**Poster Abstracts**

**BSTB: Cancer Genetics**

**P2-001**

**BSTB: Cancer Genetics Posters, Tue, Sept 4**

**FHIT Gene Expression in Lung Cancer Cell-lines and Evaluation of Its Effects with Chemotherapeutic Agents on Apoptosis.**

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Genetic alterations are well known phenomena in lung cancer. Application of gene therapy for better survival is a challenge for the future. The fragile histidine triad (FHIT) gene located at the 3p14.2 locus plays an important role in the pathogenesis of lung cancer. We have earlier reported that loss of FHIT expression is common in biopsy tissue of lung cancer patients and methylation of its promoter is one cause of gene activation (Mol Cancer Res, 2006). In the present study, two human lung cancer derived cell-lines, NCI 520 and NCI H460, were screened for the presence of FHIT expression and the effect of transfection of FHIT gene along with chemotherapeutic agents on apoptosis. Loss of heterozygosity was observed at all the three loci using three microsatellite polymorphic markers (D3S1300, D3S1312 and D3S1313) as was used for the tissue sample analysis in our earlier study. In the NCI H460 cell-line, we evaluated and compared the effects of FHIT re-introduction on cell proliferation and apoptosis. Further, the parental and FHIT-reintroduced NCI H460 cell-lines were compared with respect to apoptosis induced by three chemotherapeutic drugs namely, gemcitabine, irinocam and paclitaxel.

RT-PCR analysis demonstrated that both the cell-lines showed complete absence of FHIT transcript. Loss of heterozygosity analysis in both cell-lines revealed LOH with respect to D3S1300 and D3S1313 markers in NCI 520 cell-line whereas the NCI H460 cell-line showed complete deletion of these three loci. The NCI H460 cell-line also showed total absence of FHIT transcript or protein expression. Therefore, it was used as a model for the re-introduction of FHIT cDNA. The FHIT cDNA isolated from normal human cervical tissue was cloned into pCR 3.1 mammalian expression vector. This FHIT cDNA corresponded to the coding region of the FHIT gene. The NCI H460 cells were transfected stably with the vector containing FHIT cDNA. The transfected cell-line expressed FHIT protein at the moderate levels which was confirmed by western blotting. Soft agar colony formation assay showed that the FHIT transfected cell-line showed smaller and fewer number of tumor colonies as compared to the untransfected cell-line reconfirming the tumor suppressor properties of FHIT. Next we looked at apoptosis induction by chemotherapeutic drugs in the FHIT transfected and untransfected cell-lines. Apoptosis was measured by the cell death detection ELISA and the WST-1 cytotoxicity assay. Overall, the effect of gemcitabine in inducing apoptosis was more than irinocam and paclitaxel. Further, the FHIT transfected cell-line showed greater induction of apoptosis as compared to untransfected cell-line with all three drugs tested. The degree of increase in apoptosis induction due to the re-introduction of FHIT was modest. This experimental study implicates a pro-apoptotic function for the FHIT protein which is also corroborated by the tissue analysis earlier (Mol Cancer Res, 2006). Molecular based gene therapy using FHIT may supplement conventional therapy which may result in better survival. However, the observations from the bench top to the bedside remains a challenge and a hope for a better future for patients with lung cancer.

**P2-002**

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**MicroRNAs Potentially Important in Lung Cancer**

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**Background:** MicroRNAs (miRNAs) are a family of small, non-coding RNA species regulating expression of numerous target genes including tumour suppressor genes and oncogenes. Investigation of miRNAs and their regulation of target genes relevant to lung cancer will likely contribute to understanding of tumourigenesis as well as potentially providing a basis from which to design novel therapeutics.

**Methods:** To identify candidate miRNAs of functional significance in lung cancer, 474 miRNA genes listed in miRBase (http://microrna.sanger.ac.uk) v9.0 were mapped to positions in Agilent 44B high resolution arrayCGH data obtained in 132 subjects with primary lung cancer (60 squamous cell carcinomas, 72 adenocarcinomas) treated by resection. The group included 40 women and 10 never smokers, and had a mean age of 65.4 (±9.2) years. Fold change in gene copy number for probes immediately flanking the positions of miRNA genes was computed from the signal log ratios of tumour DNA to normal female DNA in arrayCGH experiments.

**Results:** 97 miRNAs were mapped to regions of abnormal copy number in this data. Applying this approach to other published lung cancer arrayCGH datasets revealed 22 miRNAs in common, and the mature miRNA of one of these (miR-218) had also been reported as significantly underexpressed in lung cancer. miR-218 therefore emerged as a candidate miRNA with possible tumour suppressor function in lung cancer. Reduced expression of mature miR-218 was confirmed by qRT-PCR (Ambion) in a set of 22 lung cancers - mean expression relative to normal lung = 0.31±0.34.

**Conclusions:** Our approach yielded a list of candidate miRNAs that may contribute to lung tumourigenesis and identified a miRNA (miR-218), underexpressed in lung cancer, which could have tumour suppressor function. In a larger number of tumours we now intend to confirm reduced expression of mature miR-218 and check that the mechanism is low gene copy number using an independent method (qRT-PCR), before proceeding to functional transfection studies in model systems. Support: Queensland Cancer Fund, National Health and Medical Research Council, The Prince Charles Hospital Research Foundation Note: Also submitted to the forthcoming 2007 miRNAs and Cancer Keystone Symposium