioid adenocarcinoma of the ovary. Over 20% of all cases with ovarian cancer in Japan are classified as CCC and for unknown reasons this percentage is markedly higher (by approximately two-fold) than in Europe and the United States. The purpose of our study was to identify a candidate protein which is associated with chemoresistance of CCC, and to investigate the specific mechanism of chemoresistance conferred by the identified protein.
Materials and Methods: Two human ovarian cancer cell lines (OVISE-CCC/ OVSAHO-serous adenocarcinoma) were used for proteomic analysis. Enhanced expression of Annexin A4 was identified in ovarian CCC cells using 2-D differential gel electrophoresis (2D-DIGE) and mass spectrometry. Annexin A4 expression was further evaluated by real time RT-PCR and Western blot analysis using several ovarian cancer cell lines. Immunohistochemical analysis of Annexin A4 was performed in 126 epithelial ovarian cancer tissue samples. Chemoresistance (IC_{50} values) and intracellular platinum accumulation, following carboplatin treatment of Annexin A4-transfected non-CCC cells and empty vector control cells, were analysed by modified MTT assay and atomic absorption spectrophotometry.
Results: Annexin A4 levels were elevated in CCC cells compared with non-CCC cells as determined by real time RT-PCR and Western blot analysis. Immunohistochemical analysis demonstrated significantly high expression of Annexin A4 protein in ovarian CCC tumors compared with serous and endometrioid adenocarcinoma tumors ($p < 0.01$). Annexin A4-transfected cells were more resistant to carboplatin ($IC_{50} = 42$ mM) than control cells ($IC_{50} = 23$ mM). Intracellular platinum levels were significantly lower in Annexin A4-transfected cells compared with control cells following carboplatin treatment ($p = 0.0020$) and following an additional 360 min of carboplatin-free incubation ($p = 0.0004$).
Conclusion: Expression of Annexin A4 is elevated in ovarian CCC tumors and is associated with chemoresistance in cultured ovarian cancer cells. These results demonstrate that Annexin A4 confers chemoresistance in ovarian cancer cells in part by enhancing drug efflux. Annexin A4 may thus represent a novel therapeutic target of chemoresistance in patients with ovarian CCC.

Poster presentations (Tue, 22 Sep, 14:00−17:00)
Gynaecological cancer

8008 POSTER

Expression of inhibin/activin subunits (alpha, betaA and betaB) in normal and carcinomatous cervical tissue

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Background: Inhibins are dimeric glycoproteins composed of an alpha-subunit and one of two potential beta-subunits, (βA or βB), showing substantial functions in human reproduction and in endocrine-responsive tumours. In this study the expression of these different subunits was examined in normal and pathological cervical tissue.
Materials and Methods: Normal cervical tissue ($n = 10$) and cervical adenocarcinomas ($n = 6$) in archival specimens were examined by immunohistochemistry.
Results: Immunoreactivity of inhibin-α could be demonstrated in glandular cervical epithelium, while squamous epithelia cells did not express this subunit. Interestingly no analyzed cervical adenocarcinoma showed any staining reaction of this subunit. Both inhibin-βA and -βB subunits were seen in glandular epithelium of both normal and pathological cervical tissue. However, squamous epithelia cells also expressed these subunits, but with a lower intensity.
Conclusions: In this preliminary study we demonstrated an immunohistochemically detected expression of inhibin-α, -βA and -βB subunits in normal as well as in pathological cervical specimens. Possibly inhibin molecules are useful serological markers in cervical cancer. The subunits are expressed immunohistochemically to a certain amount, thus suggesting possible functions in normal and pathological cervical tissue. Moreover, inhibin-alpha is considered a tumour suppressor in several gynaecological malignancies, including endometrial and ovarian cancer. If this holds also true for cervical cancer will be evaluated in future studies.

8009 POSTER

Human papillomavirus type16 (HPV16) E6 gene variations in cervical intraepithelial lesion from Thai women

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HPV16 infection is found as a major risk factor for cervical cancer in Thai women. Variations of HPV16 E6 gene that lead to amino acid changes may be associated with increased oncogenicity. This study aimed to investigate

the HPV 16 E6 gene variations and amino acid changes in cervical intraepithelial lesions. HPV 16 positive formalin-fixed, paraffin-embedded tissue (FFPE) that consisted of 30 cases of squamous cell carcinoma (SCC), 30 cases of high-grade squamous intraepithelial lesion (HSIL) and 10 cases of low-grade squamous intraepithelial lesion (LSIL) were used. Three gene specific primers were used to amplify E6 gene by PCR and PCR products were directly sequenced. The results showed that nucleotide (nt) variation at position 178, from T to G (T178G) was most commonly found in SCC (66.7%) then HSIL (63.3%) and only 40% in LSIL. In contrast, European (E) prototype was found in 10%, 16.7% and 40% in SCC, HSIL and LSIL respectively. The other minor amino acid changes were detected including E6 L83V, E6 R10I, E6 R10T, E6 R10K, E6 L12V, E6 Q14D, E6 Q14H, E6 E18K, E6 K34R, E6 H78Y, E6 R141K, E6 R147K. These nt variations were classified as European variant (E350G) found in HSIL and LSIL(10% and 20% respectively), American Asian (AA) variant, African 2 (Af-2) variant and Javanese135C variant found in 13.3% VS 6.7%, 6.7% VS 3.3% and 3.3% VS 0% of SCC VS HSIL, respectively. This study concluded that HPV 16 Asian(As) variants may be an oncogenic risk of cervical cancer progression in Thai women. To further study, analysis of its oncogenic potential should be more investigated for its clinical significance and interpretation of clinical outcome.

8010 POSTER

P53 codon 72 polymorphism does influence cervical cancer development in a German population

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The aim of our study was to assess the impact of p53 codon 72 polymorphism in HPV related cervical lesions.
Methods: In this study, 117 healthy pregnant women previously tested for PAP smears and 111 HPV-positive patients with different cervical lesions were enrolled. The study group included 47 patients with benign lesions, 17 CIN I and CIN II diagnosed patients, and 47 patients with CIN III and invasive cervical carcinoma. For the detection of p53 codon 72 polymorphism, PCR amplification was carried out. The PCR products of p53 and HPV were all sequenced. We analyzed HPV- DNA using following primers: HPV 1/2 and GP 5/6.
Results: When the samples were analyzed using the primer pair HPV 1/2, 36 samples out off 105 were negative, same samples being positive when the PCR was performed with GP5/6 primer pair. Our study showed that 34.29% of the HPV PCR were false negative or undetected by PCR that uses only HPV 1/2. Sixty eight per cent of homozygous Arg in the high-risk HPV-group developed CIN III and invasive carcinoma compared to only 52.3% of the heterozygous and 42.9% homozygous Pro. Sequencing detects *high-risk HPV* ignored by ELISA which distorts results needed for accurate conclusion (8.2 % were added to *high-risk HPV-group*).
Conclusions: Our data suggest that women homozygous for Arginine are more susceptible for developing HPV 16/18-related high-risk cervical lesions. Using primer pair HPV GP5/6 increases sensitivity in HPV-PCR undetected when using HPV 1/2 primer pair.

8011 POSTER

The synergistic effects of nedaplatin and cispaltin on the proliferation and apoptosis of human ovarian carcinoma Skov-3 and cervical carcinoma hela cell line

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Background: To study the synergistic effects of nedaplatin(NDP) and cispaltin(DDP) on the human ovarian carcinoma Skov-3 and cervical carcinoma Hela cell line.
Materials and Methods: The inhibition effects were evaluated by MTT assay. Cell apoptosis was detected by flow cytometry. The changes of Ki-67, Bax and Bcl-2 in mRNA and protein level were quantified by RT-PCR and Western blot.
Results: The growth inhibition of Skov-3 was dose-dependent after exposure to the NDP or DDP alone. The interaction of the two drugs was synergistic at higher concent rations according to the Median-effect principle. The inhibition rate of NDP, DDP and combinative treatment group was 39.04±1.26%, 45.04±1.45%, 56.21±1.44% (Skov-3) and 44.76±2.11%, 46.90±0.99%, 56.63±1.83% (Hela) respectively and the cells apoptotic rate was tended to increase. Compared with the NDP or DDP alone treatment group, the combinative treatment group's Ki-67 and bcl-2 mRNA (protein) expression were decreased but the expression of Bax mRNA (protein) were increased.
Conclusions: Compared to the effects of NDP or DDP alone at high concentrations, combination of NDP and DDP at low concentrations proves to be much more effective in the inhibition of the proliferation and the induction of the apoptosis of Skov-3 and Hela cell line.

8012 POSTER

Combined effects of cyclooxygenase-1 and cyclooxygenase-2 selective inhibitors on the growth of ovarian carcinoma in vivo

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Background: Nonsteroidal anti-inflammatory drugs (NSAIDs) are known to be potent inhibitors of the cyclooxygenases. The present study was designed to investigate the combined effects of cyclooxygenase (COX)-1 and cyclooxygenase (COX)-2 selective inhibitors on the growth of carcinoma in in SKOV-3 ovarian carcinoma xenograft-bearing mice.
Material and Methods: Human ovarian SKOV-3 carcinoma cells xenograft-bearing mice were treated with SC-560, a COX-1-selective inhibitor, 6 mg/kg alone and celecoxib, a COX-2-selective inhibitor, 50 mg/kg alone i.g. daily for 21 days. The expression of COX-2 and COX-1 at protein and mRNA levels in the control groups was detected by immunohistochemistry and reverse-transcription polymerase chain reaction (RT-PCR). Angiogenesis of both COX inhibitors was measured by Wester blotting. In addition, prostaglandin E_2 (PGE_2) levels was determined by ELISA.
Results: In combination therapy with SC-560 and celecoxib, tumor volumes was significantly reduced compared with that of control group ($P < 0.05$). In treatment groups, both COX inhibitors significantly reduced intratumor PGE_2 levels (all $p < 0.01$). SC-560, administered in combination with celecoxib inhibited the COX associated up-regulation of VEGF. COX-1 and COX-2, mRNA, and protein levels are elevated in tumor tissues.
Conclusions: These studies demonstrate synergism between two COX inhibitors and that COX-1 and COX-2 may to some extent contribute to tumor formation independently and inhibitor combination treatment thus has particular potential for chemoprevention of ovarian cancer.

8013 POSTER

PDCD6 and ovarian cancer metastasis, findings of a proteomic study

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Background: To identify proteins involved in ovarian cancer metastasis and to evaluate the clinical significance of our finding, we analyzed the proteomics of ovarian cancer cells and verified the results in tumor samples.
Materials and Methods: A comparative proteomic analysis involving two-dimensional gel electrophoresis and mass spectrometry identification was performed to identify proteome alterations between HO-8910, a human ovarian cancer cell line, and its highly metastatic subline HO-8910PM. Differences in protein expression between the two cell lines were further validated with western blot. Immunohistochemistry and RT-PCR were also performed to confirm the in vitro findings in tumor tissues and to analyze their associations with clinical and pathological features of ovarian cancer.
Results: Twenty-one spots with significant difference in expression (two-fold increase or decrease) were detected, and of them, 17 proteins were successfully identified and characterized. Programmed Cell Death 6 (PDCD6) was one of the proteins whose overexpressoin in HO-8910PM as compared to HO-8910 was further confirmed by western blot analysis. Compared to primary ovarian cancer and normal ovarian tissues, cancer cells metastatic to lymph nodes had significantly increased expression of PDCD6 (100% vs. 72.5% vs. 40%, $P = 0.01$). PDCD6 was mainly located in the cytosol of normal ovarian cells or ovarian cancer cells, whereas in metastatic cells, PDCD6 was mostly translocated to the nucleus. Furthermore, *PDCD6* mRNA expression was significantly correlated with clinical stage, tumor grade and histology. Patients with stage III or IV disease had higher *PDCD6* mRNA expression than patients with stage I or II disease, $p = 0.005$; *PDCD6* mRNA was highly expressed in patients with grade 3 compared to those with grade 1 or 2, $p = 0.016$; serous ovarian cancer expressed more *PDCD6* than non-serous ovarian cancer, $p = 0.014$.
Conclusions: The study demonstrated that PDCD6 overexpression was related to metastatic ovarian cancer cells and aggressive ovarian tumors. As such, PDCD6 may be a useful marker for predicting tumor metastasis and/or a therapeutic target for ovarian cancer treatment.