8014 POSTER

Genes differentially expressed in therapeutic response and non-response cervical carcinoma

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Background: Cervical cancer is the second most common cancer in women worldwide. The most important risk factor in the development of cervical cancer is persistent infection with a high-risk human papillomavirus. The standard regimen for treatment of advanced stage cervical cancer is radiotherapy. Although combination of radiotherapy and cisplatin-based chemotherapy is better than radiotherapy alone, 5-year overall survival remains only 52% suggesting that intrinsic and acquired tumor resistance impedes the improvement of standard treatment. To date there are no markers for predicting treatment outcome in cervical cancer. We thus investigated differential gene expression profiling in therapeutic response and non-response cervical carcinoma using the human whole genome microarrays.
Materials and Methods: Total RNA was prepared from cervical tissues obtained from 22 normal and 15 cervical cancer patients, FIGO stage IIIB which were separated into 2 groups based on response to therapy; 7 responses and 8 non-responses. Total RNA in each group was pooled and used to determine gene expression profiles by microarrays. Genes differentially expressed were uploaded and generated the networks using the Ingenuity Pathways Analysis (IPA) software.
Results: The top 10 up-regulated genes expressed in non-responses compared to responses were *REG1A, PLA2G2A, SAA2, LGALS4, IGLL1, CXCL10, FCGBP, IL1B, OVOL1*, and *CHI3L1*. The network generated by IPA with top functions involved in connective tissue disorders, inflammatory diseases, and skeletal and muscular disorders. The top 10 down-regulated genes were *IVL, LCE3D, SPRR3, SLURP1, KRT4, CNFN, CLIC3, SPRR2A, RHCG*, and *CRABP2*. The network generated by IPA with top functions related to cell death, skeletal and muscular disorders, and cellular development.
Conclusions: Genes differentially expressed in non-responses compared with responses in our study are involved in poor prognosis or clinical outcomes of cancer patients suggesting their role as predictive biomarkers for treatment outcome in cervical cancer patients.

8015 POSTER

NKTR-102 demonstrates nonclinical and phase 1 clinical anti-tumor activity in ovarian cancer

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Background: NKTR-102, a novel prodrug of irinotecan (IRI), is currently in phase 2 development in patients with platinum resistant ovarian cancer. NKTR-102 uses Nektar's novel small-molecule advanced polymer conjugate technology to improve the pharmacokinetics and tumor distribution of IRI and its active metabolite SN38. Objective: To investigate the nonclinical and clinical anti-tumor activity of NKTR-102 in metastatic platinum resistant ovarian cancer.
Methods: Mice bearing A2780 ovarian tumors that are minimally responsive to cisplatin, received NKTR-102 or IRI in 3 weekly doses of 50, 100, or 150 mg/kg. Anti-tumor efficacy was evaluated based on tumor growth delay (TGD) in mice and response rate in mice and humans. In the phase 1 study of NKTR-102, 5 patients with ovarian cancer were enrolled in weekly $\times 3$ q4w, q14d and q21d regimens. Patients with measurable disease were assessed for tumor response using RECIST 1.0 every other cycle.
Results: In mice, NKTR-102 and IRI were equally well tolerated. Control tumors grew rapidly and uniformly to the 2000 mm^3 endpoint in a median of 14 days. IRI administered at 50, 100, and 150 mg/kg resulted in TGD of 12, 15, and 16 days, respectively, with one partial response (PR) at the highest dose. NKTR-102 at IRI-equivalent doses resulted in TGDs of 33, 32, and 34 days, respectively, with 100% regression response rate (PRs + CRs) in each group. Increasing NKTR-102 doses were associated with increased numbers of CR responses (5, 8, and 9 CRs, respectively). NKTR-102 was superior to the equivalent IRI dose at all doses tested and the lowest dose of NKTR-102 was superior to the highest IRI dose. In the phase 1 clinical study, tumor response could be assessed in 2 of 5 patients with ovarian cancer. Of these two patients, one patient receiving 145 mg/m^2 q14 (sixth line) had an unconfirmed partial response (37% reduction in target lesions) but terminated from the study prior to confirmation, and one patient on the weekly regimen receiving 172.5 mg/m^2 had a mixed response that included a 21% reduction in target lesions.
Conclusions: NKTR-102 shows superior activity compared to IRI in the A2780 ovarian tumor model, inducing a 100% response rate at all doses and dose-related increases in CRs versus PRs. Anti-tumor activity was observed in heavily pre-treated patients with ovarian cancer. A phase 2 study in patients with platinum resistant ovarian cancer is ongoing

8016 POSTER

Gene expression profile as a diagnostic tool for synchronous ovarian/endometrial cancer

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Background: Since the histological diagnosis of synchronous endometrioid ovarian/endometrial epithelial tumors lacks of sufficient reliability, the aim of this study was to demonstrate that gene expression profiling can provide additional support to traditional diagnostic procedures.
Material and Methods: Within our patients' cohort of 24 women affected by endometrioid epithelial ovarian cancers (EOC-END), ten patients had received a pathological diagnosis of synchronous ovarian/endometrial epithelial cancer. Genetic profiles of flash-frozen tissue biopsies from 22 ovarian, 2 matched ovarian/endometrial adenocarcinomas (EC-END), 15 normal endometria (NE) and 14 human ovarian surface epithelium (HOSE) short-term cell cultures were generated using Affymetrix U133 plus 2.0 oligonucleotide microarrays. The GCOS1.3 algorithm was used to convert array intensities into expression signals and Present calls. Probe sets were retained for comparisons of EOC-END to HOSE or NE if the higher-expressing group had >75% samples called Present and an average log2(signal) >6. EOC-END cases were compared separately to HOSE and NE controls using the two-class-unpaired procedure in SAM Version 3.05. Probe sets were significant by SAM if expression changed >4.0$\times$ with $q < 5\%$. Probe sets significant in both comparisons were used to cluster the samples hierarchically via average linkage, and the two EC-END samples were included in the clustering to examine their relationship with EOC-END samples.
Results: Three hundred forty probe sets met the significance criteria in both SAM analyses and were used to cluster the samples. The hierarchical clustering cleanly divided the EOCs, NEs, and HOSE samples into three major branches, and both EC-END samples clustered with EOCs. Of interest, one EC-END formed a two-member cluster with its ovarian counterpart, indicating that the two cancers from this patient were very similar to each other. By contrast, the other EC-END clustered much closer to other EOCs than it did to its ovarian counterpart, indicating a relative lack of similarity between the two cancers from this patient. These clustering results strongly suggested the presence of metastatic disease in the first patient and of multiple tumors in the second one. Since in both cases pathology reports were ambiguous, our findings demonstrate that cancer genetic profiles can help to clarify the final diagnoses.
Conclusions: Genetic analysis may represent a powerful additional tool for synchronous ovarian/endometrial cancer diagnosis.

8017 POSTER

Tspan13 expression in epithelial ovarian cancer

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Background: Epithelial ovarian cancer (EOC) is a major cause of gynaecologic cancer mortality in the western world. The lack of specific markers and the few early symptoms that characterize EOC, cause that 80% of patients are diagnosed at advance staged of the disease, when the percentages of survival are very low.
Methods: An affinity-purified polyclonal rabbit antibody against tspan13 was raised using a peptide corresponding to amino acids 116−128 in the large extracellular region of the tspan13 protein (sequence accession number: NP_055214) (Pacific Immunolgy, San Diego, CA, USA). Paraffin embedded ovarian cancer tissues were cut at 5 μm serial sections and were deparaffinized in xilene, rehydrated in graded ethanol (100−80%) and then boiled in 10 mM sodium citrate buffer containing 0.05% Tween-20 for antigen retrieval. After incubating the sections in blocking solution, were

transferred to a humidified chamber and incubated overnight at 4°C with antibody solution at 1:25 dilution. After blocking endogenous peroxidase with 3% H_2O_2, bound antibody was detected using goat anti-rabbit coupled to peroxidase and 3-amino-9-ethyl carbazole as chromogenic substrate.
Results: Public data from multiple DNA microarrays analysis of EOC was collected, standarized, and analyzed using web based programs. Among the 350 genes deregulated we had identified in EOC, TSPAN13 was overexpressed in the most common subtypes of EOC: papillary serous, endometrioid and mucinous. To study protein levels of tspan13 in these subtypes of EOC, we performed immunohistochemistry analysis. These studies revealed that tspan13 was overexpressed in all the samples analyzed, although the inmunostaining intensity depended on the subtype: strongest intensity was found in endometrioid tissues while the mucinous ones were the weakest.
Conclusions: Our studies in epithelial ovarian cancer show overexpression of TSPAN13, which codes for a protein, tspan13, that is also overexpressed in all the subtypes of EOC. The obtained results may provide the basis for its potential use as a novel marker for epithelial ovarian cancer.

8018 POSTER

CTNNB1 promoter methylation in ovarian carcinomas is associated with loss of beta catenin expression and poor patient survival

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Background: Beta catenin, links E-cadherin to alpha catenin, together forming the E-cadherin/catenin complex, which plays a crucial role in the organization and maintenance of epithelial integrity and suppression of tumour cell invasion and metastasis. In addition, beta catenin is involved in the regulation of gene transcription by the Wnt signalling pathway.
DNA promoter methylation is an important mechanism for gene silencing and loss of protein expression. The purpose of this study was to investigate whether methylation occurs at the CTNNB1 promoter in ovarian carcinomas showing loss of beta catenin protein expression by immunohistochemistry and determine its significance in relation to overall survival.
Methods and Patients: Real-time quantitative methylation specific PCR (QMSP) was employed to examine the DNA methylation status in the tissues of 93 patients with ovarian carcinomas. Absence of b-catenin protein expression was observed in 32 carcinomas and presence of b-catenin protein expression was observed in 61 carcinomas.
Results: Aberrant methylation in the promoter region of the 5′-CpG island of CTNNB1 was identified in 22/32 (68%) tumours showing loss of beta catenin expression by immunohistochemistry.*CTNNB1* promoter methylation was not identified in the 61 tumours showing positive beta catenin expression at the cell membrane. A significant correlation between poor overall survival and *CTNNB1* methylation was observed in patients with ovarian carcinomas. The 5-year survival was 22% for patients with CTNNB1 methylation compared with 58% for patients without CTNNB1 methylation ($P = 0.02$).
Conclusion: These results indicate that aberrant methylation in the promoter region of the 5′-CpG island of CTNNB1 occurs merely in tumours showing loss of beta catenin protein by immunohistochemistry and is an important mechanism for gene silencing in ovarian carcinogenesis. The correlation between loss of beta catenin expression and CTNNB1 methylation were useful in identifying and predicting a particular subpopulation of patients with ovarian carcinomas characterized by an unfavourable outcome.

8019 POSTER

Ki-67, survivin and E-cadherin expression in early stage cervical carcinoma

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Background: Multiple scientific efforts are made to detect the markers for prediction of cervical precancer progression. In this study the authors investigated the immunohistochemical expression of Ki-67, survivin and E-cadherin in cervical intraepithelial neoplasias (CIN I-CIN III) and invasive squamous-cell cervical carcinomas (CC) to evaluate their prognostic significance.
Material and Methods: Immunohistochemistry using avidin-biotin indirect immunoperoxidase method was used to study the protein expression of Ki-67 (marker of proliferation), survivin (inhibitor or apoptosis) and E-cadherin (component of the cell-cell adhesion complex) in 44 CIN cases (14 CIN I, 15 CIN II and 15 CIN III) and 27 carcinomas (14 microinvasive CC and 13 invasive CC of IB-IV FIGO stage).
Results: Active Ki-67 expression was found in 14% of CIN I, 46% of CIN II and 80% of CIN III cases suggesting that Ki-67 may be used as a marker of CIN proliferation. However, there was no significant difference in Ki-67 expression between CIN III cases, microcarcinomas (77%) and invasive CC (86%) and Ki-67 could not be used as a marker of cervical cancer progression. Survivin expression was not detected in normal squamous cervical epithelium, but was found in 67% of CIN I, 73% of CIN II, 93% of CIN III, 92% CC of IA stage and in 77% of invasive CC cases. Frequency of cases with active nuclear survivin staining increased with CIN grade, perhaps expression of survivin may be a predictive marker of unfavorable CIN prognosis. E-cadherin was expressed in all cellular membranes in normal squamous epithelium and frequency of cells with active membrane expression diminished with CIN progression. In was found in 77% CINI, 54% CIN II, 20% CIN III, 15% CC IA stage and 10% of invasive CC IB-IVstage ($P < 0.05$). Negative E-cadherin expression was shown in 13% CIN III, 8% CC of IA stage and 30% invasive CC. That is why weak or negative E-cadherin expression in precancer is unfavorable prognostic CIN marker.
Conclusions: Complex imunohistochemical analysis Ki-67, survivin and E-cadherin that are markers of different features of cell transformation may be useful for predictive evaluation of cervical precancer lesions.

8020 POSTER

Survival and clinicopathologic characteristics of invasive adenocarcinoma of the uterine cervix

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Background: Carcinoma of the uterine cervix is one of the most common malignancies among women. The relative proportion and absolute incidence of cervical adenocarcinoma (AC) compared with squamous cell carcinoma (SCC) has increased; in the 1950s 5% of all cervical carcinomas were adenocarcinoma, in the 1990s this proportion increased to 25%. It remains controversial whether or not patients with adenocarcinoma have a worse prognosis; questions remain about whether cervical adenocarcinoma metastasizes earlier or is detected later by the cervical Papanicolaou test, or whether a poorer response to radiotherapy or the inclusion of special subtypes (such as clear cell carcinoma, which is known for its dismal prognosis) could account for an apparent poorer prognosis.
Material and Methods: This retrospective study was done in the Clinical Oncology Department of the Brazilian National Cancer Institute with 278 cases of primary AC of the uterine cervix diagnosed between 2002 and 2004. Clinical and pathological data were reviewed; survival was analyzed according to the Kaplan- Meier method.
Results: Median age at presentation was 48 years; 50.4% of the patients were married and 66.9% were white. On histologic evaluation pure adenocarcinoma was found in 80.9% of the cases (n = 225), adenosquamous carcinoma in 14.4% (n = 40), clear cell carcinoma in 2.9% (n = 8) and other variants in 1.8% (n = 5). At diagnosis anemia was found in 29.5% of the patients, but 61.5% had metrorrhagia; 6.8% had hydronephrosis and 7.6% had high creatinine levels. Using FIGO stage 38.8% were stage I, 38.8% stage II, 17.9% stage III and 4.5% stage IV. Surgical treatment was used in 93 cases, radiochemotherapy combination in 69 cases and brachitherapy 129 cases. The median survival time was 34.9 months; 1-year, 3-year and 5-year overall survival rates were 81.2%; 67.2% and 54% respectively. The median survival time for the FIGO stage I, II, III and IV was 38.1; 35.4; 18.5 and 3.6 months.
Conclusions: Adenocarcinomas are becoming more common, this report shows a similar survival to that found in previous reports and demonstrates a high rate of early FIGO stages.