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