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Advances in ABO gene expression regulation

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ABSTRACT

The ABO blood group system is vital to blood transfusion and organ transplantation. ABO antigens are the most important of all blood group antigens in clinical practice, and are not only present in red blood cells and platelets, but also in most secretions and epithelial tissues. ABO antigens are known to undergo drastic changes during the development, differentiation, and maturation of normal cells. Profound changes have also been documented in pathological processes such as tumorigenesis. To elucidate the molecular basis of how ABO genes are controlled in cell type specific expressions, such as normal cell differentiation or in cancer cells lacking A/B antigens, it is essential to understand the regulatory mechanisms of ABO gene expression. In this review, current knowledge concerning the regulatory mechanisms of ABO gene expression was summarized.

Keywords: ABO gene; transcriptional regulation; promoter; intron; exon; DNA methylation

INTRODUCTION

The ABO blood group system was discovered by Karl Landsteiner at the beginning of the 20th century and is of great importance to blood transfusion and organ transplantation. The ABO system comprises of two carbohydrate antigens, designated A and B, which give rise to four phenotypes, A, B, AB and O. In the O phenotype, neither A nor B is produced. ABO antigens are widely distributed and are present not only in red cells and platelets but also in body fluids and tissue/cell surfaces, including epithelial cells, sensory neurons, and the endothelia of blood vessels. The term histo–blood group ABO is often used to reflect the wide distribution of ABO antigens. They are, however, absent from connective tissue, muscle, and the central nervous system. ABO antigens are present in the fetus as early as 5–6 weeks gestation, but are not fully expressed until 2–4 years of age. ABO antigens are known to undergo drastic changes during the development, differentiation, and maturation of normal cells. When erythroid cells differentiate in vitro, ABO is expressed at an undetectable level in the early phase, increases subsequently, and then decreases later. The ABO gene transcript is barely detectable in CD133–CD34+ cells freshly purified from blood, whereas ABO transcripts become apparent in *ex vivo* culture[1]. In addition to these physiological processes, profound changes have also been documented in pathological processes, such as tumorigenesis. Reduction or complete deletion of A/B antigen expression in carcinomas has been reported. To elucidate the molecular basis of how ABO genes are controlled in cell type specific expression, such as in during normal cell differentiation, or in cancer cells lacking A/B antigens, it is essential to understand the regulatory mechanism of ABO gene transcription.

ABO GENE PROFILE

The ABO blood group involves three carbohydrate

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antigens: A, B and H. H antigen is the precursor of both A and B antigens. The functional A and B alleles at the ABO genetic locus, encode glycosyltransferases α1→3GalNAc transferase (A-transferase) and α1→3Gal transferase (B-transferase), respectively. A-transferase transfers a GalNAc residue from UDP–GalNAc to the precursor H substrate, producing A antigens. Similarly, B-transferase catalyzes the transfer of Gal from UDP-Gal to the same H substrate, producing B antigens[2]. The genomic structure of human ABO genes was reported in 1990 [3–4]. These initial molecular genetic studies demonstrated that the ABO gene is located on chromosome 9q34.1–q34.2, contains 7 exons, and that the coding sequence in the 7 coding exons spans over 18 kb of the genomic DNA. The exons range in size from 28 to 688 bp, with most of the coding sequence lying in exon 7. The ABO gene shows considerable polymorphisms in exons and introns, plus the upstream region and downstream region. Several polymorphisms affect the specificity of the gene product(glycosyltransferase) and explain the occurrence of blood group A and B. The common ABO alleles, ABO*A1.01, ABO*B.01 and ABO*0.01, differ by only a few single-nucleotide polymorphisms [5]. There are four amino acid substitutions between A and B transferases (on positions 176: for arginine; 235: glycine; 266: leucine; and 268: glycine, in A transferase and glycine, serine, methionine, and alanine in B transferase). O alleles contain a single nucleotide deletion (G residue at nucleotide 261) which causes frame shifting of codons, which results in a protein without enzymatic activity of either A or B transferase. Among the ABO gene polymorphisms, various lethal mutations result in blood group 0.0 ther mutations are presumed to alter activity rather than the specificity of the enzyme and result in weaker A or B blood group phenotypes [6].

THE REGULATORY FUNCTION OF THE 5´-UPSTREAM REGION OF THE ABO GENE

Gene expression is driven by promoters, enhancers, transacting factors, and other cis-regulatory elements. Studies of the human ABO genes using cultured cells have demonstrated transcriptional regulatory elements. Firstly, Kominato *et al*. determined two transcription start sites just upstream from the initiation codon by the 5´-RACE technique using human pancreatic cDNA as a template[7], starting at the –32 and the –8 position of the initiation codon. Several GC boxes were found just upstream of these transcription initiation sites, however neither TATA nor CAAT boxes were found close to this region. The characterization of the 5´-upstream sequence of human ABO genes demonstrated that the proximal promoter was located between –117 and +31 in both erythroid and epithelial cell lineages [8], whereas the distal promoter showed cell-type specificity, being located between –832 and –667 relative to the transcription start site in Exon 1 of the ABO gene. The levels of transcripts starting from the proximal promoter were much higher compared with those from the distal promoter.

Like many housekeeping genes, the ABO gene contains a typical CpG island that extends from 0.7 kb upstream to 0.6 kb downstream from the transcription start site in exon 1. Expression of ABO genes was shown to be repressed upon DNA methylation of the CpG island in the promoter region [9]. The utilization of multiple promoters and transcription start sites is a frequently used mechanism to create diversity and flexibility in the regulation of gene expression. An alternative promoter exon 1a, located 682 bp upstream of the original exon 1, was identified in AC133–CD34+ cultured cells obtained from peripheral blood [10–11]. The alternative erythroid transcript accounted for 2 percent of transcripts in these cells. Exon 1a is utilized as the transcription starting exon by cells of both erythroid and epithelial lineages. A report by Tan and coworkers[11] identified alternative first exons in 35 percent of erythroid genes and proposed that they are important for gene regulation. Both ABO promoter regions contain CpG islands that can initiate transcription. Although exon 1a transcripts lack exon 1 and thus lack the translation start site at position ABO nucleotides 1 to 3, it leads to production of a functional glycosyltransferase, probably by utilizing downstream alternative start codons[12].

The Sp1 binding site (–22 to –14) in the ABO promoter, is capable of binding to Sp1 or Sp1-like protein(s). The introduction of binding-deficient mutations at the site results in a decrease in promoter activity in erythroleukaemia cells and gastric cancer cells [13]. The expression of the ABO promoter is influenced by the binding of Sp1 or Sp1-like protein(s) in both erythroid and epithelial cell lineages. ABO promoter mutation, –35 to –18 del, was found in a B3 variant individual, relating to mixed-field weak A/B antigen expression on RBCs. This mutation was located on the SP1 binding site and thus influenced the promoter activity of the ABO genes[14].

Transcription from the ABO proximal promoter is in part controlled by silencer elements just upstream of the promoter, with negative elements for ABO gene transcription suggested to be in the region between –275 and –118[1, 10]. Kominato and colleagues conducted a transient transfection experiment in KATOIII cells with a luciferase reporter plasmid carrying a mutated

N box at −196 to −191, demonstrating that the N box is a negative regulatory element. The N box binds with a nuclear factor, RACP. Repression of transcription from the ABO proximal promoter is partially dependent on the N box.Down regulation of RACP may relieve the repression, thereby leading to the expression of ABO genes during the maturation of cells in the epithelial lineage as well as the erythroid lineage. As early as 1997, Japanese scholars proved that the transcription of human ABO genes was dependent upon the binding of transcription factor CBF/NF-Y (CCAAT−binding factor) to a minisatellite sequence. The reported minisatellite sequence was in the 43−bp unit, located 3.8 kb upstream of exon 1[8, 15]. The number of repeats in the upstream CBF/NF-Y-binding enhancer region, which contained four 43−bp repeats in $A^2/B/O^1/O^{1v}$ but only one 43−bp unit in A^1/O^2alleles, determined the transcription rate. The fourfold unit had approximately 140 times more activity than the single segment [16−17]. Some weak blood group B phenotypes may be caused by variations in the CBF/NF−Y enhancer region of the ABO genes[18].

THE REGULATORY FUNCTION OF 3′−DOWNSTREAM REGION AND INTRON 1 IN THE ABO GENES

Gene expression is driven by promoters, enhancers, transacting factors, and other cis-regulatory elements located on the core promoter, the region proximal to the core promoter, and the more distant sequences of the gene. Studies on the regulation of human ABO gene transcription have focused mainly on the 5′ region, including the core promoter and the region proximal to it. Sano and colleagues, using luciferase reporter assays,examined the involvement of the 3′ region in the transcriptional regulation of ABO genes. They found that the 3′ flanking region of human ABO histo-blood group genes was involved in negative regulation of gene expression[19]. Further study indicated that the proximal downstream region of ABO genes functioned as a negative regulator in a manner that was independent of orientation, promoter, or position, while the distal downstream sequence repressed transcription in a manner that was dependent on position. The sequence of the 3′ region of ABO is characterized by a high degree of repetition[16]. However, transfection experiments in KATOIII and K562 cells showed that regions other than the highly repetitive sequences in the proximal 3′ region were involved in the repression of ABO gene transcription. Several years later, Sano and colleagues identified another downstream regulatory element in the 3′ flanking region of human ABO genes[20]. This was a positive regulatory element, designated the +22.6−kb site, which was shown to enhance ABO promoter activity in an epithelial cell−specific manner. Expressions of ABO and B-antigen were found reduced in gastric cancer KATOIII cells by biallelic deletion of the +22.6−kb site. EMSA and ChIP assay demonstrated that the site bound to an epithelial cell-specific transcription factor, Elf5. Mutation of the Ets binding motifs to abrogate binding of this factor reduced the regulatory activity of the +22.6−kb site. Furthermore, ELF5 knockdown was found to cause reduced endogenous B-antigen expression in KATOIII cells. Thus, Elf5 appeared to be involved in the enhancer potential of the +22.6−kb site.

As genome wide approaches for the discoveries of enhancers have become available, regulatory elements are often characterized by the presence of DNase I-hypersensitive sites (DHSs), which can mark positions where transcription factors bind to DNA[21−22]. On the basis of DHSs in genomic DNA, a novel positive regulatory element in intron 1 of ABO was identified, named the +5.8−kb site[23]. The element appeared to enhance the activity of the ABO promoter in an erythroid cell-specific manner. This regulatory activity was shown to depend on the binding of transcription factors GATA−1/2 and RUNX1[24−25]. Because deletion and disruption of the GATA and RUNX sites abrogated the erythroid cell-specific enhancer activity of the +5.8−kb site, it was suggested that these mutations might downregulate transcription from B and A alleles, leading to a reduction in B- and A-antigen expression in cells of erythroid lineage, but not in mucus secreting cells, resulting in B_m and A_m, respectively. Sano and his colleagues detected the genomic DNA from 382 Japanese individuals with B_m or AB_m. 5.8−kb deletion was found in 380 individuals, and disruption of the GATA motif in intron 1 was found in one individual, and a 3.0-kb deletion was demonstrated in the remaining individuals[26]. Similar to those of the B_m phenotype, mutations were also found in positive regulatory element in intron 1. A 23−bp deletion, including a RUNX1 site in the +5.8−kb site of genomic DNA, obtained from individuals with A_m [27]. Mutations in the erythroid cell-specific regulatory element in intron 1 can also result in weak phenotypes, beside A_m/B_m phenotypes. Chinese scholars found a single point mutation of RUNX1 motif (+5904 C>T) in the +5.8 kb site, which may lead to Bel or Ael phenotypes [28]. Japanese scholars found two single point−mutations in the +5.8−kb site on the A-allele in A3 individuals [29].

OTHER MECHANISMS OF ABO GENE EXPRESSION REGULATION

ABO genes contain a typical CpG island[9]. CpG

islands are almost always maintained in unmethylated states, unlike the CpG sites in the remainder of the genome. However, methylation of CpG islands can occur on an inactive X chromosome, in promoters of imprinted genes, along with oncogenesis, and during aging. In all these cases, methylation of CpG islands spanning the promoter regions is strongly associated with transcriptional silencing. Expression of ABO genes in epithelial and erythroid cells lines was shown to be dependent on the methylation status of the proximal constitutive promoter, encoding most of the ABO transcripts. An inverse relationship was found between promoter hypermethylation and ABO gene expression[9]. Treatment of cells with the demethylating agent, 5-aza–2´-deoxycytidine, can result in demethylation of the ABO promoter region and restore transcriptional activity. Kominato and colleagues examined the methylation status of the upstream region in ABO genes by bisulfite genomic sequencing. They found that in cells expressing ABO genes, DNA hypermethylation was observed in the repetitive elements region, however the ABO CpG island was hypomethylated. The distal promoter region appeared to be located 3´ adjacent to the methylated region of the repetitive elements. In the non-expressing cells, hypermethylation extended from the repetitive elements region through the distal promoter region to the proximal promoter region [10]. Changes in ABH antigen expression have been documented in tumorigenesis. Reduction or complete deletion of A/B antigen expression in carcinomas and myeloid malignancies has been reported [10, 30–31], and the loss of ABH antigens has been correlated with tumor progression of various carcinomas[32–35]. Neoplastic cells simultaneously harbor widespread genomic hypomethylation and regional areas of hypermethylation[36]. Many studies have demonstrated that a loss of ABO expression in carcinomas and myeloid malignancies is strongly associated with DNA methylation of the ABO promoter [37–39].

Epigenetic regulation is an important component of gene expression regulation. Histone acetylation, as with methylation, is a main component of the epigenetic mechanism[40]. Kominato and his research group found that ABO transcription seemed to be regulated by histone modifications[41], firstly by noticing that sodium butyrate, one of the histone deacetylase inhibitors (HDACIs) promoted the differentiation of erythroid–lineage cells [42], resulting in the speculation that HDACIs might suppress ABO expression, leading to a reduction in ABO antigens. Subsequently, they examined the effects of HDACIs on K562 cells and KATOIII cells and observed a subsequent reduction of ABO transcripts in both cell types, finding that histone deacetylase inhibitors suppress ABO gene expression.

Endogenous antisense transcription occurs in mammals. Natural antisense transcripts can regulate key gene expression by mechanisms including translational regulation, genomic imprinting, RNA interference, alternative splicing, X inactivation, RNA editing, gene silencing, and methylation [43]. Antisense RNAs also existinthe human ABO genes, designated as ABOAS, of which the transcript is approximately 2.0 kb in length and intronless. The expression of ABOAS appears to be correlated with ABO expression in various cultured cells and normal tissues. Repression of the ABO exon 1 promoter, however, results in an increase of ABOAS transcript in the cells expressing the ABO genes. The ABO and ABOAS transcript pair are regarded as a divergent structure, whose overlapping region involves the ABO gene´s 5´-UTR, exon 1, and intron 1. Experiments have shown that the addition of mithramycin A, which is a well-known antibiotic that binds to GC-rich regions in chromatin and interferes with the transcription of genes that bear GC-rich motifs in their promoters by selectively displacing transcriptional activators to KATOIII cells[44], results in the reduction of transcription from ABO exon 1, while at the same time increasing the ABOAS. So ABOAS transcribed from the opposite strand of the ABO genes might be involved in the regulation of ABO gene expression.

Glycosyltransferase A (GTA) and glycosyltransferase B (GTB) convert H antigen to A and B antigen using a UDPGalNAc or UDP-galactose donor. GTA and GTB are transmembrane proteins, both of which contain a short cytoplasmic domain, a transmembrane domain, a stem region and a catalytic domain [45]. The crystal structure is an important influence factor in GTA´s and GTB´s catalytic ability. Some mutations in the ABO genes cause the gene products´ 3-D structure to become altered. Some weak ABO subgroups result from changes of GTA/GTB structure. Recent research concerning ABO blood antigen expression focuses on this phenomenon. Cai et al. [46]identified a mutation c.538C>T (p. R180C) of B allele in a blood donor and his father who had the ABw phenotype. In silico analyses found that the residue Arg180 on the internal flexible loop, next to the entry of the binding pocket may have its long side chain salt-bridged with the highly flexible C-terminal carboxyl, contributing to the catalysis of H to B antigen conversion. So the mutation p.R180C does not affect expression of glycosyltransferase B, but instead impairs H to B antigen conversion. A similar study conducted by Lee *et al*[47] found a novel A subgroup allele (c.538C>T

p.Arg180Cys), showing a weak A phenotype by affecting the structure of the glycosyltransferase A (GTA). Given that the internal loop region (177–195) was flexible in the open conformation of wild–type GTA, the side chain of Arg180 forming hydrogen bonds with other residues was presumed to stabilize the helical structure in the closed form of GTA. However, no hydrogen bonds were observed at residue 180 in the p.Arg180Cys and p.Arg180Pro mutants. Haobo Huang and his coworkers identified ten novel weak ABO subgroup alleles. Among them, the GTA mutant p.L339P may lead to a weak A phenotype, by changing the local conformation of GTA and reducing its stability [48].

XiaohongCai found a missense mutation c.28G>A (p.G10R), which occurred at the last nucleotide of ABO gene Exon 1. This mutation was relatively common in B3 or AB3 individuals in the Shanghai area[14]. A further in vitro expression study revealed that GTB mutant p.G10R had a normal GTB transfer capacity and was able to facilitate B antigen formation on the cell surface. This c.28G>Amutation decreased the ABO gene´s mRNA level by affecting the splicing of the ABO gene´s RNA[49], resulting in an altered B–antigen expression.

CONCLUSION AND FUTURE PERSPECTIVE

ABO blood group antigens are carbohydrate antigens, defined by terminal sugars on glycolipids and glycoproteins. H antigen is the precursor of A and B antigens, which are determined by FUT1 (H) and FUT2 (Se) genes[50]. So, the ultimate expression of A and B antigens on the red cell surface is dependent on not only ABO genes but also other related genes, for example FUT1 and FUT2 genes. Information on how the genes encode the transcriptional regulation of glycosyltransferases involved in the synthesis of ABO blood group antigens is fragmentary and superficial, and future studies in this field will most likely focus on regulatory mechanisms which ensure the cooperative expression of these genes. In addition, the expression of ABO antigens is influenced by other factors. For example, antigen density on the erythrocyte surface is probably dependent on the amount of enzymes available at the biosynthetic site. Apart from any influence due to subcellular localization, this amount depends on the transcription of the gene and also on the degradation of the ABO-mRNA, as well as the active enzyme. Although some information is available, neither process is fully understood.

ABO antigens are widely expressed, not only in red blood cells but also in body fluids and tissues. In the last few years, many investigators have studied the association between the ABO blood group and the risk of various diseases, such as infectious diseases, arterial and venous thromboembolism and certain kinds of cancer[33, 50–52]. The significance of A/B transferases and the biological functions of A/B antigens have not until now been clearly demonstrated, but it would be expected that loss of these functional proteins in patients would have some deleterious consequences. In addition to study on how ABO genes are controlled, further functional investigations are recommended to clarify the exact role of ABO antigens in physiological and pathological processes.

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