602 Poster

Tumor markers in lung adenocarcinoma-associated cytologically negative pleural effusions

C.C. Su[1], W.W. Huang[2], S.M. Tsao[3], C.L. Lai[4], C.E. Tseng[4]
[1]Buddhist Dalin Tzu Chi General Hospital, Department of Clinical Pathology, Chiayi County, Taiwan; [2] Buddhist Dalin Tzu Chi General Hospital, Department of Family Medicine, Chiayi County, Taiwan; [3] Chung Shan Medical University Hospital, Division of Chest Medicine Department of Internal Medicine, Taichung, Taiwan; [4] Buddhist Dalin Tzu Chi General Hospital, Division of Chest Medicine Department of Internal Medicine, Chiayi County, Taiwan

Background: Cytology fails to detect neoplastic cells in approximately 40–50% of malignant pleural effusions, which commonly accompany lung adenocarcinomas. Diagnostic accuracy of various tumor markers in lung adenocarcinoma-associated cytologically negative pleural effusions has been poor. This study aimed to maximize diagnostic efforts in distinguishing lung adenocarcinoma-associated cytologically negative pleural effusions from benign pleural effusions.

Materials and methods: Pleural effusion samples were collected from 74 lung adenocarcinoma patients with associated cytologically positive (41) and negative (33) effusions, and from 99 patients with benign conditions including tuberculosis (26), pneumonia (28), congestive heart failure (25), and liver cirrhosis (20). We evaluated the diagnostic sensitivity, specificity and optimal cutoff points for tumor markers Her-2/neu, Cyfra 21-1, and carcinoembryonic antigen to distinguish lung adenocarcinoma-associated cytologically negative pleural effusions from benign pleural effusions.

Results: The cutoff points for Her-2/neu, Cyfra 21-1 and were optimally set at 3.6 ng/mL, 60 ng/mL, and 6.0 ng/mL; and their accuracy levels ranged from 73.48%, to 81.06%, to 90.91%, respectively. Carcinoembryonic antigen combined with Cyfra 21-1 increases diagnostic sensitivity to 66.7%. False-positive rates of these markers in benign effusions were 6.1%, 2.0% and 0%, respectively.

Conclusions: Ours is the largest known study, describing 33 cases. Combining carcinoembryonic antigen with Cyfra 21-1 will provide the best differentiation between lung adenocarcinoma-associated cytologically negative pleural effusions and benign pleural effusions with two tumor markers to date, and allows early diagnosis and early treatment for two-thirds of affected patients.

603 Poster

Biological prognostic factors for disease free survival in breast cancer patients treated with adjuvant anthracycline chemotherapy

B. Biesaga[1], J. Niemiec[1], Z. Ziobro[2], J. Wysocka[3], A. Kruczak[3]
[1]Maria Sklodowska Curie Institute, Department of Radiation Biology, Krakow, Poland; [2] Maria Sklodowska Curie Institute, Department of Medical Oncology, Krakow, Poland; [3] Maria Sklodowska Curie Institute, Department of Pathology, Krakow, Poland

Background: In about 60 % of breast cancer patients ((T1-T2, N1-N2, M0) after surgery and adjuvant treatment with anthacyclines local reccurance is observed. These differences in treatment outcome indicate the need to identify biological markers for probability of patients' disease free survival (DFS). The aim of this study was to assess the influence of tumour proliferation rate, microvessel density (MVD), apoptosis level, expression of HER-2, oestrogen (ER) and progesterone (PR) receptors, and expression of topoisomerase II (TOPOII) and P53 protein on 5-year DFS in the group of breast cancer patients treated radically with surgery and adjuvant chemotherapy with anthracyclines.

Material and methods: The study was performed in the group of 94 breast cancer patients (mean age: 50.5 years; range: 27 – 69). Proliferation rate (labelling index of Ki-67 - Ki-67LI), MVD (CD34 antibody) and expressions of HER-2, ER, PR and P53 protein were studied immunohistochemically before treatment. These data were correlated with DFS estimated by Kaplan-Meier method. Data concerning apoptosis level and expression of TOPO II will be presented during the conference.

Results: Among 94 tumours, 83.9% were positive for ER, 82.8% expressed PR and in 48.0% expression of HER-2 was detected. The mean values of Ki-67LI, P53LI and MVD were 23.0% ±1.3 (SE), 10.1%±3.4 and 156.0 vessels/mm2±6.6, respectively. All women (n=13) with tumours characterized by positive expression of ER and higher proliferation rate (optimal cut off point Ki-67 LI >16.5%) survived 5 years without any evidence of cancer, whereas in patients having slower proliferating tumours and lack of oestrogen expression, DFS was significantly lower (40.0%; p=0.003). No other significant relation was found between the assessed biological parameters and DFS.

Conclusion: On the basis of oestrogen status and tumour proliferation rate, we are able to identify breast cancer patients without risk of cancer progression during 5 years after completing of anthracycline treatment.

This study is supported by Polish Ministry of Education and Science; grant number N401 173 31/3808.

604 Poster

Hormonal regulation of breast cancer associated chemokines

S. Potter[1], R.M. Dwyer[2], E. Hennessy[1], D.G. Griffin[3], M.J. Kerin[1]
[1]Department of Surgery Clinical Science Institute, National University of Ireland Galway, Co Galway, Ireland; [2] Department of Surgery and Regenerative Medicine Institute, National University of Ireland Galway, Co Galway, Ireland; [3] Department of Clinical Biochemistry, University Hospital Galway, Co Galway, Ireland

Introduction: Chemokines such as Stromal Cell-Derived Factor-1a(SDF-1a) and Monocyte Chemotactic Protein-1 (MCP-1), are chemotactic cytokines that have been implicated in breast cancer progression. This laboratory previously reported elevated systemic levels of SDF-1a and MCP-1 in breast cancer patients. In the case of SDF-1a, these elevated levels correlated with clinical prognostic indicators including tumour grade and epithelial subtype. The aim of this study was to investigate potential regulation of circulating chemokines by endogenous hormones, as knowledge of these regulatory mechanisms is essential for chemokines to be valid targets in breast cancer management.

Methods: Plasma and serum samples were collected from 36 premenopausal healthy females, on a weekly basis for four consecutive weeks, i.e. 144 samples in total. Measurement of Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), Oestradiol and Progesterone were preformed using a Bayer ADVIA® Centaur Immunoassay system. Simultaneous measurements of plasma SDF-1a and serum MCP-1 were performed using ELISA. Demographic data including age, date of last menstrual period and current medications was also collected on each volunteer.

Results: Menstrual cycles of all subjects were found to be ovulatory, that is, each showed an LH surge followed by an appropriate mid-luteal peak of Progesterone. Nine patients were taking an oral contraceptive pill at the time of the study, and this was found to have no influence on SDF-1a and MCP-1 levels. Plasma SDF-1a was significantly lower in the mid-luteal phase (2157 ± 60 pg/ml, $p<0.05$) than other phases of the menstrual cycle, late luteal/early follicular (2387 ± 69 pg/ml), mid-follicular (2267 ± 65 pg/ml), and mid-cycle (2349 ± 71 pg/ml). SDF-1a displayed a significant positive correlation with Oestradiol ($r =0.213$, $p<0.05$) throughout the cycle. MCP-1 levels did not differ significantly across the menstrual cycle and did not show any significant correlation to menstrual hormones.

Conclusion: The results presented here are an important first step in elucidation of the relationship between menstrual hormones and chemokines, which play an important role in breast cancer progression. Further understanding of the mechanisms of control, and mode of action of these chemokines, will support development of novel therapeutic strategies and may influence timing of surgical intervention for premenopausal breast cancer patients.

605 Poster

Diagnosis value of EGFR gene expression for early diagnosis in oral leukoplakias

G. Aparicio Gallego[1], S.m. Díaz Prado[2], V. Medina Villaamil[1], L.m. Antón Aparicio[2], J.l. López Cedrún[3], X. Collado López[3], M. Valladares Ayerbes[4], R. García Campelo[4], I. Santamarina Caínzos[1], M. Haz Conde[1]
[1]Complejo Hospitalario Universitario Juan Canalejo, Unidad De InvestigaciÓn, Coruna, Spain; [2] Universidade Da Coruña, Dpto. Medicina, Coruna, Spain; [3] Complejo Hospitalario Universitario Juan Canalejo, Servicio Cirugía Maxilofacial, Coruna, Spain; [4] Complejo Hospitalario Universitario Juan Canalejo, Servicio De Oncologia Médica, Coruna, Spain

Background: Field cancerization theory has been proposed as an explanation for the increased risk of transformation in upper airway-digestive tract. Oral cancer development involves several steps previously to malignant progression through increasing levels of dysplasia as a result of the accumulation of diverse genetic alterations. Oral premalignant lesions include leukoplakias. Since there is evidence that EGFR participates in tumorigenesis, according to analysis of RNA and protein from squamous cell carcinoma (SCC) mucosa, routine molecular study of EGFR gene expression would contribute to an improved diagnosis and treatment of premalignant oral epithelial lesions. Objective: Study the utility of EGFR gene expression as diagnosis factor in oral leukoplakias by means of Quantitative Real Time PCR (qPCR). Materials and Methods: Expression levels of EGFR gene, in 20 unique freeze samples from 20 patients with leukoplakia, were measured. From each patient, 2 samples were obtained: opposed lateral oral mucosa and leukoplakia mucosa. As control, a pool of healthy human oral mucosa from healthy donors (n=4) was used. Quantitative Real Time PCR (qPCR) experiments were performed on a LightCycler 480 Instrument (Roche) using LightCycler 480 SYBR Green I Master (Roche). A constitutively expressed gene, HPRT, was used as internal control. Results: The expression levels of EGFR were higher in opposed lateral oral mucosa and leukoplakia both from patient,

with regard to the pool of healthy human oral mucosa from healthy donors. When comparing opposed lateral oral mucosa and leukoplakia from the same patient, 60% of samples showed higher EGFR expression in opposed lateral oral mucosa than in leukoplakia. This could be explained by the "field cancerization" hypothesis in oral cavity. Conclusions: Although it was previously demonstrated that EGFR was over-expressed in head and neck cancers these results confirmed that it is also up-regulated in more initial phases of the precancerous lesion. These results indicate that EGFR may contribute to carcinogenesis. Therefore EGFR could represent an attractive diagnosis factor in oral leukoplakias. Support: S. Díaz Prado is beneficiary of an Isidro Parga Pondal contract from Xunta de Galicia (Spain).

606 Poster

combining epigenetic and metronomic chemotherapy is feasible and effective in chemotherapy refractory tumors

L. Van Daele[1], S. Vandewaeter[1], F. Geurs[1]
[1]St. Mariaziekenhuis, Medical Oncology, Halle, Belgium

Epigenetic treatment of human cancers, with histone deacetylas inhibitors (HDACi)has received much attention. Valproic acid (VPA) is a well known anti-epileptic with substantial HDACi activity. Combination with chemotherapy is widely started in phase I trials.

No experience exists on combining metronomic ('low dose -continuous') and epigenetic treatment in refractory tumors.

Patients and methods: From 15/8/2007 to 10/3/2008 we treated 15 patients who presented with refractory solid tumors, most 2nd to 5th line. All these patients had PS 0-2 and no end organ failure.. Median age was 72y (55-90y).Tumor types: breast 4, prostate 3, nsclc 1,sclc 1,melanoma 2, H&N 1,ovarian ca 2, cervical ca 1. Median number of prior CT regimens: 3. Patients received concomitant valproic acid 40mg/kg and paclitaxel 20mg/m² daily from Monday to Friday, 2-3weeks per month.

Results: Disease stabilisation was seen in 9 pts, with tumor marker decrease in 4. Two patients with melanoma had partial response and 4 pts progressive disease. There were 2 cases of febrile neutropenia and 2 patients needed transfusion. No other gr3-4toxicities

Conclusion:

1. combining metronomic and epigenetic treatment is feasible and has no prohibitive side effects
2. in a substantial amount of patients this leads to prolonged disease stabilisation and even decrease of Tumor markers. Many tumors remained stable for > 3months
3. The responses in melanoma patients are provocative and seem to confirm the in vitro data on the usefullness of combining epigenetic and angiogenic treatment in this tumor type
4. although febrile neutropenia did occur, it was mostly short lived, in patients with extensive prior chemotherapy

607 Poster

Optimization of cancer vaccination schedule looking for better responses to EGF vaccine in NSCLC patients

B. Garcia[1], E. Neninger[2], S. Pereda[1], I. Leonard[1], G. Gonzalez[1], Z. Mazorra[1], A. Lage[1], T. Crombet[1]
[1]Center of Molecular Immunology, Clinical Trials, Havana, Cuba; [2] HHA Hospital, Chemotherapy, Havana, Cuba

Purpose: An optimized schedule of EGF-based vaccine was performed to increase the immunological and clinical response in NSCLC patients.

Patients and methods: Twenty advanced NSCLC patients were immunized with 2 biweekly doses of an EGF-based vaccine (200ug/dose) before first line chemotherapy. One month after of platinum based chemotherapy (4 to 6 cycles), monthly vaccination were reinitiated. Toxicity and humoral EGF specific immunity was evaluated.

Results: Vaccination was safe and well tolerated. The antibody titres against EGF were significantly higher than the values obtained post chemotherapy in the previous phase II clinical trial (50ug/dose, initiated one month after chemotherapy). Serum EGF concentrations decreased to undetectable levels in all vaccinated patients. A higher number of patients (85%) showed an immunodominant antibody response against the loop B on the EGF molecule as compare to 46% showing this pattern with the previous schedule of treatment. The percentages of EGF/EGFR binding inhibition were higher and positively correlated to a high antibody response against the loop B. The overall survival was positively associated to high antibody titters after vaccination and showed a trend to increase with a high antibody response against loop B at 7 months post chemotherapy. Moreover, longer survival times were reached in the subgroup of elderly patients (more than 60 years old) as compared to the results obtained in the previous phase II trial.

Conclusion: The new changes on the vaccination schedule rendered both, an amplified antibody response and better survival times of NSCLC treated patients than in previous trials. Surprisingly, clinical benefits were obtained for elderly patients.

608 Poster

Expression of CXCR7 in p-stage I non-small cell lung cancer increases the risk for postoperative recurrence at the distant site and correlates with poor disease free survival

S. Iwakiri[1], M. Sonobe[1], S. Nagai[1], T. Takahashi[1], N. Mino[1], R. Miyahara[1], H. Wada[1], H. Date[1]
[1]Kyoto University Hospital, Thoracic Surgery, Kyoto, Japan

BACKGROUND: The expression of chemokine receptors has been implicated in metastatic bahavior in different models of cancer. In the present study, we evaluated which chemokine receptors are expressed in lung cancer cells, and whether the chemokine receptor expression is associated with clinical features in completely-resected non-small cell lung cancer (NSCLC).

MATERIAL and METHODS: We quantitatively examined gene expression of chemokine receptors (CCR1–11, CXCR1–7, XCR1, and CX3CR1) in 12 cell lines of lung cancer, and examined gene expression of CXCR3, CXCR4, and CXCR7 in surgical specimens resected from NSCLC patients. A total of 127 consecutive patients from May 2001 to December 2002 were included.

RESULTS: Quantitative real-time reverse transcription-polymerase chain reaction revealed substantial expression of CXCR3, CXCR4, and CXCR7 mRNA in all the NSCLC cell lines. The CXCR7 expression values in patients with squamous cell carcinoma were significantly higher than that with adenocarcinoma (P=0.009). In p-stage I NSCLC, CXCR4 and CXCR7 expression values in patients with postoperative recurrence at the distant site were significantly higher than those with no postoperative recurrence (P=0.003 and P=0.007, respectively). In p-stage II-III NSCLC, CXCR3 expression values in patients with postoperative recurrence at the local site were significantly higher than those with no postoperative recurrence (P=0.049). In p-stage I NSCLC, the 5-year disease free survival rate of high CXCR7-expressing patients (63.2%) were significantly lower than those of low CXCR7-expressing patients (84.8%) (P=0.033). On the other hand, there was no difference in the 5-year overall survival rate according to the CXCR7 expression status (P=0.243). According to the CXCR3 and CXCR4 expression, there was no difference in the 5-year disease-free survival rate and overall survival rate. A multivariate analysis confirmed that high CXCR7 expression was an independent and significant factor predicting a poor disease free prognosis in p-stage I NSCLC (P=0.041).

CONCLUSIONS: Higher levels of CXCR7 appear to be associated with postoperative recurrence at the distant site and poor disease free prognosis in patients with p-stage I NSCLC.

609 Poster

Basaloid squamous cell carcinoma of the head and neck - A heterogeneous group with different cytokeratin immunoprofiles from conventional squamous cell carcinoma

K.J. Cho[1], S.W. Lee[2], J.L. Roh[3], S.H. Choi[3], S.Y. Nam[3], S.Y. Kim[3], S.B. Kim[4]
[1]Asan Medical Center, Pathology, Seoul, Korea; [2] Asan Medical Center, Radiation Oncology, Seoul, Korea; [3] Asan Medical Center, Head & Neck Surgery, Seoul, Korea; [4] Asan Medical Center, Medical Oncology, Seoul, Korea

Basaloid squamous cell carcinoma (BSCC) is a rare high grade variant of squamous cell carcinoma (SCC) with distinct clinicopathologic characteristics. Its histogenesis is not fully understood, and has been suggested to be from a totipotent primitive cell in the basal cell layer of surface epithelium or in the proximal duct of secretory glands. Cytokeratin immunoprofiles of BSCC have been reported in only a small number of cases, with variable results. Eighteen new cases of head and neck BSCC in 10 year period of Asan Medical Center, Seoul, Korea were subjected to immunohistochemical study for cytokeratin (CK) subsets (CK5/6, CK7, CK19) using tissue microarrays. CK5/6, a basal cell marker, was expressed in 14 cases (78%), and CK7, a ductal marker, in 6 cases (33%), while CK19, a squamous epithelial marker, only in 4 cases (22%). Based on the CK immunoprofiles, BSCC could be classified as basal (11), ductal (3), mixed (3), and null (1) phenotypes. The heterogeneous CK immunoprofiles of BSCC suggests complex pathogenesis and clinical behavior. The phenotypes, however, were not correlated with patient age (32-72), gender (14 males; 4 females), tumor location (5 larynx; 5 hypopharynx; 3 oropharynx; 1 nasopharynx; 1 nasal cavity; 1 maxillary sinus; 1 external auditory canal; 1 salivary gland), tumor morphology (presence of nuclear palisading; comedonecrosis; cribriform pattern; glands; mucin; desmoplasia; combined SCC), or clinical outcome (10 deaths; 2 diseases; 6 no evidence of diseases) in this study. The present immunohistochemical results are interesting to demonstrate a group of ductal phenotype, which