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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.

P2-137 BSTB: Tumor and Cell Biology Posters, Tue, Sept 4

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

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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

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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.

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Improved efficacy of targeted kinase inhibitors may be achieved by targeting the dominant activating mutations present.

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Targeted inhibition of wild type and mutated MET receptor variants in the sema, juxtamembrane and kinase domain

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Background: MET belongs to the semaphorin superfamily of signaling proteins, containing three protein families (semaphorins, plexins, MET and RON) that have central roles in cell signaling. The MET receptor tyrosine kinase is involved in regulating cell growth/proliferation, survival, angiogenesis, cell scattering, cell motility and migration. Mutations in MET have been identified in various human cancers including lung cancer and papillary renal cell carcinomas. MET mutations occur within the extracellular seven-blade β-propeller fold sema domain (E168D, L229F, S325G, N375S), the juxtamembrane domain (R988C, T1010I), and the kinase domain (M1268T). We hypothesized that these mutations would have differential effects on the kinase inhibition.

Methods: We modeled the various MET mutations from different functional domains of the receptor using Cos-7 transfection cell system to determine their effect on MET signaling and sensitivity to a selective MET kinase inhibitor SU11274. Sensitivity to SU11274 inhibition was assayed by phospho-immunoblotting using phospho-specific antibody against the major tyrosine kinase phosphorylation epitopes pY1234/1235 of the MET kinase in vitro.

Results: First, we identified that mutations in the sema and juxtamembrane domain were activating as defined by ligand-independent constitutive receptor activation. SU11274 was capable of inhibiting ligand induced signaling through the wild-type MET as well as mutant MET receptors harboring mutations in the sema, juxtamembrane and tyrosine kinase domain. However, SU11274 inhibition of mutant MET was mutation-dependent, with the juxtamembrane domain mutations R988C and T1010I resulting in a receptor form that was less sensitive to SU11274. Mutations in the sema and kinase domain also resulted in varying sensitivity to inhibition by SU11274 inhibition. Interestingly, the kinase domain L1243R mutation in MET (homologous to the epidermal growth factor receptor L858R-EGFR sensitizing mutation in lung cancer) substantially sensitized the mutant MET receptor to SU11274 inhibition with complete p-MET inhibition at 0.5 µM compared to 5 µM as in wild type. On the other hand, the activating M1268T mutation in the kinase domain was less sensitive to SU11274 than the wild type receptor.

Conclusion: Mutations in the sema and juxtamembrane domain of MET result in receptor activation. The small molecule inhibitor SU11274 is active against wild type and various mutated MET receptor. Further studies to characterize the signaling effects and the mechanism of sensitivity and resistance of MET mutations to specific inhibitors are crucial in the successful development of therapeutic MET inhibitors in personalized cancer therapy.

P2-140 BSTB: Tumor and Cell Biology Posters, Tue, Sept 4

GSTT1 polymorphism and cytogenetic changes in peripheral blood and tissues of patients suffering from lung cancer

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Introduction: One of the basic exogenous factors of lung cancer incidence is smoking. On the other hand, hereditary predisposition in particular, genetic polymorphism of enzymes, plays an important part in development of oncologic diseases. The important role in oncopathology emergence is assigned to glutathiontransferases (GST), because these enzymes are involved in detoxication of reactive metabolites. Mutations in detoxication genes are also known to be accompanied by the change in the cytogenetic parameter level, which are attributed to cytogenetic markers of early carcinogenesis stages.

The interrelation between GSTT1 polymorphism and the chromosome aberrations level was analyzed in peripheral blood lymphocytes (PBL) and lung tissues of non-small-celled lung cancer (NSCLC) patients.

Materials and Methods: The frequencies of GSTT1 gene polymorphic alleles were investigated using a polymerase chain reaction in 49 NSCLC patients.

For cytogenetic research the interphase analysis of somatic cells without cultivation was used. The frequency of cells with micronuclei was estimated in PBL and in lung cells from nontumour tissues of lung cancer patients.

Results and Discussion: Analysis of GSTT1-gene polymorphism has shown that GSTT1(-) genotype occurred significantly more often in NSCLC patients (26,6%) than in the control (4%). The study on genetic GSTT1 polymorphism with due account of the smoking factor has demonstrated that the GSTT1(-) genotype was observed with statistical significance 1,5-fold more often (36,4%) in the group of non-smoking patients than among smoking ones - 23,7%. It is interesting that such regularity was revealed by other researchers for many others genes of xenobiotic biotransformation enzymes.

The obtained data of the cytogenetic analysis in different tissues of oncologic patients were estimated by us with due account of the smoking factor and the genotype. In non-smoking patients, the level of cells with micronuclei in PBL was twice as high in the patients with the GSTT1(+) genotype (0,43±0,13%) as in the patients with the GSTT1(-) genotype (0,21±0,07%). In smoking patients the level of cells with micronuclei in PBL did not differ significantly in carriers of the GSTT1(+) genotype (0,32±0,05%) and the GSTT1(-) genotype (0,29±0,09%). Some authors associate the low number cells with chromosome aberrations in smoking patients and GSTT1 (-) carriers with higher enzyme expression, involved in repair of DNA damages.

Statistically significant excess of the group average level of cells with micronuclei was shown in nontumoral tissue of smoking patients with the GSTT1(-) (0,57±0,08%) over those with the GSTT1(+) (0,33±0,04%). In non-smoking patients, the level of cells with micronuclei did not differ in GSTT1(-) - 0,30±0,02% and GSTT1(+)- 0,38±0,14%. Damaging effect of tobacco smoke carcinogens directly on lung tissue in smokers is one of explanations of this fact that in combination with availability of mutant gene alleles of detoxication enzymes results in high probability of emerging cytogenetic injuries just in lung tissue.

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