The role of age related methylation as an underlying cause of cancer development

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INTRODUCTION/AIMS

Cancer is often associated with the turning off and on of specific genes. These are called epigenetic mechanisms, an example of which is CPG island methylation. CPG island methylation involves the addition of a methyl group to the carbon 5 position on a cytosine residue that is paired to a Guanine (Hence CpG) and has the effect of repressing transcription.

My project is to investigate the underlying epigenetic mechanisms that take place as we age and their associations with cancer.

The main aims of my project were:

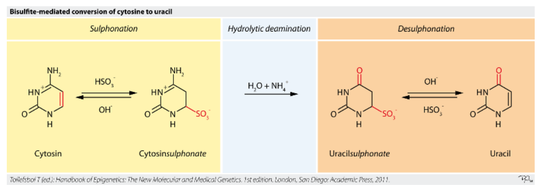
- To determine whether the relationship between age-related methylation and cancer is age dependent or seen across all ages of cancer patients.
- To investigate whether age-related methylation is isolated mainly to Acute Lymphoblastic Leukaemia (ALL) or seen in other non-haematological malignancies.

I focused mainly on the genes HOXA4 and Twist2 which have been seen to be hyper methylated in cancer. I studied samples from patients with ALL, liver cancer and oesophageal cancer.

METHODOLOGY

Modification

In order to prevent a false positive during pyrosequencing the unmethylated cytosine residues must be converted to uracil. This is done by bisulphite modification. The process involves sulphonation, hydrolytic deamination and desulphonation.

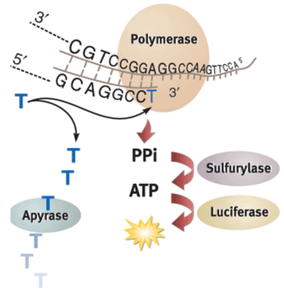


PCR

PCR is used to amplify the target loci so that there is a sufficient volume of the required DNA to sequence. PCR is a series of reactions that amplifies target DNA using the enzyme polymerase, nucleotides, forward and reverse primers, and differing magnesium concentrations according to which gene is being amplified.

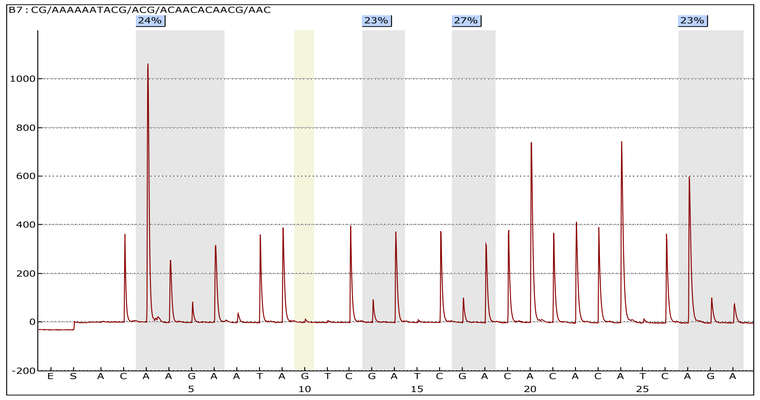
Pyrosequencing

Pyrosequencing was used to analyse the average methylation levels of each sample. Pyrosequencing uses the light produced during the luciferase reaction to analyse the number of nucleotides added in order to determine the sequence and levels of methylation.



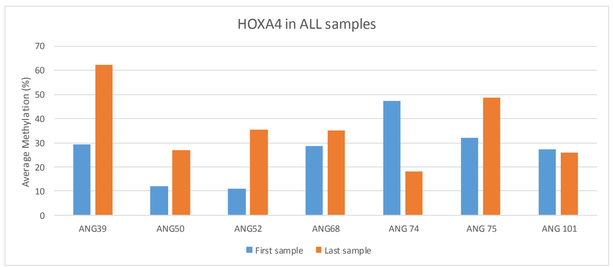
As a base is incorporated into the DNA sequence, pyrophosphate is released. This is converted to ATP by sulfurylase. The volume of ATP produced is proportional to the number of bases added. This ATP is used as a substrate for luciferase and converted to light which is recorded by a camera in the pyro-sequencer.

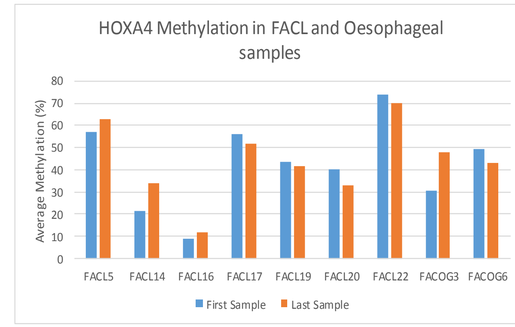
A program is then produced like the one below. The peak heights are then checked in accordance with the expected sequence and the average CPG site methylation values are found.



RESULTS

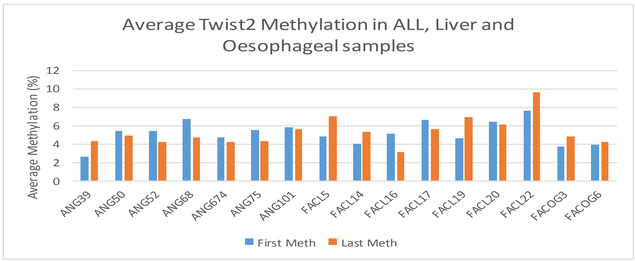
Firstly I have produced data for HOX in my ALL samples that is concurrent with previous research. My research showed an increased average methylation level across the samples except for in one sample.





My results from the ALL, liver and oesophageal samples address the aims of my project proving that methylation is not just age-related as both childhood (ALL) samples and older patient (Liver) samples show high levels of methylation in HOXA4.

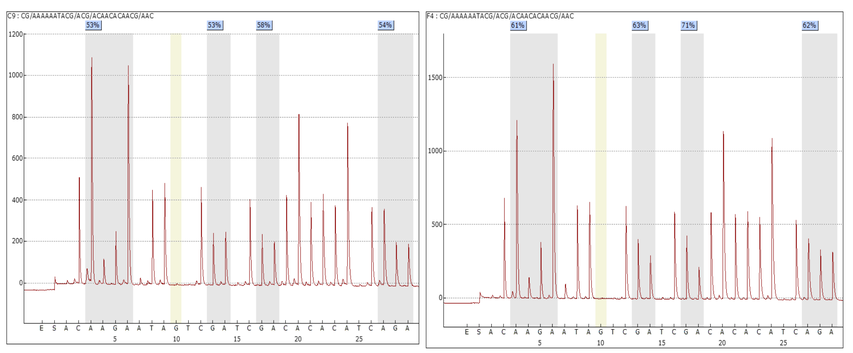
It was more difficult to identify a pattern in the data I collected for TWIST2. However on average there was a slight increase in methylation in the later samples.



SUMMARY/ DISCUSSION

Methylation in genes such as HOXA4 has been linked with the down regulation of tumour suppressor genes leading to cancers such as ALL, Liver cancers and Oesophageal cancer. Therefore as cancer progresses you would expect to see increases in methylation of these genes.

The greatest difficulty I found was in obtaining repeats that were closely matched (<5% difference per sample). I would record accurate data but my entire results would be biased higher or lower by 10%. I attempted to solve this by adjusting both my PCR and Pyro methods but to no effect.



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