The use of methylation profiling to identify genes involved in relapse of adult ALL.

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Introduction

In healthy cells CpG islands that are based at a gene promoter are usually methylation free. In cancerous cells these same regions frequently exhibit hypermethylation, leading to stable gene inactivation.

It is known that genes important in the development of cancer are switched off due to this process and therefore identifying altered DNA methylation patterns can lead to the identification of genes important in cancer development.

This project focussed specifically on genes that might be important in causing relapse in acute lymphoblastic leukaemia (ALL). Based on previous genome wide screening for changes in methylation and gene expression a set of 12 genes had been identified as candidates for a role in the development of relapse (based on their methylation levels in diagnostic and relapse samples and a negative association between methylation and expression). These included the *MSC1* and *TTC12* genes.

Aims

- To investigate the methylation patterns of the genes MSC1 and *TTC12* in paired patient samples at diagnosis and at relapse
- Compare the methylation level in these samples looking for changes in methylation between diagnosis and relapse

Acknowledgements

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Methods

- The conditions for the primers of each gene were found by carrying out PCR at multiple annealing temperatures (58-63°C) and varying $MgCl_2$ levels (1-4mM). The final conditions were 63°C and 2mM for *MSC1* and 58°C and 3.5mM *TTC12*

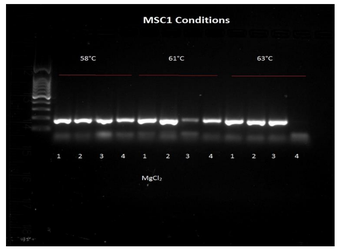
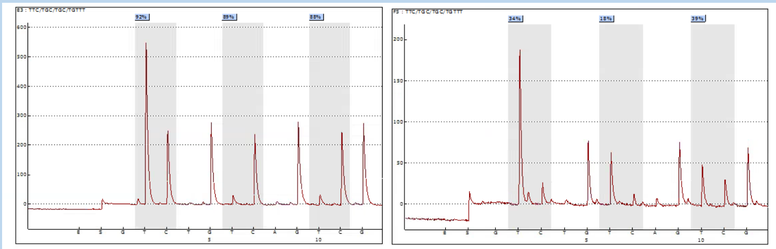


Figure 1 and 2 show electrophoresis gel images of the conditions using gel red staining. The brightest band suggests the most PCR product and therefore shows which conditions are optimum for the primers.

- The patient genomic DNA samples were modified by bisulphite modification which allows subsequent assessment of methylation status by PCR based methods
- PCR was carried out to amplify the regions of interest in the modified DNA Samples
- Pyrosequencing was then carried out to determine the methylation levels at specific CpG sites

Results

- For the *TTC12* locus good quality pyrograms were achieved which allowed determination of methylation levels in most of the sample set
- However, for the *MSC1* locus the pyrograms produced were frequently weak and inconsistent. Further optimisation of conditions were unable to generate consistent results for this locus and so investigation was not continued



Figures 3 and 4 show two pyrograms produced from a patients modified diagnostic and relapse DNA samples. The number in blue is the percentage of methylation at that specific CpG site. The final value for each samples is determined by averaging the values at the 3 CpG sites covered by the assay.

- After comparing the results for *TTC12* there are some samples that show variation however the majority only have a small difference between diagnostic and relapse.

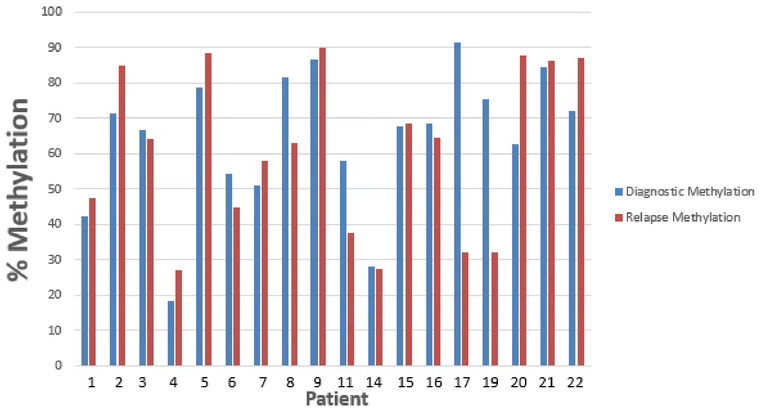


Figure 5 shows a bar chart comparing the levels of methylation between the diagnostic and relapse samples of patients. Although some individual samples do show variation between diagnosis and relapse, overall there is no clear pattern of increased or decreased *TTC12* methylation between diagnostic and relapse samples (p=0.70, paired T-test)

Conclusions

- The *TTC12* loci is frequently methylated in ALL samples at booth diagnosis and relapse. While methylation levels can vary within paired samples there was no apparent association with disease progression