MOLECULAR ANALYSIS OF CHANGES IN ABO BLOOD GROUP ANTIGEN EXPRESSION IN HAEMATOLOGICAL MALIGNANCY

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Summary

Loss of expression of the A and B antigens of the ABO blood group system from the red cells of patients with haematological malignancy was first observed in the 1950s. Loss of antigen expression *per se* is probably not of direct importance in disease progression, but reflects a genetic alteration that has occurred in the malignant stem cell, as both the leukaemic cells and erythrocytes can be derived from the same precursor.

The recent cloning of the coding region of the ABO gene has made it possible to investigate loss of ABO antigens at the molecular level. This thesis describes the development of techniques to genotype and simultaneously assess allele dosage at the ABO locus using PCR and allele-specific restriction enzyme digestion. Fifteen patients with a variety of haematological malignancies and loss of A and/or B antigen expression were analysed. Loss of antigen expression in two cases was not due to changes occurring at the ABO locus. Four of the remaining thirteen patients had abnormal allele dosage of the ABO gene; one with physical loss of the A allele, one patient with two copies of the O allele and one A allele, and two patients that had lost the O allele. As the O allele of the ABO gene is non-functional, and loss of a non-functional allele can not provide a growth or other advantage to the cell, it was concluded that the changes seen at the ABO locus are an indication of other genetic events occurring in the same chromosomal region.

Analysis of the relative expression of the ABO alleles using RT-PCR and restriction enzyme digestion revealed that the A allele was transcriptionally silenced in four patients. However, these four patients, all of whom genotyped as AO, expressed the O allele. As monoallelic expression of autosomal genes usually indicates imprinting, studies of the patients and their parents were carried out to determine if there was any parental bias in the contribution of the affected allele. Including data from the literature, 11 of 12 patients with loss of A or B antigen expression had lost

expression of the maternally inherited allele of the ABO gene. Therefore, it seems highly likely that imprinting is involved in the etiology of this phenomenon.

Other reports show that some patients with loss of A or B antigens have markedly reduced levels of the enzyme adenylate kinase 1. As both the ABO and AK1 genes are located in chromosome band 9q34, it was possible that the AK1 gene was also affected by the silencing or loss of heterozygosity (LOH) event occurring at the ABO locus. A *Taq*1 restriction enzyme polymorphism in exon 7 of the AK1 gene was identified. Using this polymorphism, it was determined that the loss of heterozygosity event occurring at the ABO gene in one patient, does not extend to the AK1 gene. However, analysis of the allelic expression of the AK1 gene in a patient with severely reduced AK1 enzyme activity and allelic silencing of the ABO gene, revealed decreased expression of one of the alleles, although this patient had normal allele dosage at the AK1 locus. It was therefore concluded that this patient has allelic silencing of both the linked ABO and AK1 genes.

As recurrent LOH in a given region usually indicates the presence of a tumour suppressor gene, the ABL gene, which is located in between the ABO and AK1 genes, was examined. The ABL gene is involved in regulation of the the cell cycle, and therefore it can be considered a potential tumour suppressor gene. Two patients with allelic silencing of the ABO gene and no LOH at the ABO locus were examined. Although both patients had normal allele dosage at the ABL locus, expression analysis of the ABL gene was inconclusive. Although normal controls always produced repeatable results using three different PCR-based methods for the analysis, the relative allelic expression of the ABL gene in the patients was highly variable. The reasons for this are unclear, but are probably due to low mRNA copy numbers of the ABL gene in these patients.

This is the first report of LOH events occurring at the ABO locus and of allelic silencing in haematological malignancy, and represents as yet undescribed genetic and epi-genetic mechanisms for leukemogenesis.