Cancer's Special Pro-survival Genes

Potential Conditional Lethal Genes in T cell Acute Lymphoblastic Leukaemia

Gina Elsayed Abdelaal*, Timothy Barrow, Dr Gordon Strathdee

Student number: 140071292
School of Biomedical science
Email:G.Elsayed1@newcastle.ac.uk

Introduction

T cell lymphoblastic leukaemia is a rare form of blood cancer. It is responsible for 15% of adult cases and 25% of pediatric cases of lymphoblastic leukaemia. If left untreated the disease progresses quickly and may be fatal within a few months.

In the past cancer was thought to be a result of changes in the DNA sequence of a gene, however it is now understood that other factors can play a role in disease progression such as DNA methylation. A gene's CpG island acts as a switch, when it is methylated the gene will be switched off and when it is left unmethylated the gene will be switched on See figure 1.

Cancer occurs when normal cells lose control over their gene expression, which may be due to abnormal methylation. There are genes that are exclusively expressed by cancer cells known as conditional lethal genes. These genes can allow cells with genetic mutations to survive. TUSC3 is a conditional lethal gene, so it is methylated (switched off) in normal conditions. Previous work on B cell lymphoblastic leukaemia has shown that when TUSC3 is unmethylated (switched on) it allows cells that have the ETV6/ RUNX1 genetic fusion to survive. TUSC3 has a similar role in T cell lymphoblastic leukaemia as it allows cells with the SIL-TAL1 fusion escape cell death. This project will mainly focus on 2 potential conditional lethal genes : PTPRK and FAT1.

Aims

- Obtain the methylation levels of 2 conditional lethal genes FAT1 and PTPTRK in T cell patient samples and cell lines
- Compare results to known methylation levels of 2 other conditional lethal genes SPI1 and TUSC3
- See if there is any connection between methylation levels and the presence of the SIL-TAL1 gene fusion.

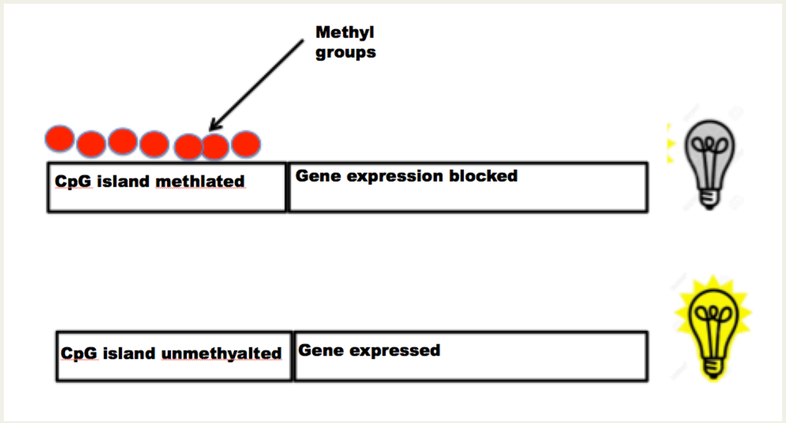

Figure 1: When CpG island is methylated gene is expressed, when CpG is unmethylated gene is not expressed.

Methods

Bisulphite modification

The DNA must be bisulphite modified. The aim of this technique is to deaminate unmethylated cytosines using sodium bisulphite . A methylated cytosines remain a cytosine. The unmethyaled cytosine becomes a thymidine.

Assay Optimisation and PCR

PCR amplifies the section of DNA we are investigating. New primers were designed using primer design software. The purpose of the optimization assay is to find the conditions (MgCl2 concentration and annealing temperature) that give the largest quantity of DNA.

Pyrosequencing

Pyrosequencing is used to quantify methylation along the amplified DNA. It is sequencing by synthesis technique. The sequence is entered into the pyrosequencing software. The end result is a pyrogram which gives the methylation levels of the DNA sample. See figure 2 for pyrogram.

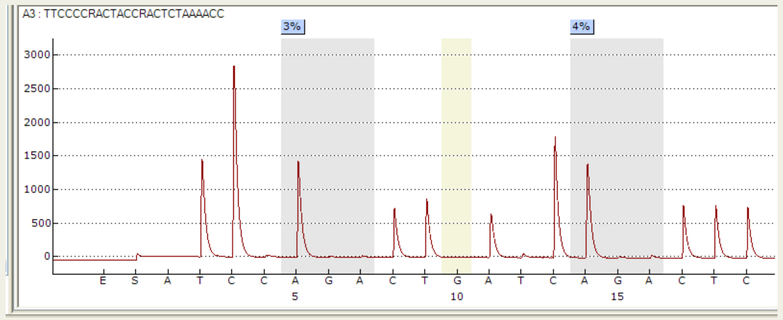

Figure 2: Pyrogram for FAT1 gene. 3% and 4% represent the levels of methylation at the gene's first and second CpG sites

Results

For both genes there was a lot of variation in the methylation levels; some samples had higher methylation statuses and some had lower as seen in figure 3.

FAT1 gene results

Results for the correlation test for FAT1 showed that there was a weak negative correlation between FAT1 and TUSC3 and a weak positive correlation between FAT1 and SPI1, both were statistically insignificant. When looking at the samples with the SIL-TAL1 fusion there was a stronger positive correlation between FAT1 and SPI1 and a stronger negative correlation between FAT1 and TUSC3, but it was still statistically insignificant. 3 out of 5 of the samples with lower methylation levels had a deletion in in the CDKN1B gene, this may be just a coincidence or it may have some significance.

PTPRK gene results

Results for PTPRK show a positive correlation with TUSC3 and a negative correlation with SPI1 both were statistically insignificant. The results were the same for the SIL-TAL fusion samples.

Without SIL-TAL fusion

FAT1	TUSC3	SPI1
84.81	70.13	5.065
35.4775	71.85	5.405
88.675	20.94	10.415
1.875	61.615	11.185
12.865	84.89	12.77
12.25	70.725	22.24
49.82	58.3	24.17333333
6.6075	54.04	33.555
39.9025	78.39	58.28
71.3	27.1	66.97
62.67	38.53	75.405
33.22	65.49	78.10666667
79.5	65.37	84.25
81.79	78.46	86.795
28.55	6.55	88.21
5.9075	4.35	91.53

With SIL-TAL fusion

FAT1	TUSC3	SPI1
38	6.695	85.075
43.59	13.16	70.225
61.335	30.21	24.67
81.8275	25.01	61.01
88.8375	8.025	87.14125
39.775	29.22	32.86

Without SIL-TAL fusion

PTPRK	TUSC3	SPI1
3.964	61.615	11.185
4.924	54.04	33.555
5.04	71.85	5.405
14.82	6.55	88.21
20.16	65.37	84.25
21.112	4.35	91.53
29.592	78.46	86.795
48.708	84.89	12.77
71.508	78.39	58.28
78.882	20.94	10.415
80.132	38.53	75.405
88.39	70.13	5.065

With SIL TAL fusion

PTPRK	TUSC3	SPI1
7.934	85.075	6.695
20.476	61.01	25.01
25.878	70.225	13.16
39.63	24.67	30.21

Figure 3 :Tables show the results for FAT1 and PTPRK. Methylation levels are colour coded: Green= low methylation, yellow=intermediate methylation, red= high methylation

Conclusion

The two potential conditional lethal genes have a correlation with TUSC3 and SPI1, but it was not statistically significant, so it might be due to chance. This means that the methylation levels for these genes may have no effect on the survival of cells with the SIL-TAL fusion.

Limitations of this project were that T cell ALL is a rare disease, so few samples were available for testing. To confirm findings this needs to be repeated in a larger study, which may give a higher statistical significance.

The results that may be expected based on this project are that FAT1 expression is blocked as it has a negative correlation with TUSC3 a conditional lethal gene, and PTPRK expression is enhanced as it has a positive correlation with TUSC3. Potentially we could take advantage of this while designing a targeted cancer therapy.

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