PP 89

The prognostic value of plasma TIMP-1 in resectable colorectal cancer: a prospective validation study

H.J. Nielsen, N. Brünner, I.J. Christensen. *Hvidovre Hospital, University of Copenhagen, Hvidovre, Denmark*

Background: Results from retrospective studies show that preoperative plasma TIMP-1 and CEA levels carry independent prognostic information of patients with primary CRC. The purpose of the present, prospective study was to validate the prognostic value of preoperative plasma TIMP-1 and CEA in patients with primary CRC.
Materials and Methods: Blood samples were collected before surgery from 297 patients with stage I-IV disease. TIMP-1 and CEA levels were determined in ETDA plasma using an automated platform (Architect®, Abbott Laboratories, Chicago, USA). The Cox proportional hazards model was used with TIMP-1 and CEA on a continous scale (log base 2) adjusted for clinical covariates. The endpoints were overall survival (OS) and disease-free survival − time from operation to any event (DFS).
Results: Of the 297 patients 118 were females and 179 males with a median age of 70 (32−79) years. Using the TNM stage 50 had stage I, 91 stage II, 70 stage III and 86 stage IV distributed as 180 with colonic and 117 with rectal cancer. The median observation period was 6.1 (5.2−7.3) years and 162 deaths were recorded. In a multivariate analysis including age, gender, stage, localization, plasma TIMP-1 and CEA it was shown that plasma TIMP-1 had independent, significant prognostic value: HR = 2.9; 95% CI: 2.0−4.8; $p < 0.0001$, whereas the value of CEA was non-significant. Restricting the analysis to stages II and III and patients not receiving adjuvant chemotherapy plasma TIMP-1 had independent, significant prognostic value: HR = 2.9; 95% CI: 1.3−6.8; $p = 0.013$, whereas the value of CEA was non-significant. Analysis including those patients, who received adjuvant chemotherapy, showed that neither plasma TIMP-1 nor CEA had any prognostic value. Similar analysis of patients with stages II and III and focus on DFS as the endpoint could not demonstrate significant results.
Conclusion: The present results achieved in a prospective study confirm that preoperative plasma TIMP-1 has independent prognostic value. In addition, the results suggest that patients with stage II or III and high plasma TIMP-1 values have particular benefit of adjuvant chemotherapy. The results must however be confirmed in prospective studies with inclusion of sufficient numbers of patients to confirm the results.

PP 95

Targeted therapy for mesothelioma using anti-podoplanin antibody NZ-1 via ADCC

Y. Nishioka, S. Abe, M. Kishuku, M. Hanibuchi, S. Kakiuchi, K. Kawazoe, K. Minakuchi, M.K. Kaneko, Y. Kato. *Department of Respiratory Medicine and Rheumatology, Institute of Health Biosciences, University of Tokushima Graduate School, Tokushima, Japan*

Background: Malignant pleural mesothelioma (MPM) is a neoplasm arising from the mesothelial cells lining the pleura, which is caused by asbestos exposure and shows thoracic tumors and malignant pleural effusion. Podoplanin/Aggrus is 36 kDa type I membrane protein and is known to be highly expressed in MPM. In the present study, we examined whether anti-podoplanin antibody NZ-1 showed antitumor effects mediated by antibody-dependent cellular cytotoxicity (ADCC).
Materials and Methods: We used human MPM cell lines and tissues. Expression of podoplanin was examined by using flow cytometry and immunohistochemistry. ADCC activity was measured by ^{51}Cr-release assay. In vivo antitumor effects was examined xenograft model of human MPM cells in SCID mice.
Results: MPM cell lines in 12/15 (80%) expressed podoplanin and MPM tissues also expressed high level of podoplanin. NZ-1 showed high ADCC activity when rat NK cells were used as effector cells. Administration of NZ-1 with rat NK cells significantly suppressed the growth of human MPM cells expressing podoplanin.
Conclusion: These results suggest that podoplanin is a promising marker to target mesothelioma for immunotherapy with anti-podoplanin antibody having ADCC activity.

PP 52

Automated enrichment of circulating, cell-free DNA from high sample volumes for tumor biomarker analysis

A. Nocon, M. Horlitz, A. Schubert, M. Sprenger-Haussels. *Department of Plant Biochemistry, Heinrich-Heine-University, Düsseldorf, Dormagen, Germany*

Background: Circulating, cell-free DNA (ccfDNA), derived from tumors, is fragmented and circulates in plasma, serum and other body fluids. Because of its extremely low concentration, the extraction and detection of tumor-derived ccfDNA is technically challenging. Here, the efficiency of a new automated large volume ccfDNA extraction method was evaluated.
Materials and Methods: EDTA plasma from healthy donors (with IRB approval) was used for the development of a new ccfDNA enrichment/extraction protocol. For the manual enrichment protocol, 3 ml plasma from individual donors was processed. The ccfDNA was bound to magnetic particles with novel surface chemistry, washed and eluted in a final volume of 150 µl. The QIAamp® Circulating Nucleic Acid Kit (QIAGEN) served as reference method to determine the amount of ccfDNA; this was used to calculate the percent recovery of ccfDNA using the developed protocol. An automated version of the enrichment protocol was run on the QIAsymphony® instrument (QIAGEN) with 3 ml plasma as sample input. ccfDNA yield was quantified by qPCR, targeting a short fragment within the 18S rRNA coding region (66 bp). As internal control, DNA fragments (75, 200, 1000 bp) were added at 200,000 copies per sample and the recoveries were measured by qPCR.
Results: Manual enrichment protocol: The median recovery of ccfDNA (18S target; compared to the reference method) was 94% (N = 8; range 69–132%). The median recoveries of the added DNA fragments were 127% (77–162%) for the 75 bp, 119% (94–128%) for the 200 bp and 87% (62–103%) for the 1000 bp fragment. Automated enrichment protocol (early version): The median recovery of ccfDNA (18S) was 57% (N = 12; 16–138%) and of the added DNA fragments 83% (58–102%) for the 75 bp, 78% (53−93%) for the 200 bp and 47% (23−68%) for the 1000 bp fragment.
Conclusion: The new enrichment protocol led to an overall similar ccfDNA recovery compared to the QIAamp® Circulating Nucleic Acid Kit. A slightly lower recovery of longer DNA fragments ($\geqslant$1000 bp) is unlikely to adversely affect tumor DNA detection as it is mostly shorter than 500 bp. Novel chemistry allows for the enrichment protocol to be run on the QIAsymphony® instrument without hardware changes and enables automated ccfDNA recovery from up to 6 ml sample for molecular cancer detection.

PP 70

Stathmin1 − drug sensitivity associated protein of lung cancer

M. Nomura, T. Fukuda, K. Nagase, H. Tojo, T. Nagao, N. Gotoh, T. Ohira, H. Katoh, N. Ikeda, T. Nishimura. *Tokyo Medical University, Tokyo, Japan*

Background: Primary lung carcinoma is the leading cause of cancer-related death in not only Japan but also in all over the world. In Japan, the annual number of eligible subjects for operation is only 10,000 among 80,000. Most of other patients in advanced stages, IIIB or IV, have been usually treated by chemotherapy. However, the sensitivity of chemotherapy to lung cancer is still very low except small cell lung carcinoma (SCLC). We planned to find some specific proteins related to chemosensitivity from SCLC and apply them to non small cell lung carcinoma (NSCLC).
Materials and Methods: We have selected relatively specific proteins from formalin fixed paraffin-embedded (FFPE) tissues of histologically diagnosed as neurosndocrine carcinoma, as SCLC (n = 5) or LCNEC (n = 4), by laser microdissection, liquid chromatography/mass spectrometry, semi-quantified method and quantified method. We have searched for NSCLC cell lines that express the specific protein of NE carcinoma as mRNA by PCR, knocked it out by siRNA technique and checked the change of sensitivity to usual chemotherapeutic agents, CDDP and VP16 by MTT assay.
Results: We identified around 1000 proteins as characteristic proteins of NE carcinoma and 100 proteins were expressed in both SCLC and LCNEC. Among them, stathmin1 which is involved in the regulation of microtubule filament system by destabilizing microtubules, was more expressed in SCLC compared to LCNEC by quantified method and immunohistochemical staining. Stathmin1 was also expressed in lung adenocarcinoma cell line, H838, and large cell carcinoma cell line, H1299. After knocking out of stathmin1, the sensitivity to VP-16 was decreased, while the sensitivity to CDDP was increased.
Conclusion: In this study, our findings suggested that stathmin1 may be one of proteins associated to the chemosensitivity not only NE carcinoma but also NSCLC.

PP 41

Anticancer potential of *Datura inoxia* extract against cervical cancer HeLa cells

M. Pandey, S. Saraswati. *Delhi Institute of Pharmaceutical Sciences and Research, New Delhi, India*

Background: Cervical cancer is one of the most prevalent malignancies in women in many countries worldwide. Therefore, the development and search for novel and effective anticancer agents have become very important issues. *Datura* seeds and leaves were used in the treatment

of common cold, headache and asthma. However, its role in cancer growth has not been elucidated yet. The present study is aimed at investigating the cytotoxicity of the aqueous extract of the seeds of *Datura inoxia* against cervical cancer HeLa cells.
Materials and Methods: The effect of aqueous extract of the seeds of *Datura inoxia* on cell death and intracellular targets that affect angiogenesis (VEGF), inflammation (IL-12, TNF-α), apoptosis (caspase-3, -8 & -9) and antioxidant (superoxide dismutase & catalase) were determined by MTT assay, ELISA and enzymatic activity assay. In addition, Anti-VEGF neutralization effect was evaluated alone and in combination with aqueous extract, to assess whether it could result in augmented anticancer efficacy than the single agent.
Results: Aqueous extract of the seeds of *Datura inoxia* inhibited growth of cancer cells in a dose and time-dependent manner. Experiments aiming to investigate the anti-angiogenic activity against HeLa, revealed that following the treatment, a dose-dependent decrease ($p < 0.001$) in the levels of VEGF secreted by the cells was recorded. In another set of experiments Aqueous extract potentiated ($p < 0.001$) the cell death induced by anti-VEGF antibody. VEGF and its receptors are established as major mediators of tumor cell growth and invasiveness; taken together; the results of these experiments suggest that datura possesses antiangiogenic activity. Although it appeared to decrease the levels of tumorigenesis factor, TNF-α ($p < 0.05$), it did not alter IL-12 level significantly. The pro-apoptotic effects were confirmed by significant ($p < 0.001$) increase in caspases-3 and -9 but not 8 activity. Significant increase in antioxidant enzymes (SOD, catalase) activity was also recorded.
Conclusion: Aqueous extract of the seeds of *Datura inoxia* acts via multiple albeit specific molecular targets to elicit anti-carcinogenic activity thus might be a candidate for developing multifunctional anti-cancer agent through its inhibitory activity on several aspects of tumor growth and angiogenesis.

PP 42

Correlation between cancer stem cell like cells markers and clinical assessment in breast cancer patients

I. Papasotiriou, P. Apostolou, M. Toloudi, M. Chatziioannou. *Research Genetic Cancer Center Ltd (R.G.C.C. Ltd), Filotas, Greece*

Background: Recent literature and experimental data have pointed out the predictive value of circulating tumor cells (CTCs), while other studies have demonstrated that cancer stem cell-like cells (CSCs) are included in the vast majority of CTCs. The aim of the present study is to find out the correlation between clinical assessment and CSCs' markers (Nanog, Oct3/4, SOX2, Nestin, CD34).
Materials and Methods: For the realization of this study have been identified and isolated CTCs from patients with breast cancer in different stages according TNM classification system. It has been followed quantification of cancer stem cell-like cells in CTCs cultures and molecular analysis of the above cells with RT-qPCR, by using gene-specific primers for each marker and for the housekeeping gene (18s rRNA) that has been used. The comparative Ct method ($2^{-\Delta\Delta C_t}$) has been used for the calculation of relative quantitation. Finally, has been requested the clinical assessment from physicians of each patient so far.
Results: The expression of the transcription factors was dependent on the clinical status of patients. It has been observed that SOX2 is overexpressed in cases of complete response, while Nestin is expressed much more than the other factors in cases of stable disease. In stable disease it has been also observed the decrease of CD34 transcription factor. High levels of Nestin combined with overexpression of Oct3/4, SOX2 have been noted when patients had progress of disease. The expression of Nanog transcription factor also seems to vary, depending of the clinical status.
Conclusion: This study is an attempt of correlation of clinical evaluation with the transcription factors responsible for cancer stem cells in patients with breast cancer. The results show that the expression pattern of transcription factors is proportional to the clinical assessment of patients. However, worth noting is that the CSCs which were studied, were isolated before starting treatment. Therefore, might be able to avoid cases of relapse which are not seemed prima facie. However, it is necessary to perform numerous studies to a greater range and number of samples in order to be applied at clinical level.

PP 61

Chromosomal aberrations in group of PTCL, NOS − own experience

A. Pastwinska, J. Rygier, R. Woroniecka, B. Grygalewicz, P. Krawczyk, B. Pienkowska-Grela. *M. Sklodowska-Curie Memorial Cancer Centre and Institute, Cytogenetic Laboratory, Warsaw, Poland*

Background: Cytogenetic investigations play important role in the diagnosis of lymphomas, however T-cell neoplasms remain poorly characterized by genetic alterations. The low availability of diagnostic material for testing and its intractability in obtaining cell divisions during in vitro culture is one of the major research problem.
Materials and Methods: We investigated group of 32 cases of PTCL, NOS (peripheral T-cell lymphoma, not otherwise specified), diagnosed in M. Sklodowska-Curie Memorial Cancer Centre and Institute in 2007–2010. Lymphoma cells were obtained mostly by fine needle aspiration biopsy (FNAB) of lymph nodes or extranodal tumours, peritoneal fluid, and from bone marrow and peripheral blood. In order to identify cytogenetic aberrations G-banding and FISH analyses were performed (commercial unique probes for TRA@/TRB@, TRG@, TRB@ and CDKN2A/CEP9 genes).
Results: In analysed group we detected aberrant karyotypes in 6 and normal karyotypes in 7 cases. No metaphases were obtained in 8 cultures, while other materials (11 cases) was not diagnostic. Among the aberrant karyotypes we found the presence of 3 cases with hiperdiploid karyotype, 2 cases with pseudodiploid and 1 with hypodiploid set of chromosomes. Structural changes affecting a number of chromosomes were detected, but the only recurrent aberration was: der(11)del(11)(p13)del(11)(q21). We also revealed two repeatable breakpoints: 6p21 and 10p13. T-cell receptor rearrangements were sporadic in FISH analysis. We detected only one case with rearrangement of TRA@/TRD@ (5.2%), while the changes in copy number of TRG@ and TRB@ were found thrice (20%). In one case we identified three copies of TRG@ and TRB@, probably because of trisomy 7, while in second − four copies of TRG@ was visible. Third patient had loss of one copy of TRG@. Monoallelic deletion of CDKN2A was detected in two cases (12.5%).
Conclusion: A random chromosomal aberrations were seen in few cases, but only a few were recurrent: der(11)(del(11)(p13)del(11)(q21) and breakpoints: 6p21 and 10p13. Both rearrangements of T-cell receptor genes and CDKN2A are sporadic in PTCL, NOS.

PP 106

Integration of clinical, histopathological, radiological and biomolecular data for prediction of oral squamous cell cancer (OSCC) recurrence: the NeoMark project's interim results

T. Poli, D. Lanfranco, C. Copelli, D. Ardigò, E.M. Silini, R. Perris, N. Bertani, E. Derlindati, C. Azzoni, E. Sesenna. *Maxillofacial Surgery, Head and Neck Department, University Hospital of Parma, Parma, Italy*

Background: Neoplastic diseases are at large the second cause of death in the western world. In the last decade, the continuous improvement in treatment protocols has substantially increased the number of patients who achieve a complete disappearance of the disease (remission) after treatment. NeoMark Project − ICT Enabled Prediction of Cancer Reoccurrence performs research in the integration of heterogeneous clinical, laboratory, biomolecular and imaging data to develop a data integration environment facilitating multiscale and multilevel modeling, aimed at advancing models and methods currently in use to predict neoplastic reoccurrences, and to apply it to the study of oral cancer.
Materials and Methods: The NeoMark concept will be tested and validated through a clinical observational study in a relevant number (100–150 patients) of OSCC cases treated with conventional surgery ± radiation and followed up for at least 12 months. Clinical and imaging data, as well as blood cell genomics will be collected at the time of diagnosis, after treatment, and during follow-up. Cancer specimen will be collected intra-surgery for histological and gene expression analysis. All patients showing a complete remission of the disease from a clinical and laboratory point of view after first line treatment will be selected for the follow-up phase. Primary end-point of the study will be the clinical evidence of recurrence during follow-up.
Results: The presented interim results of the Neomark Project obtained from the Baseline Data Analysis with the current state of the dataset are quite encouraging; however, as more and more patients' records become available, the data will be analyzed again in order to infer more reliable results.
Conclusion: The main contributions that NeoMark expects to bring to research is a better understanding of the correlations between biological factors (personal for each patient and specific for OSCC) that are characteristic for oral cancer and fostering reoccurrences. The emphasis given by NeoMark partners to the evaluation and processing of integrated data from each patient and from many patients with similar tumours is expected to lead us to the identification of some prominent markers which will be experimentally analysed on a prototype medical diagnostic device based on RNA micro-array techniques and on chip-based RT-PCR technologies.