



# **Loss of ABO Antigens in Haematological Malignancies**

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## Abstract

This thesis describes the investigation of the alteration of ABH antigen expression on the surface of red blood cells in patients with haematological malignancies. ABH antigens are complex carbohydrates present on the surface of erythrocytes. These antigens are generated by the stepwise addition of monosaccharides, with each step catalysed by a specific glycosyltransferase. The *A* and *B* alleles of the *ABO* locus encode the A and B glycosyltransferases, which act on the precursor H antigen to produce the A and B antigens. The precursor H antigen is determined by a fucosyltransferase coded for by the *FUT1* locus.

A flow cytometric technique was developed to analyse the red blood cells of patients for changes in ABH antigen expression. In normal individuals flow cytometric profiles were associated with particular *ABO* genotypes. Nearly 50% of patients had some alteration of the A, B or H antigens.

RT-PCR was used to investigate *ABO* mRNA expression in bone marrow (BM) cells of patients with alterations in ABH antigens. Of eleven patients with loss of A or B antigens detected by flow cytometry, only 3 had loss at the mRNA level. The lack of correlation between loss on the flow cytometer and loss at the mRNA level was not surprising as the population with loss was often small and the predominant normal population would mask any losses.

The expression of *FUT1* mRNA was also investigated using RT-PCR. *FUT1* has three transcripts arising from three different transcription initiation start sites. The individual expression of each transcript was assessed by RT-PCR in normal individuals and in patients with loss of ABH antigens. It was found that the exon 2 transcript is the most important in determining H antigen expression.



The molecular mechanisms underlying loss of ABH antigens were investigated by assessing CpG methylation of the *ABO* and *FUT1* promoters, and by assessing loss of heterozygosity (LOH) of genes adjacent to *ABO*. Treatment of leukaemia cell lines, which did not express *ABO*, with a demethylating agent led to re-expression of *ABO*. There was no association between patients with loss of ABH antigens and *ABO* methylation. However, of 7 patients with loss of ABH antigens detected in a previous PhD study, 5 were methylated in this study. This difference may be attributable to a greater majority of cells (>50%) with loss of ABH antigens in the samples detected previously compared to the samples in this study.

LOH of genes adjacent to *ABO* on the chromosomal region 9q34 was investigated. This chromosomal region has been shown to be frequently lost in acute myeloid leukaemia. Single nucleotide polymorphisms in various 9q34 loci, were assessed for LOH by a PCR-RFLP method. Samples from 42% of patients showed loss of expression in the tumour suppressor gene *DAPK1*. Two less well-known genes, *POMT1* and *PPP2R4*, had allele dosage alterations in patient samples.

In conclusion, loss of ABH antigens in haematological malignancies is a frequent event. Loss of cell surface ABH antigen expression is a marker for alterations at other 9q loci besides *ABO*, suggesting that this region harbours potential tumour suppressor genes or that 9q undergoes regional silencing.