P60. HYPOXIA-REGULATED PROTEINS IN GASTRIC CANCER: CORRELATION WITH DISSEMINATED TUMOR CELLS AND CLINICAL OUTCOME

S. Osinsky[a], D. Gluzman[a], G. Olijnichenko[c], L. Bubnovskaya[a], I. Ganusevich[a], D. Osinsky[b], S. Merentsev[a], L. Gumenjuk[a]. [a]*Inst. Exp. Pathol. Oncol. Radiobiol., NASU, Ukraine;* [b]*Inst. Oncology, AMSU, Ukraine;* [c]*Municipal Oncological Hospital, Kiev, Ukraine.*

Background: It is known that tissue hypoxia has a positive impact on malignant progression. Our study was aimed to examine the correlation between hypoxia-regulated proteins (HRP), disseminated tumor cells (DTC) and clinical outcome in gastric cancer (GC).
Methods: One hundred and thirty three naïve patients with primary GC who underwent surgery were included in the study. All patients have been informed about investigation. Hypoxia within tissue was evaluated using ^{31}P NMR spectroscopy (PME/Pi), expression of hypoxia-inducible factor-1α (HIF-1α) and CD34 (microvessel density – MVD) in tissue were assessed using immunohistochemistry, gelatinases (MMP-2 and -9) activity was determined with zymography. DTC in bone marrow (BM) were detected using immunocytochemistry. Statistical analyses were done using NCSS/PASS package (NCSS, UO, USA).
Results: High hypoxia levels (HLs) were found in 29% of pts., and low – in 71% of pts. Strong nuclear expressions of HIF-1α were found in 7%, moderate – in 80%, and weak – in 13% of pts. Strong MVDs were observed in 54% and moderate – in 46% of pts. It was revealed a close association of HL in tumor both with expression of HIF-1α in tumor cells ($P < 0.01$) and MVD rate ($P = 0.02$). The HIF-1α expression correlated with histological grade of tumor ($P < 0.05$). HL and MVD in tumor correlated with clinical stage ($P < 0.05$). It was shown that increased PME/PDE ratio in tumor may be associated with the early development of distant metastases. It was also shown that tumor cells in BM are detected in 40% of patients with M_0 stage. It was observed that tumor in patients with negative BM is characterized both by high MMP-9 activity and low level of hypoxia. At the same time tumor in patients with positive BM is characterized both by high activity of MMP-2 and high level of hypoxia. High tumor HL positively correlated with decreased overall survival ($P = 0.044$). For overall survival, HL and HIF-1α expression were independently predictive in multivariate analysis for lymph-node negative patients.
Conclusion: Statistical analysis has indicated that PME/Pi and PME/PDE ratios as well as HIF-1α expression in tumor tissue may be used as an independent prognostic factors of clinical outcome in patients with GC. It is supposed that hypoxic profile of tumor can be a favourable basis for the appearance of DTC and formation of distant metastases.

doi:10.1016/j.ejcsup.2006.04.120

P61. PECULIARITIES OF GASTRIC CARCINOMAS IN PATIENTS UNDER 50 YEARS OF AGE

C. Schildberg, T. Horbach, S. Merkel, T. Papadopoulos, A. Dimmler, W. Hohenberger. *Erlanger, Germany.*

Introduction: Primary gastric carcinomas more commonly appear in advanced age; the portion of younger patients with this (<50 years) is about 7–15%. To discover possible clinical and pathologic peculiarities between the two groups, our clinic's patients who were under 50 years of age were compared to patients who were over 50 ($n = 481$).
Materials and Methods: The study's time-frame spanned from 01/01/1994 to 12/31/2002. The younger-aged group (median age 44 years) was composed of 73 afflicted patients; the older-aged group (>50 years) was composed of 408 operated patients (median age 68 years).
Results: Younger patients reported symptoms preoperatively more often than older patients (99% vs. 92%, $p > 0.05\%$). The diffuse gastric carcinoma predominated the younger collective of patients (82% vs. 49%, $p = 0.0001$). In differentiating the tumor stages, there were minimal differences between the two groups ($p > 0.05\%$). Postoperative complications in the sense of insufficient anastomoses was remarkably higher in older patients (5%) as compared to younger patients with 1% ($p = 0{,}22559$). There were remarkable differences in survival (5-year survival rate) in favor of the younger patients even after statistical age corrections were performed (54.1% vs. 41.1 %; $p = 0{,}0414$).
Conclusion: In younger patients, the diffuse type predominates, which could point to differences in genesis. It is often only initially found in a late stage (IV) and is always associated with clinical symptoms. The treatment results in younger patients are more favorable than in the >50 group, not only in the view of the surgical complications, but also in lethality and 5-year survival. This is also true in consideration of the different life expectancies and gender differences.

doi:10.1016/j.ejcsup.2006.04.121

P62. SEMI-QUANTITATIVE GENE EXPRESSION PROFILING FOR THERAPY PREDICTION IN A BREAST CANCER NEOADJUVANT THERAPY STUDY APPLYING DOCETAXEL/EPIRUBICIN/CYCLOPHOSPHAMIDE (TEC)

U. Vogt, C.M. Schlotter, U. Bosse, U. Bonk, B.H. Brandt. *European Laboratory Association, Ibbenbueren, Germany; Clinical Centre, Ibbenbueren, Germany; Institute of Pathology, Osnabrueck, Germany; Institute of Pathology, Bremen, Germany; Institute of Tumor Biology, Hamburg, Germany.*

Background: Currently there are no tests to assist in selecting the optimal preoperative chemotherapy (PST) regimens for breast cancer patients. Primary study goals are pathologically confirmed tumour response and the rate of breast conserving therapy. Secondary study goals are to find histopathologic and gene profiling patterns best correlating with tumour remission in a TEC based neoadjuvant setting as well as to evaluate cytostatic toxicity and quality of life.
Metohds and Patients: In this neoadjuvant phase II study of totally 40 eligible patients with histologically confirmed invasive breast cancer Human Genome Survey Microarray (HGSM) expression profiling is preformed on jet-biopsy sample basis. The protocol was elaborated for the treatment of breast cancer patients suffering from a primary tumour greater than 1.5 cm or

inflammatory breast cancer with 6 cycles of TEC (3-week) prior to the surgical treatment.
Results: Tumour response (pCR, pPR) of more than 70 % can be achieved using neoadjuvant TEC-regimen. The preliminary expression profiling results shown here indicate a subset of 148 genes that classifies all patients with a complete remission (pCR). A comparable separation of the groups could not achieved by established tumor factors, e.g. ER, PgR, HER2, uPA, which are measured simultaneously on the HGSM and also statistically evaluated.
Conclusions: HGSM semi-quantitative expression profiling is promising to have the potential to figure out genes that are related to cancer progression and chemotherapy resistance, especially in primary systemic chemotherapy.

doi:10.1016/j.ejcsup.2006.04.122

P63. EXPRESSION OF TISSUE FACTOR IN PANCREATIC ADENOCARCINOMA

Stephan L. Haas[a], Ralf Jesnowski[a], Michael Steiner[b], Frank Hummel[a], Jörg Ringel[a,c], Christine Burstein[b], Horst Nizze[d], Stefan Liebe[e], J.-Matthias Löhr[a]. [a]*Department of Medicine II, University of Heidelberg at Mannheim, Germany;* [b]*Department of Clinical Chemistry and Pathobiochemistry, University of Rostock, Germany;* [c]*Department of Medicine, Martin Luther University at Halle-Wittenberg, Halle/Saale, Germany;* [d]*Department of Pathology, University of Rostock, Germany;* [e]*Department of Medicine, University of Rostock, Germany.*

Background: Pancreatic cancer is the tumor with the highest risk for thromboembolic complications. Tissue factor represents the principal initiator of coagulation. The aim of this study is to study expression of tissue factor (TF) in pancreatic cancer.
Methods: TF expression was studied in human pancreatic carcinoma cell lines by Northern blot and immunofluorescence. Expression of alternatively spliced TF (asTF) was assessed by RT-PCR. TF expression was determined by immunofluorescence in tissues of pancreatic adenocarcinoma (PCa), chronic pancreatitis (CP) and normal controls. Plasma samples (30 PCa-patients, 13 CP-patients and 20 controls) were investigated for soluble TF levels and coagulation activation markers (thrombin-antithrombin III complex [TAT], prothrombin fragment 1 + 2 (F1 + 2)).
Results: All pancreatic carcinoma cell lines expressed TF (8/8) and most of them asTF (6/8). All but two pancreatic cancer tissue samples stained positive for TF (17/19). In all samples of cP weak staining was restricted to pancreatic duct cells. TF and TAT levels in PCa patients were significantly elevated whereas elevated F1 + 2 levels did not reach statistical significance compared to controls. In CP patients TAT and F1 + 2 levels proved to be significantly elevated compared to controls, although TAT elevation was less pronounced than in PCa patients.
Conclusion: We conclude that in addition to the upregulated expression of TF on the cell membrane soluble TF might be pivotal for activation of coagulation in pancreatic cancer.

doi:10.1016/j.ejcsup.2006.04.123

P64. PROTEIN-BOUND POLYSACCHARIDE PSK INDUCES GROWTH INHIBITION IN PANCREATIC TUMOR CELLS

Ralf Jesnowski, Matthias Löhr. *German Cancer Research Center, CCU Molecular Gastroenterology, Heidelberg, Germany.*

Background: Pancreatic carcinoma is particularly aggressive tumor, it has a poor prognosis and at the time of diagnosis, the tumor is generally in an advanced stage no longer suitable for resection. Moreover, this tumor is virtually resistant to conventional radio-/chemotherapy. Thus alternative therapy modalities are urgently needed. Morphologically pancreatic adenocarcinoma is characterized by a dense stromal reaction comprised of activated pancreatic stellate cells. This stroma may protect the tumor cells against the patients immune system and may in part be responsible for the chemoresistance of the tumor cells. Polysaccharide-K (polysaccharide-Kureha; PSK), also known as krestin, is a unique protein-bound polysaccharide derived from the CM-101 strain of the fungus Coriolus versicolor, which has been used successfully as a chemoimmunotherapy agent in the treatment of various cancers in Asia for over 30 years, however there is only one publication concerning the combined treatment of two pancreatic cancer patients with Cisplatin, PSK and UFT. PSK not only boosts the immune system of patients but also has direct antineoplastic effects. So it has been shown, that PSK reduces the invasiveness of a pancreatic tumor cell line by downregulating TGFbeta1 and MMP.
Aim and Methods: In order to analyze, if the reduction of the invasive potential by PSK is a general phenomenon in pancreatic tumor cells, we treated a panel of different pancreatic tumor cell lines with PSK and subsequently analyzed changes in gene expression using RT-PCR. Moreover, the activity of MMPs was analyzed using zymography, proliferation of the cells after PSK treatment was analyzed by WST-1 assay.
Results: Treatment of the pancreatic tumor cells with PSK for up to six days resulted in a short term induction of the cell cycle inhibitor p21/WAF1 at 4 and 24 h, after that expression returned to basal levels. In contrast PCNA and cycD1 expression gradually decreased during the PSK treatment, reaching maximal repression after about three days, which persisted for the rest of the treatment period. In contrast to the published results PSK did not change MMP expression, neither on the RNA level analyzed by RT-PCR nor on the protein level as analyzed by zymography. Moreover, PSK dose dependently decreased the proliferation of all pancreatic tumor cells investigated, reaching a plateau of about 40% decrease of proliferation at a concentration of 250 μg/ml PSK. A further increase of PSK concentration had only marginal additional effects on the proliferation of the cells.
Conclusion: Our results demonstrate a direct antineoplastic effect of PSK on pancreatic tumor cells by decreasing the proliferation of the cells, pointing on the possibility for the use of PSK in the treatment of pancreatic cancer.

doi:10.1016/j.ejcsup.2006.04.124