Mechanisms controlling survival and induction of apoptosis following 12-lipoxygenase (LOX) inhibition in non small cell lung cancer (NSCLC)

Campbell, Vikki\textsuperscript{1} Lysaght, Joanne\textsuperscript{1} Gately, Kathy\textsuperscript{1} Kay, Elaine\textsuperscript{3} Reynolds, John\textsuperscript{2} Pidgeon, Graham P.\textsuperscript{1} O’Byrne, Kenneth J.\textsuperscript{2}

\textsuperscript{1} Trinity College, Dublin, Ireland \textsuperscript{2} St. James Hospital, Dublin, Ireland \textsuperscript{3} Royal College of Surgeons Ireland, Dublin, Ireland

Background: Platelet-type 12-Lipoxygenase is an arachidonic acid metabolising enzyme that results in the formation of 12(S)-HETE. 12(S)-HETE is proangiogenic, and has been shown to stimulate tumour cell adhesion, invasion and metastasis. Inhibitors of 12-LOX are currently undergoing extensive investigation, and a detailed examination of the effects of these agents in lung cancer is warranted. In this study we examined the expression profile of 12-LOX in human lung cancer cell lines and resected tissue. We also examined the mechanisms responsible for apoptosis following selective inhibition of 12-LOX with baicalein.

Methods: A549 (adenocarcinoma), SK-MES1 (squamous cell lung carcinoma), H460 and H647 (large cell lung carcinoma) were grown in serum depleted media (0.5%) and screened for 12-LOX expression by RT-PCR and western blot analysis. Cells were treated with baicalein (10\textmu M), a selective inhibitor of 12-LOX, or 12(S)-HETE (100ng/ml) and cell survival / proliferation determined by BrdU assay. Apoptosis was determined using the multi-parameter apoptosis kit and In-cell Assay Kit. DNA analysis was also performed by FACS. Gene alterations following 12-LOX inhibition in both A549 and SKMES-1 cells were assessed by quantitative PCR arrays and validated by RT-PCR. A panel of retrospective resected lung tumours were stained for 12-LOX expression by immunohistochemistry.

Results: All lung cancer cell lines expressed moderate levels of platelet-type 12-LOX, which was reduced following treatment for 24h with increasing concentrations of baicalein. Baicalein decreased lung cancer survival in all cell lines, while 12(S)-HETE increased cellular proliferation. Inhibition of 12-LOX induced apoptosis in a dose dependent manner, with decreased f-actin filaments and loss of mitochondrial membrane potential. Induction of apoptosis was also confirmed by DNA laddering and Annexin-V FACS labelling. QPCR array data implicated a number of genes regulating these effects which were validated by RT-PCR, many of which control apoptosis and angiogenesis. The subset of genes downregulated included bcl-2, VEGF, integrin o2 and o4. 12-LOX expression was observed in a variety of human lung cancers with different histological subtypes. We are currently silencing 12-LOX expression in these cells, using shRNA technology, to further examine these mechanisms.

Conclusions: 12-LOX is a survival factor in NSCLC. 12-LOX inhibition decreased NSCLC survival, inducing apoptosis through mechanisms including downregulation of the bcl family of proteins, integrin receptor and angiogenic growth factors. Expression of 12-LOX in fresh resected and retrospective tissue suggests that inhibition of this enzyme is a potential therapeutic strategy in the treatment of NSCLC.

Epigenetic changes of the tumor suppressor genes SHP1, SHP2, SOCS1, SOCS3 and the transcription factor STAT1 in human lung cancer

Gullibo, Joachim\textsuperscript{1} Bergqvist, Michael\textsuperscript{1} Sooman, Linda\textsuperscript{1} Ericsson, Peter\textsuperscript{1} Lennartsson, Johan\textsuperscript{2} Brattström, Daniel\textsuperscript{2} Bergström, Stefan\textsuperscript{2} Ekman, Simon\textsuperscript{2}

\textsuperscript{1} ORKI, Uppsala, Sweden \textsuperscript{2} Ludwig Institute for Cancer Research, Uppsala, Sweden \textsuperscript{3} Karolinska University Hospital, Stockholm, Sweden

Background: Abberant DNA methylation, including hypermethylation of tumor suppressor genes or hypomethylation of oncogenes, is a hallmark of cancer and can be found in almost all cancer types. Information about which methylation events are disease specific and also have effect on gene expression has a great potential in diagnostics and drug development. The aim of this study was to investigate the methylation status of the tumor suppressor genes SHP1, SHP2, SOCS1 and SOCS3 and the transcription factor STAT1, and its effect on protein expression and tumor biology in lung cancer cell lines. Their activities are required for a functional regulation of cell growth and these genes have previously shown hypermethylation-associated tumor occurrence in several other types of cancers.

Methods: To study methylation patterns, bisulfite treatment of total DNA followed by PCR amplification and Pyrosequencing analysis was employed. The gene regions to be analyzed were determined according to the regions that previously had shown aberrant methylation patterns in other types of cancers and psoriasis. Expression levels of the different proteins were evaluated by immunoprecipitation using antibodies directed against each protein, followed by SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE) and Western blotting.

Five NSCLC cell lines and five SCLC were used in the studies.

Results: The methylation studies showed that the genes SHP1, SOCS1 and SOCS3 are strongly methylated in some of the analyzed lung cancer (LC) cell lines, whereas SHP2 and STAT1 were not significantly methylated in any cell line. The protein expression studies showed that the observed methylation levels in SHP1 were associated with a reduction of protein expression in the LC cell lines. Furthermore, the promoter region 1 (SHP1 A) of SHP1 demonstrated varying degrees of methylation throughout the LC cell lines, whereas promoter region 2 (SHP1 B and C) was highly methylated in all cell lines. The epigenetic regulation of SHP1 has earlier been attributed to promoter region 2. In this study, on the other hand, the reduced protein expression seems to be associated with methylation in promoter region 1. SHP1 has earlier been reported to be methylated in promoter region 2 in normal epithelial cells, but to our knowledge there have been no reports on methylation in promoter region 1. The importance of the reduced SHP1 expression in the regulation of proliferation, migration and invasion are being analyzed in the lung cancer cell lines and further data will be presented at the meeting.

Conclusions: The tumor suppressor genes SHP1, SOCS1 and SOCS3 are highly methylated in lung cancer cell lines, making them potential diagnostic markers for lung cancer. Furthermore, methylation of the SHP1 promoter region 1 is associated with a strongly reduced protein expression.
Experimental Design: We evaluated EphA2 and ephrin A1 using immunohistochemistry in 41 cases (1:14, III:27) with long-term follow up. Additionally the relationship among EphA2 expression, MVD(microvascular density), clinicopathological parameters, and E-cadherin expression was assessed.

Results: EphA2 expression was identified in 28 cases(68.3%). Stage I cases displayed significantly lower levels than stage III cases(p=0.017). Survival curve determined by the Kaplan Meier method demonstrated that strong immunoreactivity for EphA2 was associated with overall survival (p=0.028). The MVD in EphA2 positive group was significantly higher than that in EphA2 negative group (p=0.006). In immunohistochemical study EphA2 expression was related to loss of E-cadherin (p=0.041), but not to p53 on serial sections.

Conclusions: EphA2 expression was associated with tumor progression, angiogenesis and loss of E-cadherin.

P2-044
BSTB: Molecular Targets Posters, Tue, Sept 4

Lapatinib increases cytotoxicity against gefitinib-resistant T790M lung cancer cells by inhibiting active heterodimerization of EGFR and HER-2

Kim, Hwang-Phill1 Kim, Tae-You1 Han, Sae-Won2 Im, Seock-Ah1 Bang, Yung-Ju2
1 Cancer Research Institute, College of Medicine, Seoul National University, Seoul, Korea 2 Cancer Research Institute, Seoul National University College of Medicine, Department of Internal Medicine Seoul National University Hospital, Seoul, Korea

Somatic mutations in epidermal growth factor receptor (EGFR) tyrosine kinase domain predicts the response to EGFR tyrosine kinase inhibitor (TKI) in non-small cell lung cancer (NSCLC). The emergence of acquired resistance to EGFR TKI has been recently described in patients whose tumor initially responded to gefitinib. Lapatinib is a dual inhibitor of ErbB1 (EGFR) and ErbB2 (HER-2) tyrosine kinases that has shown promising efficacy in HER-2 overexpressing breast cancer. However, its therapeutic role is not fully studied in NSCLC. Here we report that lapatinib enhances cytotoxicity against gefitinib-resistant NSCLC by blocking active heterodimerization of EGFR and HER-2. We identified a secondary T790M mutation in a NSCLC tumor with a gefitinib-sensitive L858R mutation that eventually progressed after initial response. Acquired T790M mutation conferred resistance to L858R mutant cells sensitive to gefitinib. Of the various molecules downstream of EGFR, signal transduction and activator of transcription 3 (Stat3) signaling was continuously activated in L858R/T790M cells, and the inhibition of Stat3 suppressed gefitinib-resistant cell growth. As for Stat3 inhibition, we found that lapatinib inactivated Stat3 and inhibited cell growth in gefitinib-resistant NSCLC. Finally, lapatinib was also found to block the heterodimerization of EGFR and HER-2, and this led to the inactivation of Stat3 in gefitinib-resistant T790M cells, whereas the active heterodimerization of EGFR and HER-2 was maintained when these cells were treated with gefitinib. Taken together, our data suggest that lapatinib may overcome gefitinib resistance in NSCLC. A clinical investigation of the use of lapatinib in gefitinib-resistant NSCLC is strongly warranted.