Results: The resistance indexes of A549/Gem and H460/Gem are stably about 120 respectively which had exceeded 160 before had acquired steady gemcitabine resistance in the process of inducement. Expression of CDA, RRM1, PTEN and ERCC1 varies according to the changing trend of resistance indexes of gemcitabine, but expression of dCK does not change apparently. Since wild type promoter can amplify the frequency of genome in different derivational stages of A549/Gem and H460/Gem, but allelotype not, the gene type of A549/Gem, H460/Gem and their parental cells are still wild type.

Conclusion: Compared with their parental cells, expression of CDA, RRM1, PTEN and ERCC1 in human gemcitabine-resistant non-small cell lung cancer cell lines A549/Gem and H460/Gem rise, and dCK change is not obvious; the gene type of them are all wild type.

P2-135 **BSTB: Tumor and Cell Biology Posters, Tue, Sept 4**

Establishments of gemcitabine-resistant cell lines A549/Gem and NCI-H460 and studies about their biological characters

Wang, Weixia[1] Liu, Xiaoqing[1] Liu, Guangxian[2] Zheng, Xiaoling[2] Zhu, Yunfeng[2] Li, Xiaobing[2]

[1] Lung Cancer Department in Tumor Center of PLA, Affiliated 307 Hospital, Academy of Military Medical Sciences, Beijing, China [2] Affiliated 307 Hospital, Academy of Military Medical Sciences, Beijing, China

Background: To establish human gemcitabine-resistant non-small cell lung cancer cell lines and discuss their biological characters so as to elaborate the possible mechanisms of gemcitabine resistance.

Methods: Two human Gemcitabine-Resistant non-small cell lung cancer cell lines A549/Gem and H460/Gem were established by repeated clinical serous peak concentration then low but gradually increasing concentration of gemcitabine and 2/3 clinical serous peak concentration gemcitabine intermittent selection from their parental cells human lung adenocarcinoma cell line A549 and human large cell lung cancinoma cell line NCI-H460 who are sensitive to gemcitabine respectively. During the course of inducement, we had monitored their morphology, checked their resistance indexes and resistant pedigree by MTT method, gathered their growth curves and calculated their doubling time, examined their DNA contents and cell cycles by FCM; at the same time, we had measured their expression of P53, EGFR, c-erb-B-2, PTEN, PCNA, c-myc, VEGF, MDR-1, Bcl-2, nm23, MMP-9, TIMP-1 and CD44v6 Proteins.

Results: The resistance indexes of A549/Gem and H460/Gem to gemcitabine are 1.644 and 129.783, respectively, and the cell lines also exhibits respectively cross-resistance to vinorelbine, docetaxel, etoposide, cisplatin and taxol, etoposide, cisplatin. Compared with their parental cells, A549/Gem and H460/Gem are mixed with giant cells of different sizes that are larger and more irregular. The doubling time of A549/Gem is shorter and figures in G0-G1 phase are increased. Meanwhile, H460/Gem have developed contrary changes. Furthermore, they produced different results in different checkpoints. The farther studies indicated that compared with A549, PTEN expression of A549/Gem had been (-) and then rises, MMP-9 expression rises, EGFR TIMP-1 and c-myc (+), P53 c-erb-B-2 and bcl-2 drops, nm23 rises and then (-), and PCNA, MDR-1 (-). Compared with NCI-H460, H460/Gem had exhibited TIMP-9 (+) and P53 CD44v6 (-), then c-erb-B-2 (+), increased expression of nm23 bcl-2 MDR-1 and decreased expression of MMP-9 VEGF P53.

Conclusion: The human gemcitabine-resistant non-small cell lung cancer cell lines A549/Gem and H46/Gem have achieved multi-drug resistance and great changes of biological characters compared with their parental cells. And these changes possibly participate in the formation of multidrug resistance.

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Discrepancy of lung cancer cell growth in bone microenvironments

Zhang, Jie[1] Liao, Mei-Lin[2] Zhao, Yi[2] Chen, Hai-Quan[1] Xiang, Jia-Qing[1] Tan, Qiang[2] Lu, Shun[3]

[1] Shanghai Cancer Hospital, Fudan University, Shanghai, China [2] Shanghai Chest Hospital, Shanghai Jiaotong University, Shanghai, China [3] Shanghai Jiaotong University, Shanghai, China

Background: Disseminated cancer cells may initially require local nutrients and growth factors to thrive and survive in bone marrow before developing into overt metastasis. Bone seems a milieu particularly favorable for the growth, vascularization and survival of lung cancer. In order to explore the mechanism how bone stromal factors contribute to the bone tropism, we therefore use serum of bone marrow aspiration (BM) and condition medium from bone stromal cells(BSC) to study their effects on lung cancer cells.

Materials and Methods: Lung cancer cell lines, A549, H460, gastric cancer cell line SGC-7901 were obtained originally from ATCC. SPC-A1 lung cancer cell line, tong cancer cell line T8113 and primary bone stromal cells were established in our lab before, and reported in many publications. These cells were cultured in pheno- red -free DMEM / 10% fetal calf serum. Regular medium(RM), condition medium from fibroblast cell(FC) were chosen as controls. MTT assay was applied to analyze these different treatments on proliferation of lung cancer cells and controls. Flow cytometry analysis of DNA content was performed to assess the cell cycle phase distribution using propidium iodide fluorescence. Adhesion assay was used to compare cells adhesion on the plates coated with different medium.

Results: (1) In our cancer center, disseminated cancer cells were found in 35% lung cancer patients, 41.7% breast cancer patients, and 39.6% gastric cancer patients. Bone metastasis was often in breast/lung cancer patients, but very rare in gastrointestinal cancer patients. (2) Both supernatants from BM and BSC significantly increased the proliferation of H460 lung cancer cells, which had widespread skeletal metastatic potential Yang M, Cancer Res. 1998). This activity was not shown in A549 and SPC-A1 lung caner cells, which had low bone metastasis potential according to literal reports and our animal study. BM did not improve the proliferation of tong cancer cells T8113, which had very low bone metastasis potential, and even inhibited the growth of SGC7901 gastric cancer cells. (3) Bone- stromal- cell- induced proliferation in H460 cells was blocked by Zometa (biphosphanate), but not by PI3-K inhibitor Wartmannin in time-course and doze dependent studies. (4)BM and BSC also induced cell cycle progression in H460 cells, but not A549 and SPC-A1 cells shown by flow cytometry. (5) Some factors literally related to bone metastasis were compared. ELISA showed the levels of TGF-b, HGF, IGF-1, IL-6 and PDGF were identical in BM, BSC, RM and FC. (6) Similar numbers of H460 cells adhered to BM, BSC and RM coated plates. There were also no significant difference for SPC-A1 and A549 cells in adhesion capability to different medium.

Conclusion: Although a supporting role of bone stromal factors on metastatic breast or prostate cancer cells have been repeatedly proposed, it is largely unknown the mechanism of bone tropism for lung cancer. In our study, we found bone stromal factors favored the growth of cancer cells with bone tropism by increasing proliferation, cell cycle

progression, but not adhesion capability. Bone stromal cells should secret some growth factors although not identified by ELISA, which promoted the proliferation of high bone metastasis potential cells. The high through-out assay, 2D gel followed by mass spectromics, is currently ongoing to recognize the effective factors.

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Rosiglitazone prevents the progression of preinvasive lung cancer in a murine model

Lyon, Christopher M.[1] Belinsky, Steven A.[2] Klinge, Donna[2] March, Tom[2] Keith, Robert[3] Stidley, Chris[4]

[1] Lovelace Respiratory Research Institute, Albuquerque, NM, USA [2] LRRI, Albuquerque, NM, USA [3] University of Colorado, Denver, CO, USA [4] University of New Mexico, Albuquerque, NM, USA

There is a clear need to develop effective chemoprevention strategies to reduce the high incidence of lung cancer. The purpose of this study was to evaluate the effectiveness of two targeted approaches, reversal of gene silencing through demethylation therapy and the induction of apoptosis through activation of the PPAR-γ pathway. A chemoprevention study was designed in which A/J mice, which are highly susceptible to lung cancer, were treated with the tobacco carcinogen NNK. Animals were held for 42 weeks to allow the development of pre-invasive lesions: alveolar hyperplasias and adenomas. Then were separated into 12 groups of 15 mice and treated for 6 weeks with individual or a combination of the agents [hydralazine (Hyd), selenium (Se), sodium phenylbutyrate (PheB), valproic acid (VPA), iloprost (Ilo), and rosiglitazone (Ros)]. Following sacrifice, the number of lesions was determined for each animal by evaluating H&E stained sections that included all lung lobes. Lesions were classified as hyperplasia, adenoma, or carcinoma. The most dramatic effects on tumor progression were seen in the groups in which Ros was administered. The number of hyperplasias increased by 52%, while the number of adenomas was decreased by 31% ($p < 0.05$) with Ros treatment alone compared to sham. The combination of Ros with Se, Hyd, and PheB resulted in a decrease in adenomas by 38%. The area and volume of hyperplasias and adenomas from sham and treatment groups were then quantified. Volume of the hyperplasias and adenomas decreased by 42% and 67%, respectively ($p < 0.005$) in the combination therapy group. Additionally, a companion study using nude mice was also under-taken to evaluate the effects of Ros on tumor growth rates. In this study 72 nude mice were divided into 4 groups of 18 mice and inoculated with one of 4 cancer cell lines (IO33, CL25, H23, and H125). Within these groups the mice were divided into 6 sub-groups of 3 mice each according to treatment plans (Sham, Se, Ros, PheB, Ros+PheB, and Ros+PheB+Hyd). Tumor growth, in terms of total area of tumors, for each sub-group was charted over a 4 week period after which the mice were sacrificed. Preliminary statistical studies have already shown high degree of correlation with our previous work. Of note is that within the H125 cell line; the combination of Ros+PheB retards the progression of tumor growth compared to sham with a difference in average total area of -286 mm^2 ($p< 0.02$; CI: -513.3 to -58.7 mm^2). These studies provide a strong indication that Ros treatment alone and in combination with PheB can dramatically affect lung tumor development by blocking the progression of hyperplasias to adenomas and through affecting the clonal expansion of these lesions. These findings could ultimately lead to a new chemoprevention strategy for lung cancer. (Supported by a fellowship from IASLC).

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Disruption of a conserved ion pair E884-R958 in EGFR differentially alters kinase inhibitor sensitivity and downstream signaling

Tang, Zhe[1] Jiang, Shan[1] Du, Runlei[1] Dietrich, Sascha[1] Petri, Edward T.[2] Boggon, Titus J.[2] Halmos, Balazs[3] Kern, Jeffrey A.[4] Ma, Patrick C.[3]

[1] Case Western Reserve Unviersity, Cleveland, OH, USA [2] Department of Pharmacology, Yale University School of Medicine, New Haven, CT, USA [3] Division of Hematology/Oncology, Univeristy Hospitals of Case Medical Center, Case Comprehensive Cancer Center, Case Western Reserve University, Cleveland, OH, USA [4] Division of Pulmonary, Critical Care and Sleep Medicine, University Hospitals of Case Medical Center, Case Western Reserve Unviersity, Cleveland, OH, USA

Background: Development of molecularly targeted kinase inhibitors against epidermal growth factor receptor (EGFR) represents a major therapeutic advance in treatment of non-small cell lung cancer (NSCLC). Kinase domain mutations of the EGFR gene, most commonly L858R (exon 21) and short in-frame deletions in exon 19, have been shown to confer general sensitivity towards the EGFR kinase inhibitors gefitinib and erlotinib. Other less frequently identified mutations are much less well characterized. We recently described a unique somatic mutation, E884K (exon 22), that occurred in combination with the L858R mutation (L858R+E884K) in a patient with erlotinib-refractory stage IV NSCLC. This patient's leptomeningeal metastases had a striking response to gefitinib monotherapy. We hypothesized that EGFR kinase mutations can work in concert to differentially alter kinase inhibitor sensitivity and downstream signaling.

Method: We generated transfects of Cos-7 cells expressing the following mutant EGFR constructs for signaling and kinase inhibition studies: (1) wild type, (2) L858R, (3) E884K, (4) L858R+E884K and (5) L858R+R958D. Standard phospho-immunoblotting with phosphospecific antibodies was used in to study mutant EGFR signaling in vitro. EGFR kinase inhibition studies were performed with the EGFR variants using erlotinib, gefitinib and dual EGFR/ERBB2 small molecule inhibitors. Molecular modeling and bioinformatics sequence analysis was performed to understand the structural mechanism of the effect of E884K-EGFR.

Results: We show that E884K worked in concert with L858R and altered sensitivity to kinase inhibitors and mutant receptor signaling. Mutation of E884 to lysine disrupts an ion pair with residue R958 in the EGFR kinase domain C-lobe, an interaction that is highly conserved within the human kinome, many of which are "druggable targets", demonstrated by our sequence analysis and structural modeling. When in cis with L858R, the E884K-EGFR mutation desensitized, in a dominant fashion, the mutant receptor to erlotinib, while further sensitizing the receptor to gefitinib and an EGFR/ERBB2 dual inhibitor with corresponding inhibition of p-EGFR and downstream signaling inhibition of p-AKT and p-STAT3. Inhibitor sensitivity modulation similar to that seen with E884K was observed with the R958D mutation that should also disrupt the conserved ion pair. Both alone and in cis, the E884K mutation was activating with specific cellular phosphoproteome activation pattern and downstream signaling regulation distinct from that of L858R alone.

Conclusion: E884K-EGFR mutation worked in concert with L858R in cis to differentially alter sensitivity to specific kinase inhibitors and selective downstream phosphoproteomic signaling activation. Our findings suggest that mutations of the conserved ion pair E884-R958 may result in conformational changes that alter kinase substrate recognition.

