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

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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 H467 (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 (10uM), 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 laddering and Annexin-V FACS analysis. A panel of prospective resected lung tumours were stained for 12-LOX expression by immunohistochemistry.

Results: All lung cancer cells 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 protein expression. The subset of genes downregulated included bcl-2, VEGF, integrin α2 and α4. 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.

Conclusions: CD20 and c-kit expression are restricted to TET WHO AB and C respectively. HER2 nuclear staining pattern is of unknown significance. VEGFR 1-2 data will be presented at the meeting.

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

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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, bisulphite 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.

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 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.