

cancer. The mRNA level of AKAP12 α gene was examined by reverse transcription-polymerase chain reaction (RT-PCR) in ten lung cancer cell lines. The mRNA level of AKAP12 α gene was reduced in 6 of 10 (60%) lung cancer cell lines. The AKAP12 α expression was restored after treatment with the demethylating agent 5-aza-2'-deoxycytidine in lung cancer cell lines which have downregulated mRNA level of AKAP12 α gene. This result was further substantiated by a luciferase reporter assay, showing the restoration of promoter activities when transfected with unmethylated AKAP12 α promoter. The methylation status of CpG island 1 and 2 existed in the AKAP12 α promoter was analyzed by performing bisulfite-sequencing analysis in ten lung cancer cell lines, forty six lung cancer tissues and matched normal tissues. Methylation of CpG island 1 in the AKAP12 α promoter was detected in 30% of the lung cancer cell lines, whereas methylation of CpG island 2 in the AKAP12 α promoter was observed in the immortalized bronchial cell line and in all the lung cancer cell lines. In all of the lung tumors, the CpG island 1 in the AKAP12 α promoter was infrequently methylated. However, CpG island 2 in the AKAP12 α promoter was highly methylated in lung tumors compared with the surrounding normal tissues, and this was statistically significant ($p=0.002$). Also, the analysis in the methylation level of CpG island 2 and the patient's clinicopathological features was showed that the CpG island 2 methylation of AKAP12 α gene in male, non-recurrence subgroup was occurred more frequently than other subgroup. In summary, epigenetic AKAP12 α silencing seems to be a relevant mechanism of inactivating this AKAP12 α gene in lung cancer and the methylation rate of AKAP12 α promoter is associated with lung cancer.

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P2-058

BSTB: Others Posters, Tue, Sept 4

Multicomponent Multicellular Tumor Spheroids(MMCTS) is the new model of cancer research

Kim, Ki-Up¹ Lee, Young-Mok² Na, Dong-Jib³ Kim, Do-Jin⁴ Uh, Soo-Taek²

¹ Department of Internal Medicine, Soonchunhyang University, Seoul, Korea ² Dept. of Internal Medicine, Soonchunhyang University, Seoul, Korea ³ Dept. of Internal Medicine, Eulji University, Daejeon, Korea ⁴ Dept. of Internal Medicine, Soonchunhyang University, Bucheon, Korea

Introduction: Researches of cancer have two ways, in vivo and in vitro. Most of them are in vitro and their results are promising. However, using their results to in vivo had been disappointed because tumor contains 3 dimensional structure, complicated components, and intercellular and/or cell/matrix interactions. Multicellular tumor spheroids (MCTS) is a kind of new research area of biology but lack of stromal cells and tumor associated macrophages. Artificial organisms, which have similar cell components compare with tumor, will be more complicated and advanced model than MCTS. We tried making a multicomponent multicellular tumor spheroids (MMCTS) from 3 kinds of cells from cancer cell from cancer cell line, fibroblasts and peripheral blood mononuclear cells(PBMC) from human as a models to cancer research.

Method: A549 cell line, fibroblasts cultured from human, and PBMC were cultured on rotatory cell culture system (RCCS) singly or combined in DME media. Their formation of spheroids were observed 2 times a week by inverted microscope and got samples serially for 4 weeks. Cell components from combined spheroids were stained immunohistochemically by antibody to cytokeratin, fibroblast and CD68.

Result: Cells, cultured singly, were aggregated and formed clusters except PBMC within a few days and formed spheroids between 4 to 7 days. Fibroblasts were formed earlier than A549. The mixed cluster with A549, fibroblast and PBMC were also formed round shape. Immunohistochemical stain showed that cells from MMCTS preserved their characteristics for 4 weeks.

Conclusion: MMCTS will be a new model for tumor research.

P2-059

BSTB: Others Posters, Tue, Sept 4

Detections and identifications of the histopathological differentiation-specific proteins due to Ettan DIGE analysis of non small cell lung cancer

Kojika, Masakazu¹ Kataba, Hiroaki¹ Matubayashi, Jun² Gong, Yunbo¹ Guo, Ran¹ Serizawa, Hiromi² Mukai, Kiyoshi² Kato, Harubumi¹

¹ Department of Thoracic Surgery, Tokyo Medical University, Tokyo, Japan ² Department of Pathology, Tokyo Medical University, Tokyo, Japan

Design: We investigated histopathological differentiation-associated proteins (HD proteins) of non-small cell lung cancer (NSCLC) using 2 dimensional polyacrylamide gel electrophoresis (2DE) and attempted to classify NSCLC. We applied Ettan-DIGE system to 2DE analysis and investigated HD proteins on a single 2DE gel to compensate for the unfavorable reproducibility of 2DE analysis.

Materials and Methods: We collected samples for 2-DE from representative histological types of primary lung cancer, such as squamous cell carcinoma, adenocarcinoma and small cell carcinoma between 2003 and 2005. We selected 8 cases of well or moderately differentiated squamous cell carcinoma confirmed postoperatively to possess typical histological features of primary lung squamous cell carcinoma. The mixture that contained equal amount of these eight samples was regarded as a standard sample of lung squamous cell carcinoma (Sq standard sample). In the same way we selected 8 cases of well or moderately differentiated adenocarcinoma to possess a standard sample of primary lung adenocarcinoma (Ad standard sample). We also mixed all four cases of small cell carcinoma samples which were collected during these periods, and preferred a mixture as a standard sample of small cell carcinoma (Sm standard sample). Each standard sample of representative histopathological types of primary lung cancer was labeled with 400 pmol of a fluorescent dye (either Cy2, Cy3 or Cy5) per 50 μ g protein according to the manufacturer's protocol (GE Healthcare Biosciences). Furthermore, using mass spectrometry we identified protein molecules that were detected in 2DE analysis, followed by validation analysis by immunohistochemistry.

Results: We detected 7 spots associated with squamous cell carcinoma, 9 spots associated with adenocarcinoma and 13 spots associated with neuroendocrine carcinoma (NE-associated spots) on 2DE gel, and succeeded in identifying all histopathological associated proteins except three NE-associated proteins (Figure 1, Table 2). Of 29 identified proteins in this investigation 8 were cytokeratin (CK), which were CK5, CK6A, CK8, CK6C, CK6D, CK17, CK18 and CK19. CK5, CK6A, CK6C, CK6D and CK17 were identified as a Sq-associated protein, and also CK8, CK18 and CK19 were identified as Ad-associated protein. The other identified proteins we recognized contained napsin A and annexin A4. We tried to validate the expression of various kinds of CK using immunohistochemical analysis. In CK5, CK5/6 and CK17 the expression levels in squamous cell carcinoma were greater than in the other histopathological types. On the other hand, the expression levels of CK8 and CK18 in adenocarcinoma were greater than the other

histopathological types. On immunostaining expression pattern of CK in large cell carcinoma was similar to that of adenocarcinoma.

Conclusion: The investigations of CK expression give us additional information concerning histopathological differentiation of primary lung cancer. Though more investigations are needed, there is a strong possibility that classification based upon proteomic analysis, as well as morphological features, may reflect the biological characteristics of tumor cells.

P2-060

BSTB: Others Posters, Tue, Sept 4

Altered iron metabolism, inflammation, transferrin receptors and ferritin expression in lung cancer

Kukulj, Suzana

Clinical Hospital For Lung Diseases Jordanovac, Zagreb, Croatia

Introduction: Alterations in whole-body iron metabolism are known to occur in patients with cancer. Iron could participate in carcinogenesis and overabundance of iron is associated with increased risk of neoplasia at the site of metal deposition.

Aim: The relationship between the iron status and survival of lung cancer patients and the expression of transferrin receptors 1 (TfR1) and ferritin in tumor tissue, tumor stroma and normal lung were studied.

Patients and Methods: These findings were correlated with tumor type and clinical outcome in 111 male patients. Iron metabolism and inflammation parameters were determined by automated laboratory measurements at the time of diagnosis. TfR1 and ferritin expression were determined by immuno-histochemical methods on cancer tissue, tumor stroma and on the surrounding normal lung tissue.

Results: More than fifty percentages of patients survived less than 12 months. At the time of diagnosis approximately a half of the patients had mild anemia of chronic disease and significantly elevated serum ferritin. Nonspecific laboratory markers of inflammation were present. Tumor tissue expressed much more TfR1 and ferritin than the tumor stroma and normal lung tissue. The expression of TfR1 and the ferritin content in tumor tissue depended on the carcinoma type. TfR1 and the ferritin content in tumor tissue did not show correlation with systemic parameters of most of iron metabolism parameters. Strong ferritin expression in tumor tissue correlates only with lower transferrin saturation.

Conclusion: Higher expression of ferritin in tumor tissue is not the results of higher body iron accumulation. Elevated serum ferritin in lung cancer patients is results of inflammation and oxidative stress rather than body iron overload.

P2-061

BSTB: Others Posters, Tue, Sept 4

ProGRP and NSE for follow-up of small cell lung cancer patients with limited disease

Wojcik, Ewa Kulpa, Jan K. Sas-Korczynska, Beata

Center of Oncology - M.Sklodowska-Curie Memorial Institute, Cracow, Poland

Introduction: For some years, NSE has been known as a marker of choice for small cell lung cancer. However, its diagnostic sensitivity and specificity are not completely satisfactory, due to relatively high false negative rate in SCLC patients with limited disease and false positive rate in patients with non-malignant lung disease and non-small cell lung cancer. Recently, the usefulness of pro-gastrin-releasing peptide (ProGRP) as a tumor marker for SCLC has been investigated. Although

the diagnostic sensitivity and specificity of ProGRP was found to be higher than serum NSE, only small number of data concern its utility in disease therapy monitoring and its value in prediction of response to treatment. The aim of the study was the evaluation of ProGRP and NSE levels at the time of diagnosis and during chemo- and radiotherapy of SCLC patients with limited disease in respect to their prognosis.

Material and Methods: Studies of NSE and ProGRP were performed in a group of 52 SCLC-LD. Patients with SCLC with limited disease were treated simultaneously with chemo and radiotherapy. All of them also received prophylactic cranial irradiation between fourth and fifth course of chemotherapy. The increment ratio of tumor markers was calculated as serum concentration divided by the cut off, for assessment of prognostic value of these markers.

Results: ROC curve analysis confirmed that ProGRP was a better than NSE tumor marker for diagnostics of SCLC-LD patients (Area under curves ROC: 0.935 vs. 0.789, p = 0.000).

There were observed significant differences in the frequency of elevated NSE and ProGRP levels before each course of chemotherapy and 3 months after its finishing, respectively: 1st 57.6% vs. 78.8%, 2nd 5.8% vs. 67.3%, 3th 0% vs. 36.5%, 4th 1.9% vs. 21.2%, in restaging 6.7% vs. 15.7%. Changes in NSE levels during therapy were more intensive than for ProGRP what was reflected in tumor markers half-life (NSE: 4.6 - 11 days; ProGRP: 19-28 days) as well as in the frequency of increment of tumor markers ratio values. Patients with tumor marker levels 2 times exceeding NSE cut off and 12.5-times ProGRP cut off before treatment, and those with NSE and ProGRP having these ratio values higher than 0.4 and 0.65 during restaging 3 months after finishing therapy has shown worse prognosis.

Multivariate analysis confirmed that independent prognostic factors in SCLC with limited disease were: NSE level exceeding 2-times cut off value before treatment as well as NSE threshold 0.4 cut off and 0.65 ProGRP cut off value 3 months after therapy.

Conclusions:

- 1 Changes of ProGRP during combined therapy of SCLC-LD seem to be more adequate to actual clinical status of patients than NSE
2. NSE before treatment is a better than ProGRP prognostic factor in SCLC-LD patients however after finishing therapy both markers have similar predictive value

P2-062

BSTB: Others Posters, Tue, Sept 4

Optimizing the yield of circulating DNA from plasma and serum

Xue, Xiaoyan; Teare, M D.; Holen, Ingunn; Zhu, Yong M.; Woll, Penella J.

University of Sheffield, Sheffield, UK

Background: Low levels of circulating cell-free DNA are present in normal individuals. In cancer patients, much higher levels of circulating DNA are found. Importantly, circulating DNA in lung cancer patients demonstrates genetic alterations typical of the tumour, leading to interest in plasma and serum DNA for early clinical diagnosis, prognosis and disease monitoring. There is considerable variation among studies in the reported levels of circulating DNA and its characteristics, which may be attributable to differences in selection of the patient and control groups, and the methods used for DNA extraction and estimation of circulating DNA concentrations. Here, we compare the efficiency of different methods for extracting low-level circulating DNA from blood samples.