**Characterisation of Epigenetic Biomarkers in Chronic Lymphocytic Leukemia**

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**INTRODUCTION**

Chronic lymphocytic leukemia (CLL) is a haematological malignancy, which mainly affects the elderly. CLL has a heterogeneous clinical course which cannot be distinguished according to clinical staging systems1,2. However, prognostic biomarkers such as IGHV mutational status, ZAP 70 and CD 38 expression could be used in addition to clinical staging systems providing valuable prognostic information2. In this study we used IGHV mutational status as a prognostic marker relating to favourable prognosis (IGHV mutated) and poor prognosis (IGHV unmutated). DNA methylation is one of the main elements of epigenetic modifications and is a known factor in cancer development, as it can silence genes vital to normal cell function3,4. Differential DNA methylation in CLL, which leads to differential gene expression can play a role in the development of the heterogeneity of the disease5.

**SPECIFIC OBJECTIVES**

1. Validation: Validate the DNA methylation status and gene expression levels of significantly differentially methylated genes, using pyrosequencing and quantitative real-time PCR (qRT-PCR) techniques.
2. Correlation: Perform statistical analysis on the DNA methylation and gene expression data to determine the significance of the results. Perform correlational analysis between DNA methylation and gene expression to establish if a functional relationship exists.
3. Functionality: Treat RAMOS cell line with DNA methyltransferase inhibitor 5-aza-2’-deoxycytidine (DAC) and histone deacetylase inhibitor trichostatin A (TSA) to determine the role of DNA methylation in gene expression.

**RESULTS**

In this study significantly methylated genes, SOX3 and SOX17, selected following genome wide methylation studies were validated for differential methylation and expression between the two prognostic subsets of CLL. The results revealed that differential DNA methylation lead to differential gene expression.

SOX3 DNA methylation and Gene expression results: SOX3 was found to be significantly hypermethylated in the IGHV mutated subgroup (p<0.00001). Interestingly this lead to low gene expression in the favourable prognosis subgroup.

SOX17 DNA methylation and Gene expression results: SOX17 was found to be significantly hypermethylated in the IGHV unmutated subgroup (p<0.00001). Resulting in low gene expression in the poor prognosis subgroup.

**DISCUSSION**

In this study we found specific DNA methylation patterns which can distinguish IGHV mutated CLL patients and IGHV unmutated CLL patients. Interestingly we found that poor prognostic IGHV unmutated patients have low SOX3 methylation resulting in high gene expression. However hypermethylation of SOX17 in the poor prognosis group leads to decreased gene expression.

These results suggest that CLL patients expressing increased levels of SOX3 may suffer from a more aggressive form of the disease. In contrast increased SOX17 expression, due to decreased methylation may relate to good prognosis in CLL.

Correlation analysis of SOX17: SOX17 gene expression significantly correlated with its DNA methylation status (p=0.0137). The Spearman correlation coefficient (r= -0.3465) demonstrates the negative correlation between gene expression and DNA methylation.

**REFERENCES**