ERBB3 interacts with EGFR and is expressed in EGFR TKI sensitive NSCLC cell lines and may represent a poor prognostic marker in NSCLC. In this study we correlated the activity of the HDAC inhibitor vorinostat in NSCLC cell lines with ERBB3 expression and evaluated the synergistic effect of vorinostat with gefitinib. Further, we evaluated the interaction of ERBB3 and E-cadherin and their effect on response to gefitinib.

Methods: Apoptosis: cell lines were incubated in control medium or in the presence of various vorinostat and/or gefitinib concentrations and cell death and apoptosis were assessed using the Annexin V assay. Samples were assessed in the presence or absence of gefitinib and/or vorinostat. Gene expression analysis was done using real-time RT-PCR and microarray gene expression profiling. Protein expression and coimmunoprecipitation was evaluated with western blot.

Results: We performed detailed analysis of the apoptotic activity of vorinostat in a panel of 21 NSCLC cell lines. The normalized apoptotic to life ratio was > 2.16 in 12 cell lines (sensitive) and < 2.16 in 11 cell lines (resistant). Using microarray analysis we found that ERBB3 expression was significantly higher in vorinostat sensitive compared to its expression in resistant cell lines (p<0.005). Vorinostat also induced erbB3 expression in gefitinib resistant cell lines. When gefitinib resistant cell lines (IC50>10uM) were treated with vorinostat and gefitinib, synergistic effects were detected in 4 of the 5 cell lines tested. Co-transfection of ERBB3 and E-cadherin in a gefitinib resistant cell line, H157, showed enhanced apoptotic response to gefitinib similar to what is detected in an EGFR mutant cell line H3255.

Conclusion: ERBB3 may predict response to EGFR and HDAC inhibitors in NSCLC. For tumors with low ERBB3 and E-cadherin expression the combination increases expression of ERBB3 and E-cadherin and produce more than additive antitumor effects through increased apoptosis.

PD2-3-7 Molecular Targets and Prognostic Factors, Tue, 16:00 - 17:30

The antitumor effect caused by small molecular agents targeting EGFR, VEGFR2 and their downstream kinases in human non small cell lung cancer cells

Nakachi, Ichiro1 Naoki, Katsuhiko2 Soejima, Kenzo1 Kawada, Ichiro1 Watanabe, Hideo1 Yasuda, Hiroyuki1 Iishizaka, Akitsuh1

1 School of Medicine, Keio University, Pulmonary Department, Shinjuku, Tokyo, Japan 2 Yokohama Municipal Citizen’s Hospital, Yokohama, Japan

Background: Epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKI) such as gefitinib and erlotinib show anti-tumor activity in a subset of non-small cell lung cancer (NSCLC) patients having mutations of EGFR gene. On the other hand, clinical resistance to EGFR-TKI is commonly observed in spite of the initial response. Recent works show such resistance can be caused by a secondary mutation, leading to a T790M amino acid change in the EGFR tyrosine kinase domain. Several studies suggested the importance of VEGFR (Vascular endothelial growth factor receptor) and the downstream kinases of EGFR as potential drug targets in solid tumors.

Purpose: The aim of this study is to evaluate the efficacy of alternative small molecules, such as AEE788, RAD001 and U0126, that inhibit other targets than EGFR in NSCLC cell lines with and without T790M mutation. AEE788 is a triple TKI for EGFR, HER2 and VEGFR2, while RAD001 is an inhibitor of mammalian target of rapamycin (mTOR), which is a serine/threonine kinase located downstream of PI3K-Akt pathway, and thought to have persistent activity in EGFR-mutated cells. U0126 is an inhibitor of MEK, which is located downstream of Ras-Raf pathway.

Methods: We used three human NSCLC cell lines, namely, A549, H1650 and H1975. A549 has wild type EGFR, H1650 harbors a deletion mutation in exon 19 which accounts for a sensitive mutation to erlotinib, while H1975 possesses double mutations at L858R and T790M, which account for sensitiveness and resistance to erlotinib, respectively. We first treated these cells with erlotinib, AEE788, RAD001 and U0126 as a single agent, then tried combination with either of two agents and evaluated the effect on cell growth as well as the phosphorylation of receptors and downstream kinases.

Results: AEE788 alone inhibited the cell growth more effectively than erlotinib alone in erlotinib-resistant cell line H1975. However it failed to inhibit the autophosphorylation of EGFR. While RAD001 alone inhibited cell growth in all these three cell lines with effective blocking of phosphorylation of p70S6K, which is located downstream of mTOR. The combination of RAD001 and AEE788 resulted in more striking growth inhibition in H1975. This combination may work through inhibiting other target of AEE788, such as VEGFR, together with blocking PI3K pathway by RAD001.

Conclusions: EGFR downstream kinases and VEGFR will be possible molecular targets and double blocking of these molecules may overcome the acquired erlotinib resistance in NSCLC.

PD2-3-8 Molecular Targets and Prognostic Factors, Tue, 16:00 - 17:30

-2518 MCP-1 and Interleukin-4 VNTR polymorphisms and NSCLC risk

Coelho, Ana P.; Araújo, António; Calçada, Cármen; Nogal, Ana; Cardoso, Diana; Faria, Ana; Azevedo, Isabel; Soares, Marta; Catarino, Raquel; Medeiros, Rui

Instituto Português de Oncologia do Porto, Porto, Portugal

Background: Non-small-cell lung cancer (NSCLC) accounts for 80% of all lung cancers and is responsible for more deaths from cancer than any other tumour type in the Western world. Tumor associated macrophages (TAMs) constitute an important interface between tumor cells and the immune system and provide an environment that enhances the survival, proliferation and migration of tumor cells. MCP-1 chemokine is one of the main determinants of the macrophages content in NSCLC and accounts for the majority of the chemotactic activity in these tumors and high levels of MCP-1 correlate with poor prognosis. IL-4 is an interleukin that displays pleiotropic immunomodulatory functions and is a key player in the immune reactions of the lung, involved in monocyte differentiation in TAM and TH2 immune responses activation. -2518 MCP-1 is a biallelic G/A polymorphism in the 5’-flanking region of the MCP-1 gene and individuals with genotypes carrying the G allele produce more MCP-1 than individuals with AA genotype. The aim of our study was to evaluate the genetic influence of this polymorphism as a prognostic/predictive factor in NSCLC progression and the influence of a VNTR polymorphism in intron 3 of IL-4 gene in NSCLC progression, among -2518 MCP-1 G carrier genotypes.

Methods: DNA samples were extracted from peripheral blood cells of 223 consecutive patients with NSCLC. The -2518MCP-1 polymorphism was analyzed through PCR-RFLP (PvuII) and VNTR polymor-