Exploiting Weaknesses in Cancer Genes

- identification of methylation patterns in Leukaemia

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Introduction

DNA Methylation is a mechanism used to turn genes on and off in different cells. In cancer, the pattern of methylation can change which means some genes which are normally switched on can be turned off, and vice versa. Sometimes methylation occurs uncharacteristically at the genes which are normally responsible for regulating cell growth. This can lead to uninhibited growth or proliferation of cells, a key trait of cancer.

However, some of the methylation changes in cancer cells also create weaknesses in these cells that aren't present in normal healthy cells. If Identified, these weaknesses in cancer cells could be exploited to develop new treatments that could specifically kill only the cancer cells, whilst leaving normal healthy cells intact.

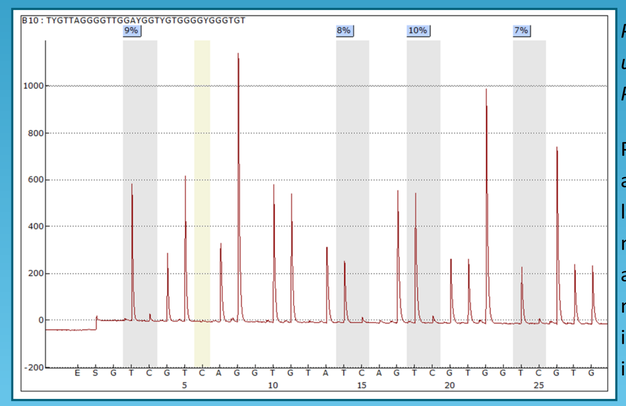
T Cell Acute Lymphoblastic Leukaemia (T ALL) is so rare, that currently, most patients with this type of cancer are grouped together and given the same treatment. However, because there are different genes and different DNA methylation changes involved, patients with T ALL can be further divided into different sub-groups which are almost like different diseases. By identifying these sub-groups of T ALL, and consequently the weaknesses specific to these groups, it is hoped to make treatment more tailored to individual groups and consequently more optimal for patients.

Aims

- To identify candidate genes likely to be functionally relevant in leukaemia development.
- To analyse methylation levels of candidate genes in DNA samples taken from ALL patients at diagnosis.
- To propose a link between differential methylation of ALL subtypes and their cytogenetic qualities.

Methods

Methods used during this project included DNA modification, Polymerase Chain Reaction (PCR), Gel Electrophoresis and Pyrosequencing.

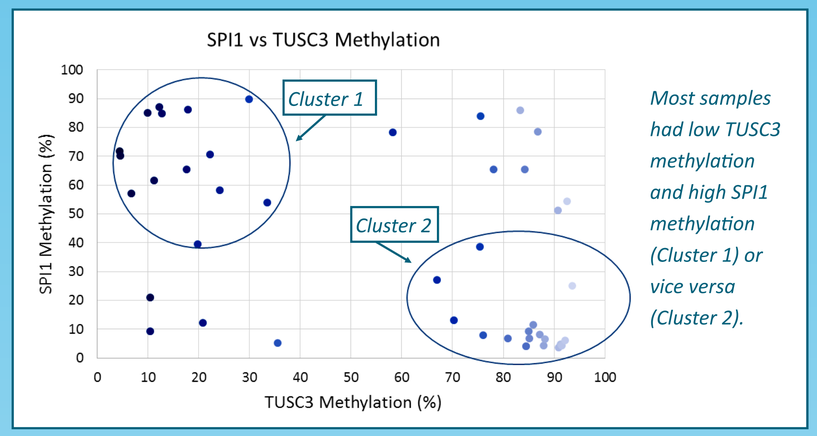


Pyrogram produced using Qaigen Pyrosequencing.

Pyrosequencing allows methylation levels to be measured very accurately for multiple CpG sites in a gene of interest.

Results - SPI1 vs TUSC 3 Correlation

The majority of methylation of TUSC3 and SPI1 was either high or low. This is illustrated by the four clusters shown in the graph (below).

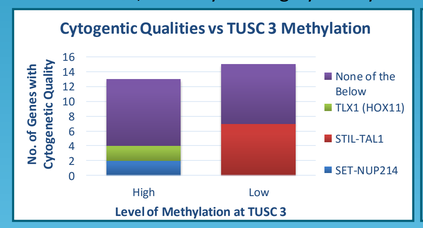


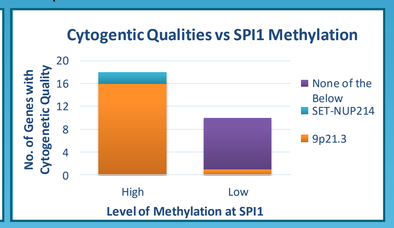
Most samples had low TUSC3 methylation and high SPI1 methylation (Cluster 1) or vice versa (Cluster 2).

There was a significant negative correlation of r = -0.486 between SPI1 and TUSC3 methylation which was not expected as SPI1 was selected from cluster analysis of many genes thought not to have a correlation with TUSC 3. This had a p-value of 0.000834.

Results - Methylation vs Cytogenetics

There appeared to be a significant correlation between low methylation in TUSC3 and a SIL-TAL1 fusion and between high TUSC 3 methylation and TLX1 (HOX11) and SET-NUP214. High methylation of SPI1 appeared to have a significant correlation with 9p21 abnormalities; the only two highly methylated samples at SPI1 had SET-NUP214 fusions.





These findings give scope for further research into the differential methylation of T ALL subgroups and the different behaviours driven by this. In the future, methylation could be used to distinguish between these subgroups; consequently, development of subgroup targeted treatments could be possible.

Conclusions

- SPI1 and TUSC 3 were identified as candidate genes for methylation level investigation.
- 61 T ALL DNA samples were tested to determine the methylation levels at both SPI1 and TUSC 3.
- There was a negative correlation between methylation of the two genes, SPI1 and TUSC3.
- High methylation in SPI1 correlated with 9p21 deletions.
- Low levels of methylation in TUSC3 correlated with the presence of a SIL-TAL fusion. This low level of methylation suggests that these leukaemia cells may be dependent on expression of TUSC3.
- TUSC3 is not required for survival of normal cells so it is possible that targeting TUSC3 may specifically kill the leukaemia cells in patients with a SIL-TAL fusion without harming healthy cells.

Acknowledgements

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