Doctoral Program of Higher Education of China (to Qinghua Zhou) (No. 20040610060) .

P2-035 BSTB: Cancer Genetics Posters, Tue, Sept 4

Double mutant P96S/S120G of Nm23-H1 abrogates its NDPK activity and motility-suppressive ability*

Zhou, Qinghua[1] Yang, Xueqin[2] Zhu, Daxing[3] Ma, Li[3] Zhu, Wen[4]

[1] Tianjin Lung Cancer Institute,Tianjin Medical University General Hospital, Tianjin, China [2] Cancer Center, West China Hospital, Sichuan University, Chengdu, China [3] Tianjin Lung Cancer Center, Tianjin Medical Unversity General Hospital, Tianjin, China [4] Key Lab. of Lung Cancer Molecular Biology of Sichuan Province,West China Hospital, Sichuan University, Chengdu, China

Background: The Nm23-H1 gene is a metastasis suppressor gene. However, its biochemical mechanism of suppressing the met static potential of cancer cells is still unknown. The previous hypothesis that a histidine protein kinase activity may contributes to the motility-suppressive effect of Nm23-H1 could not explain why the H118F mutant, a kinase-deficient mutant, still had motility-suppressive ability.

Methods: We conducted a study on the double mutant P96S/S120G of Nm223-H1 and succeeded in introducing the RP-HPLC method in NDPK assay.

Results: The results showed that double mutant P96S/S120G, when expressed in the bacteria, was completely aggregated in inclusion bodies; prompted that the deficiency of motility-suppressive fuction of S120G, P96S, and P96S/S120G mutants was due to their altered structure, which might deprive Nm23-H1 of most activities including kinase activity or interactions with other proteins.

Conclusions: Double mutant P96S/S120G of Nm23-H1 abrogates its NDPK activity and motility-suppressive ability in the human high-metastatic large cell lung cancer cell line L9981.

*This work was partly supported by the grants from the Key Project of National Natural Science Foundation of China (to Qinghua Zhou)(No. 30430300), National Natural Science Foundation of China (to Qinghua Zhou) (No. 30070333), and the Research Found for the Doctoral Program of Higher Education of China (to Qinghua Zhou) (No. 20040610060) .

P2-036 BSTB: Cancer Genetics Posters, Tue, Sept 4

Novel mechanism of collagen-tumor cell interaction by integrin alpha-11 expression by cancer associated fibroblasts in non-small cell lung cancer cells

Zhu, ChangQi[1,3] Popova, Svetlana[2] Brown, Ewan R.[1,4,6] Barsyte-Lovejoy, Dalia[4] Navab, Roya[1,4] Shih, Warren[1,4] Li, Ming[1,4] Penn, Linda[1,4] Gullberg, Donald[2] Tsao, Ming-Sound[1,4,5]

[1] Ontario Cancer Institute, Toronto, ON, Canada [2] Department of Biomedicine, Division of Physiology, University of Bergen, Bergen, Norway [3] University of Toronto, Toronto, ON, Canada [4] Princess Margaret Hospital, University Health Network, Toronto, ON, Canada [5] Medical Biophysics; Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON, Canada [6] Sir Alastair Currie Cancer Research, UK

Background: The integrin α11 subunit is commonly overexpressed in non-small cell lung carcinoma (NSCLC). α11β1 integrin is a one of the four receptors for interstitial collagen. Immunofluorescence study localized the protein mainly to the tumor stroma. α11 is also commonly overexpressed in cancer-associated fibroblasts. We hypothesized that stromal expression of α11 may play important role in regulating the tumor formation of NSCLC cells.

Methods: SV40 immortalized mouse embryonic fibroblasts established from the wild type (WT) and α11 deficient (KO) mice were tested for their tumorigenicity in immune deficient mice when co-implanted with the A549 human lung adenocarcinoma cells. Fibroblasts and A549 alone served as controls. Total cellular RNA was isolated from tumors formed and profiled using the Affymetrix U133A microarray, and differentially expressed genes were identified. Stable gene expression downregulation was accomplished by retroviral mediated transduction of short hairpin (sh) RNA.

Results: A549 co-implanted with the fibroblasts showed markedly increased tumor growth rate as compared with all control cell lines alone, which formed only small tumors. Importantly, the growth was significantly greater for A549+WT compared to A549+KO tumors. The reduced tumorigenicity was also rescued by re-expression of human α11 in KO fibroblasts. The tumor-promoting effect of fibroblast α11 was reproduced in 2 other NSCLC cell lines: NCI-H460 and -H520. Gene expression profiling indicated that IGF2 mRNA expression level was tightly regulated by alpha-11 in the fibroblasts, and A549+KO tumors expressed >200-fold lower IGF2 compared to A549+WT tumors. The shRNA downregulation of IGF2 in WT (WTsh-IGF2) fibroblasts resulted in decreased growth rate of A549+WTsh-IGF2 compared to A549+WT tumors. In the orthotopic NCI-H460 human lung cancer model, host stromal α11 expression was significantly higher in metastatic compared to the primary tumors.

Conclusion: Integrin α11 expressed on tumor stromal fibroblasts provide a novel and alternate mechanism for collagen to modulate the growth of NSCLC cells, and IGF2 is one of the mediators for such activity.

BSTB: Molecular Diagnostics and Staging

P1-001 BSTB: Molecular Diagnostics and Staging Posters, Mon, Sept 3

Reverse transcriptase polymerase chain reaction assay designed for the detection of LUNX-mRNA to compare the micrometastasis during chemotherapy in patients with non-small cell lung cancer

Huang, Yisheng; Wu, Yilong; Huang, Yujuan; Yang, Jinji; Liao, Riqiang

Guangdong Provincial People's Hospital, Guangzhou, China

Objective: To compare the micrometastasis during chemotherapy in patients with non-small cell lung cancer.

Material and Methods: To detect the expression of the lung tissue specific gene named LUNX with reverse transcriptase chain reaction method (RT-PCR) in peripheral blood of patients with non-small cell lung cancer (NSCLC), the micrometastasis of non-small cell lung cancer was diagnosed.

Result: Total 20 patients was involved in the study, the peripheral blood was sampled before and after two cycles chemotherapy. There were 7 patients with NSCLC showed LUNX gene expression in blood before chemotherapy by RT-PCR.After two cycles of chemotherapy, there were 2 patients who was positive in blood detection. The positive detection rate of in peripheral blood in the group of before chemother-

apy was evidently higher than the group of after two cycle chemotherapy. Nevertheless,there was no statistically significant difference between two groups(P=0.063).

Conclusion: Effective chemotherapy may decrease the chance of micrometastasis.But it need to further proved.

P1-002 **BSTB: Molecular Diagnostics and Staging Posters, Mon, Sept 3**

Expression of ERCC1 in normal and tumor tissues in non-small cell lung cancer

Jung, Maan-Hong[1] Park, Ki-Ryong[1] Oak, Chul-Ho[1] Jang, Tae-Won[1] Chang, Hee-Kyung[2] Park, Sung-Dal[3]

[1] Internal Medicine, Gospel Hospital, Kosin University, Busan, Korea
[2] Dept. of Pathology, Gospel Hospital, Kosin University, Busan, Korea
[3] Dept. of Thoracic Surgery, Gospel Hospital, Kosin University, Busan, Korea

Background: Cisplatin is the most widely used agent in the chemotherapy of non-small cell lung cancer. In tumor cells, excision repair cross complement 1 (ERCC1) blocks the effect of cisplatin by repairing the cisplatin-DNA adduct, so the expression of ERCC1 in the tumor cells could be a predictor of response to chemotherapy. But the significance of the expression in the normal lung tissue is not well known. In this study, the levels of ERCC1 expression in the tumor tissue and normal tissue were compared.

Materials and Methods: The level of ERCC1 was measured in the tumor tissue from the surgical specimens of 28 patients with non-small cell lung cancer, using the real-time RT-PCR. In 13 patients, the expression of ERCC1 was measured in both the normal and the tumor tissues simultaneously. The ERCC1 levels of normal tissues and tumor tissues were also analysed according to the patients' characteristics. The level was expressed as a percentage value compared to that of the A549 lung cancer cell line.

Result: The mean level of ERCC1 expressions in 28 tumor tissues was significantly higher than that in the normal tissues (192.9 % (0 - 1460.1 %) vs. 8.2 % (0 to 28.2 %)). In the 13 cases, in which ERCC1 was measured simultaneously, ERCC1 was more increased in the tumor tissues except in two cases, but the differences were not significant(p=0.233). When the upper limit of ERCC1 expression in the normal tissues (30 %) was used as the cut-off level, 13 cases (46 %) expressed more than 30 %. The differences of ERCC1 expression in the tumor tissues by the age, sex, smoking, CEA level, pathologic stages, T stages, N stages and cell types were not significant. But some tendency of increased expression of ERCC1 was observed in the groups with higher T stages and adenocarcinoma histology.

Conclusion: The expression of ERCC1 was higher in the tumor tissue than the normal tissue. However, the difference was insignificant. In 46 % of the tumor tissues, ERCC1 was expressed above the highest level of the normal tissues.

P1-003 **BSTB: Molecular Diagnostics and Staging Posters, Mon, Sept 3**

Prediction model for lung cancer with multiple serum tumor markers

Kaneda, Hiroyuki Saito, Yukihito Maniwa, Tomohiro Minami, Ken-Ichiro Imamura, Hiroji

Kansai Medical University Hirakata Hospital, Hirakatashi, Japan

Background: Tumor markers have not been generally recommended as a tool for the detection of lung cancer, because of the low specificity and sensitivity. Several reports have suggested the use of a combination of tumor markers in the follow-up of lung cancer patients, although the most useful combination remains subject to discussion. Here, using stepwise logistic regression analysis, the most useful combination with multiple serum tumor markers was examined for a prediction model in non-small cell lung cancer diagnosis.

Methods: We retrospectively reviewed our medical records of patients who had pre-operative measurements for seven serum tumor markers; carcinoembryonic antigen (CEA), carbohydrate antigen 19-9 (CA19-9), cytokeratin 19-fragments (CYFRA 21-1), sialyl Lewis X-i antigen (SLX), Squamous cell carcinoma antigen (SCC), neuron specific enolase (NSE), pro-gastrin-releasing peptide (ProGRP). We conducted 90 patients with non-small cell lung cancers and 10 with non-cancers who performed surgery for diagnosis or treatment in our department at Kansai Medical University Hospital from March 2004 to April 2005. Upper cutoff levels routinely used in our institution for CEA, CA19-9, CYFRA 21-1, SLX, SCC, NSE, ProGRP were 5 ng/ml, 37 ng/ml, and 3.5 ng/ml, 38 ng/ml, 1.5 ng/ml, 10 ng/ml, 46 ng/ml, respectively. The prediction power for the diagnosis of lung cancer was evaluated by univariate logistic regression analysis to calculate odds ratios, 95 percent confidence intervals and area under receiver-operating characteristics (ROC) curves, using JMP version 5.1. Stepwise analysis was then used to select and identify the most important independent predictors of lung cancer. To strengthen the prediction model with the serum tumor markers selected by the stepwise analysis, we additionally collected data for 50 patients with lung cancers and 8 patients who were suspected to have lung cancers, but then appeared to have non-cancer nodules by pathological findings with surgical biopsies. The selected serum tumor markers, with totally 140 patients with lung cancers and 18 patients with non-cancer nodules, revised the prediction model for lung cancer diagnosis.

Results: Two of seven tumor markers; CEA, CYFRA 21-1, had statistical significance in univariate analysis for prediction of lung cancer diagnosis (P-value; 0.0068 and 0.0012, respectively). The odds ratios were both significantly high. Area under ROC curve were 0.79 and 0.87, respectively. A stepwise logistic regression for lung cancer diagnosis using the seven serum tumor markers demonstrated that a model containing CEA and CYFRA 21-1 was the most predictive (P<0.0001, area under ROC curve; 0.91). Revised prediction model using CEA and CYFRA 21-1 with total 158 cases including additional 50 lung cancers and 8 non-cancer nodules showed statistical significance (area under ROC curve; 0.85). Using the revised model with CEA and CYFRA 21-1, a cut-off value for lung cancer diagnosis showed that sensitivity was 0.7 and specificity was 0.94, although 47 % of CEA and 74 % of CYFRA 21-1 were negative.

Conclusions: Prediction model with multiple serum tumor markers is potentially useful for lung cancer diagnosis. CEA and CYFRA 21-1 were the candidates to develop the prediction model in non-small cell lung cancer.