Human ABO gene transcriptional regulation

Yoshihiko Kominato ,[1] *Rie Sano* ,[1] *Yoichiro Takahashi,*[1] *Akira Hayakawa* ,[1] *and*
Kenichi Ogasawara [2]

The ABO blood group system, discovered by Karl Landsteiner in 1900,[1] is fundamental to the safety of blood transfusion, which requires identification of weak phenotypes or subgroups. The system is composed of two carbohydrate antigens, A and B, and their antibodies. The International Society of Blood Transfusion designates four antigens—A; B; A,B; and A1—based on the specificity of the antibodies. Biochemical and molecular genetic studies have clarified the molecular basis of the histo-blood group ABO system.[2–4] The functional A and B alleles at the ABO genetic locus encode two glycosyltransferases, α-1-3-N-acetylgalactosaminyltransferase (A-transferase) and α-1-3-galactosyltransferase (B-transferase), respectively. Initial molecular genetic studies demonstrated that *ABO* is composed of seven exons spanning approximately 19.5 kb of genomic DNA (Fig. 1A)[5] and that two critical single-base substitutions in the last coding exon result in amino acid substitutions responsible for the different donor nucleotide-sugar substrate specificity between the A- and B-transferases.[6–8] A single-base deletion in Exon 6 is considered to shift the reading frame of codons and to abolish the transferase activity of A-transferase in most O alleles.[6]

On the other hand, some aspects remain to be explored.[9,10] The ABO antigens are expressed in a cell type–specific manner[9]; the isoantigens A, B, and H of blood groups A, B, and O are not confined to red blood cells (RBCs) but are also found in most secretions and on some epithelial cells. However, they are absent in connective tissue, muscle, and the central nervous system. Moreover, ABH antigens are known to undergo drastic changes during the development, differentiation, and maturation of cells in the epithelial and erythroid lineages.[10] For example, studies of A-antigen expression during the maturation of erythroid progenitors in a two-phase liquid culture system showed that A-positive cells gradually increased during erythroid maturation.[11–13] Fluorescence-activated cell sorting analysis with monoclonal antibodies has demonstrated the expression of A-antigens on colony cells derived from blast-forming units–erythroid and colony-forming units–erythroid.[14] In addition to these physiological processes, profound changes have also been documented in pathological conditions such as tumorigenesis. Reduction or complete deletion of A- or B-antigen expression in bladder and oral cancers has been documented.[15–17] Moreover, the loss of ABH antigens has been correlated with the progression of various cancers, including lung and bladder carcinomas.[18] Finally, since Yamamoto and colleagues[6] clarified the molecular genetic basis of the ABO system, a number of weak phenotypes have been found to be attributable to single nucleotide polymorphisms in the coding exons and splicing sites and hybrid formation between common alleles,[19,20] although other weak phenotypes for which no variant apparently exists in the coding exons and splicing sites have also been reported.[21–23] Therefore, to understand the molecular mechanism responsible for the control of ABO gene expression in a cell type–specific manner, during normal cell differentiation, in cancer cells lacking A or B antigens, and in weak phenotypes, it is essential to grasp how ABO gene transcription is regulated.

The DNA sequences in and around specific genes provide the code that dictates when, where, and at what level specific genes are transcribed.[24,25] This code comprises three parts: the core promoter where RNA production initiates and is directed toward Exon 2, the region proximal to
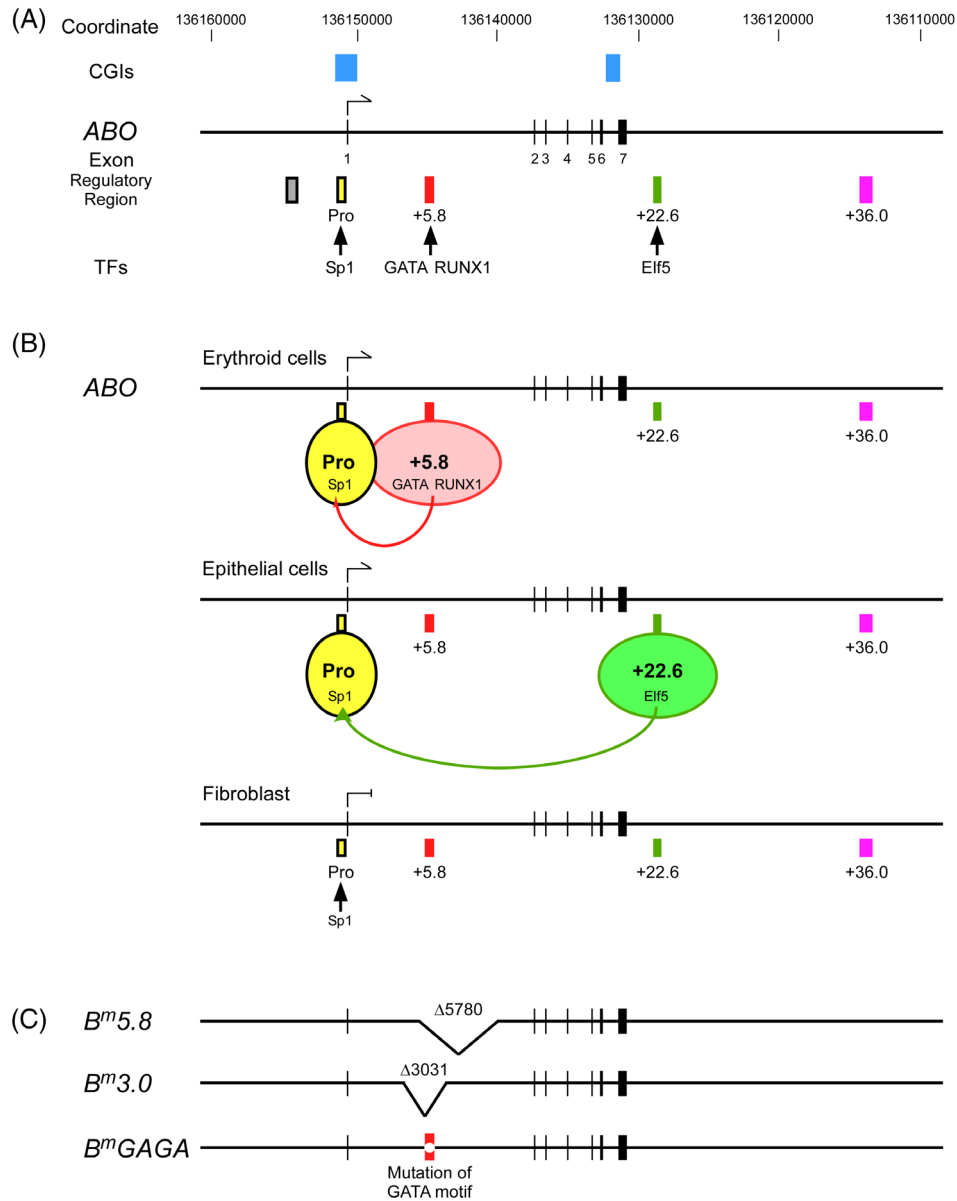
Fig. 1. Schematics of transcriptional regulation of *ABO* expression. (A) A map of the 50-kb region of genomic DNA from the upstream to downstream region of the human ABO gene. The diagram shows ABO gene Exons 1-7 represented as vertical lines with coordinates in hg19 and CGIs. Below the diagram, regulatory regions including the proximal promoter, the erythroid cell-specific regulatory element or the +5.8-kb site, the epithelial cell-specific regulatory element or the +22.6-kb site, and the DHS region +36.0 are shown. Pro, promoter: +5.8, the +5.8-kb site: +22.6, the +22.6-kb site: +36.0, region +36.0. The colored boxes are represented as follows: yellow, the proximal promoter; light red, the +5.8-kb site; dark green, the +22.6-kb site; purple, region +36.0. Also, the CCAAT-binding factor/NF-Y enhancer region is shown as a gray box. Binding of TFs to individual regions is also indicated. (B) Schematic diagram of cell-specific regulation of *ABO* expression. The top diagram indicates putative interaction of the proximal promoter with the +5.8-kb site for *ABO* expression in erythroid cells. The second diagram from the top indicates putative interaction of the proximal promoter with the +22.6-kb site for the *ABO* expression in epithelial cells. The third diagram from the top indicates the proximal promoter not interacting with either the +5.8-kb site or the +22.6-kb site without *ABO* expression in fibroblasts, in which neither *GATA-1,2* nor *Elf*5 is expressed. C. Schema of *B^m5.8*, *B^m3.0*, and *B^mGAGA*. The deletions in *B^m5.8* and *B^m3.0* are each represented by a V-shaped segment. Variant of the GATA motif in the +5.8-kb site of *B^mGAGA* is denoted as a clear circle in a red box. [Color figure can be viewed at wileyonlinelibrary.com]

the core promoter, and the more distant regulatory sequences that enhance RNA production. It has become obvious that enhancers usually work in groups (i.e., the locus control region and super-enhancers), each being bound by several transcription factors (TFs), forming a so-called enhanceosome. These enhanceosomes are nucleated

by pioneer TFs early during differentiation, and these TFs are subsequently replaced by other TFs that trigger recruitment of the preinitiation complex, involving RNA polymerase II, to the promoter. Enhancers also interact with each other through a multilooped structure. Thus, for elucidation of *ABO* regulation, it is important to reveal regulatory regions such as the core promoter, the region proximal to it, and the more distant enhancer, as well as the TFs that bind to those regions.

Although A- or B-antigen expression is dependent upon many steps including the structure of *ABO*, transcriptional regulation of *ABO*, translational regulation, modification or localization of A- or B-transferase, and H antigen expression, in this review we focus on the transcriptional regulation of the ABO gene through regulatory regions and TFs, and outline the molecular basis for weak phenotypes with variants in those regions.

REGULATORY REGIONS FOR *ABO* EXPRESSION

ABO gene regulatory regions have been identified by in vitro studies and genetic studies of weak phenotypes. The variants are described according to the HGVS nomenclature using the nucleotide sequences of accession numbers NG_006669.1 and NM_020469.1 as a reference in the genetic study sections. The relationship between the descriptions of variants in Intron 1 according to the Human Genome Variation Society (HGVS) nomenclature and those in the original reports is shown in Table 1. However, the positions that were used in the in vitro experiments described in the original papers remain unchanged. The positions reported in the original papers have been described according to the nucleotide sequences of accession number NT_035014.4 as a reference.

The proximal promoter of *ABO*

In vitro studies

Initially, Yamamoto et al.[5] demonstrated two transcription initiation sites upstream of the ATG translation start site in *ABO* using human pancreatic cDNA. Consistently, similar transcription initiation sites were found upstream of the translation start site using erythroid cells differentiated in vitro from AC133⁻CD34⁺ cells and K562 cells by us and others.[35,36]

For demonstrating the proximal promoter of *ABO*, we carried out transfection experiments into gastric cancer KATOIII cells and erythroleukemia HEL cells using *luciferase* reporter plasmids prepared from a genomic clone of human *ABO*. Those experiments defined the proximal *ABO* promoter between −150 and −2 relative to the translation start site in those cells (Figs. 1A and 2), and the promoter showed constitutive activity regardless of the cell types examined.[37,38] Electrophoretic mobility shift assays (EMSAs) demonstrated that the GC box at −56 to −44 in the promoter bound a ubiquitous TF Sp1 or Sp1-like protein(s) (Fig. 1A), whereas mutations of the recognition motif that abrogated binding of those factors reduced the promoter activity in both cell types.[38] Thus, Sp1 or Sp1-like protein(s) seemed to be important for proximal promoter activity. The nucleotide sequence in *ABO* reveals two CpG islands (CGIs) (Fig. 1A), one extending from the immediate 5′ flanking region through the first exon and into Intron 1, and the other extending from Intron 6 to Exon 7. Thus, the *ABO* proximal promoter is located within a CGI. Because a promoter within a CGI would include a few transcription initiation sites, the presence of several transcription initiation sites in *ABO* is relevant.

Genetic studies

Cai et al.[39] reported a nucleotide deletion between −35 and −18, i.e., c.−35_−18del, in the proximal *ABO* promoter in the B₃ phenotype (Fig. 2), which reduced the promoter activity in a plasmid-based reporter assay. Similarly, three single-nucleotide substitutions at −77, −76, or −68 in the *ABO*

TABLE 1. Relationship between the descriptions of variants in Intron 1 according to the HGVS nomenclature and those in the original reports*

Variants according to HGVS nomenclature Reference sequence: NG_006669.1 (NM_020469.2)	Variants in the original reports Reference sequence: NT_035014.4†, NG_006669.1‡ or KC841929§	Phenotype	Reference
c.28 + 4077_7107del	+4105_ + 7136del†	B_m	26
c.28 + 5110_10889del	+5137_ + 10914del†	B_m	27
c.28 + 5443_11354del	c.28 + 5443_29–1655del‡	A_x	28
c.28 + 5859G > C	c.28 + 5830G > C§	A_m	29
c.28 + 5861 T > G	+5890 T > G†	A_el, B_el, B_m	30,31
c.28 + 5864G > A	+5893G > A†	A₃	32
c.28 + 5865_5887del	+5892_ + 5914del†	A_m	33
c.28 + 5875C > T	+5904C > T†	B₃ or B_w	31
c.28 + 5880A > G	+5909A > G†	A₃	32
c.28 + 5885C > T	c.28 + 5885C > T‡	B₃	34

* The original reports are listed in the Reference section. The specific reference numbers are shown in the Reference column.
† Variants were described according to the reference sequence of NT_035014.4.
‡ Variant was described according to the reference sequence of NG_006669.1. It is likely that c.28 + 5443_11354del is easier to indicate the deletion size than c.28 + 5443_29–1655del.
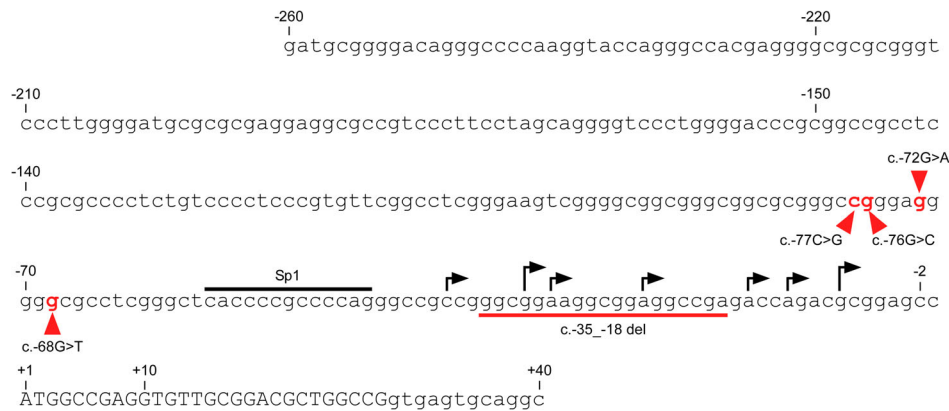§ Variant was described according to the reference sequence of KC841929.

Fig. 2. Nucleotide sequence of the 5′-flanking region in *ABO*. The sequence is given in full, from position −260 to +40, relative to the translation start ATG site of *ABO*. The upper case letters denote the coding sequence of Exon 1, and the lower case letters the noncoding genomic sequence. High arrows above the sequence indicate the transcription initiation sites that were determined by 5′-RACE using human pancreas cDNA by Yamamoto et al.,[5] and low arrows denote the transcription initiation sites that were determined by in vitro erythroid culture of AC133⁻CD34⁺ cells.[35] The proximal *ABO* promoter is located between −150 and −2 relative to the ATG translation start site.[37,38] The recognition motif for TF Sp1 is indicated by an overbar. Nucleotide substitutions at −77, −76, −72, and −68 are indicated in red, and the deletion between −35 and −18 is denoted by an underbar. [Color figure can be viewed at wileyonlinelibrary.com]

promoter, i.e., c.−77C > G, c.−76G > C, or c.−68G > T, respectively, have been reported in A₃ and B₃, each substitution reducing the promoter activity in *luciferase* reporter assays.[32,40] Recently, another single-nucleotide substitution at −72 in the *ABO* promoter, i.e., c.−72G > A, was reported in B₃.[41] These genetic variants confirm the functional significance of the proximal *ABO* promoter in vivo.

Conclusion

ABO transcription is likely regulated by the proximal promoter and cell-specific regulatory regions (Fig. 1B), because the proximal promoter shows constitutive activity regardless of cell type.[35]

Regulatory region for erythroid cell–specific expression of *ABO*

For delineation of distal regulatory regions involved in *ABO* regulation in erythroid cells, we used both in vitro experiments and genetic approaches.

In vitro studies

First, because DNase I–hypersensitive sites (DHSs) are associated with transcriptional regulatory regions including the promoter and distal enhancer, we prepared *luciferase* reporter plasmids on the basis of DHSs within a 15-kb region of genomic DNA in and around *ABO* in erythroleukemia K562 cells with publicly available data from DNase-Seq and FAIRE-seq on the University of California, Santa Cruz (UCSC) Genome Browser. Subsequent plasmid-based reporter assays demonstrated a distal regulatory region

between +5653 and +6154 relative to the translation start site, named the +5.8-kb site (Fig. 1A), in K562 cells, and the regulatory activity of this region was specific to erythroid cells.[27] According to the HGVS nomenclature, the +5.8-kb site corresponds to c.28 + 5624_6125. The same site was referred to the GATA binding site by the others.[41] EMSAs and chromatin immunoprecipitation (ChIP) assays demonstrated that the region bound hematopoietic TF GATA-1 or -2, and RUNX1 (Fig. 1A), whereas mutations of the recognition motifs that abrogated binding of those factors reduced the regulatory activities of the +5.8-kb site in K562 cells.[27,33,42] Thus, binding of GATA-1 or -2, and RUNX1 to the +5.8-kb site seemed to be crucial for the erythroid cell–specific activity of the region (Fig. 1B).

Genetic and serologic studies

Comparison of the genomic DNA in the *ABO* gene between humans and these primate species demonstrated high conservation between the ATG translation start codon and the stop codon of the *ABO* gene, except for a few regions. The +5.8-kb site is conserved among humans, chimpanzees, and orangutans, showing similar expression of the A and B antigens on RBCs. However, it is intriguing to note that the site is not conserved in rhesus monkeys and marmosets, in which the A and B antigens are expressed only slightly on RBCs. Therefore, a comparative approach could indicate involvement of the site in *ABO* expression in human erythroid lineage cells.

Genetic studies demonstrated a 5.8-kb deletion of c.28 + 5110_10889del in Intron 1 of *ABO* and a 3.0-kb deletion of c.28 + 4077_7107del in individuals with the B_m

phenotype, termed $B^m5.8$ and $B^m3.0$, respectively (Fig. 1C).[26,27] These deletions involved the +5.8-kb site. The B_m phenotype is characterized by the discrepancy of B antigen expression between RBCs and secretions[43]: B_m RBCs are not agglutinated by anti-B or anti-A,B antibody, whereas the saliva of B_m secretors contains about as much B substance as that of a normal B secretor. However, the B antigens on RBCs can only be detected by sensitive techniques such as adsorption and elution of anti-B. B_m erythrocytes contain abundant H sites, which can be converted into B sites by in vitro treatment with B-transferase derived from normal B individuals. B-transferase activity was detected in serum of B_m individuals, although the activity was distinctly reduced in all cases. The B_m trait is inherited as a rare allele at the *ABO* locus, although a few non-hereditary cases have also been reported.[44,45] Thus, deletion of the erythroid cell–specific regulatory region or the +5.8-kb site on the B^m allele could explain the discrepancy of B-antigen expression between RBCs and secretions in B_m. Further genetic studies found variants in the GATA motif of c.28 + 5861 T > G or c.28 + 5859G > C in B_m or A_m, respectively (Fig. 3).[29,30] The former was termed B^mGAGA. A_m is analogous to B_m in blood group A. Moreover, deletion of the RUNX1 binding motif of c.28 + 5865_5887del was revealed in A_m (Fig. 3).[33] A similar discrepancy of blood

Fig. 3. Alignment of variants within the +5.8-kb site found in weak phenotypes. The wild-type sequence between c.28 + 5852 and c.28 + 5891 in Intron 1 of *ABO* is shown at the top. The motifs for TFs GATA and RUNX1 are indicated by overbars. In alignment with the wild-type sequence, the variants found within the +5.8-kb site in weak phenotypes are shown. However, two reports have described variants in the coding exon of A_m,[46,47] while a number of variants of exons and splicing sites are reportedly associated with A_3 and B_3.[20] Those variants are described according to HGVS nomenclature using the nucleotide sequences of accession numbers NG_006669.1 and NM_020469.1 as a reference. Relationship between the variant descriptions according to the HGVS nomenclature and those in the original reports is shown in Table 1. Positions c.28 + 5852 and c.28 + 5891 correspond to +5881 and +5920 relative to the *ABO* translation start site, respectively, according to the nucleotide sequences of accession number NT_035014.4 as a reference, which was used in the original reports.[30,32,33] [Color figure can be viewed at wileyonlinelibrary.com]

antigen expression between RBCs and secretions is also observed in A_3 and B_3 phenotypes, where single nucleotide substitutions of c.28 + 5864G > A and c.28 + 5880A > G were found around the GATA and RUNX1 motifs in the +5.8-kb site (Fig. 3).[32] Similarly, single-nucleotide substitution of c.28 + 5885C > T was reported around the RUNX1 motif in an individual with B_3.[34] These genetic studies confirmed the regulatory significance of the +5.8-kb site for erythroid cell–specific expression of *ABO* in vivo.

Conclusion
It appears that the proximal promoter and the +5.8-kb site are required for *ABO* expression in an erythroid cell–specific manner (Fig. 1B), although it remains to be explored whether regions other than the +5.8-kb site might affect *ABO* expression in erythroid cells.

Regulatory region for epithelial cell–specific expression of *ABO*
In vitro studies
For delineation of *ABO* regulation in epithelial cells, we prepared *luciferase* reporter plasmids on the estimation of enhancers within a 50-kb region of genomic DNA in and around *ABO* in epithelial cells by publicly available data from DNase-Seq and chromatin state segmentation (ChromHMM) on the UCSC Genome Browser. Subsequent plasmid-based reporter assays indicated a distal regulatory region between +22563 and +22781 relative to the translation start site of *ABO*, termed the +22.6-kb site, in KATOIII cells (Fig. 1A), and the regulatory activity of the region was specific to epithelial cells.[48] Subsequently, we validated the significance of the +22.6-kb site with use of KATOIII cells with homozygote deletion of the site constructed by the CRISPR/Cas9 system. EMSAs and ChIP assays demonstrated that the region bound an epithelial cell–specific TF, Elf5 (Fig. 1A), whereas variant of the recognition motif that abrogated binding of the factor reduced the regulatory activity of the site in KATOIII cells. Thus, binding of Elf5 seemed to be crucial for the epithelial cell–specific activity of the region.

Conclusion
It is likely that the proximal promoter and the +22.6-kb site are required for *ABO* expression in epithelial cells (Fig. 1B), although regions other than the +22.6-kb site might also have some influence.

Other regulatory regions for *ABO* expression
CCAAT-binding factor/NF-Y enhancer region
The initial *luciferase* reporter assays with KATOIII cells demonstrated that a positive element was located between −3931 and −3650 from the translation start site where four tandem copies of 43-bp repeat units bound a positive TF, CCAAT-binding factor/NF-Y, through the CCAAT motif (Fig. 1A).[37] However, similar regulatory activity was not

observed in K562 cells and HEL cells of erythroid origin.[18,23] Thus, it was likely that the minisatellite was not involved in transcriptional regulation of *ABO* in erythroid cells. Genetic population studies revealed that both the B and O alleles are linked via four tandem copies of a 43-bp repeat unit, and that the A_1 allele is linked in the absence of this tandemly repetitive element.[49-51] Seltsam et al.[52] observed unexpected variations in the CCAAT-binding factor/NF-Y enhancer region including the repeat units in four individuals with weak B phenotypes, suggesting that those weak phenotypes might be caused by sequence variations in the enhancer region. On the other hand, Thuresson et al.[53] reported a hybrid allele between O^2 and *B* which lacked three repeat units, although the *B* transcript level was similar to that in fresh peripheral blood samples from normal controls. Thus, it remains controversial whether *ABO* transcription is influenced by the CCAAT-binding factor/NF-Y enhancer region in erythroid cells.[36,52,53]

Distal promoter of ABO

At the 5′ end of the CGI involving the *ABO* proximal promoter in cultured cells expressing *ABO*, an alternative starting Exon 1a comprising 27 base pairs was found by 5′-RACE (Fig. 1A). The level of transcription from Exon 1a was much lower than that from Exon 1.[35] The *luciferase* reporter assays demonstrated that the sequence located between −864 and −699 was responsible for transcription from Exon 1a in both erythroid and epithelial cell lineages. However, significance of Exon 1a remained elusive.

The region proximal to the proximal ABO promoter

The plasmid-based reporter assays demonstrated a negative regulatory element just upstream from the proximal *ABO* promoter in KATOIII cells and HEL cells,[54] suggesting that *ABO* transcription is regulated by negative elements in the −307 to −150 region from the translation start site. EMSAs indicated that this region bound to a nuclear factor from KATOIII cells. However, we have not identified this factor.

INFERENCES REGARDING ABO REGULATION

Cell type–specific expression

ABO transcription is regulated by a constitutive proximal promoter and a cell-specific regulatory region such as the +5.8-kb site or the +22.6-kb site (Fig. 1B). *Luciferase* reporter assays showed that the erythroid cell-specific regulatory activity of the +5.8-kb site was dependent upon binding of the erythroid cell–specific TF GATA-1 or -2.[27] In addition, variants in the GATA motif were found in B_m and A_m,[29,30] in which B- or A-antigen expression is reduced on RBCs, while a large amount of B or A substance is present in the saliva of secretors. Similarly, plasmid-based reporter assays demonstrated that the epithelial cell–specific regulatory activity of the

+22.6-kb site was dependent upon binding of the epithelial cell–specific TF Elf5.[48] In fibroblasts not expressing GATA-1 or -2, or Elf5, it is plausible that abrogation of the site's cell-specific regulatory activity contributes to lack of *ABO* expression.[48] Therefore, it is likely that the cell type–specific expression of *ABO* is dependent upon expression of cell-specific TFs binding to those cell-specific regulatory elements.

Cell differentiation–specific regulation

In vitro erythroid differentiation of $CD34^+$ cells and $AC133^-CD34^+$ cells from peripheral blood mononuclear cells indicated that *ABO* was expressed at an early stage and disappeared later,[35,42] and that the period when *ABO* was
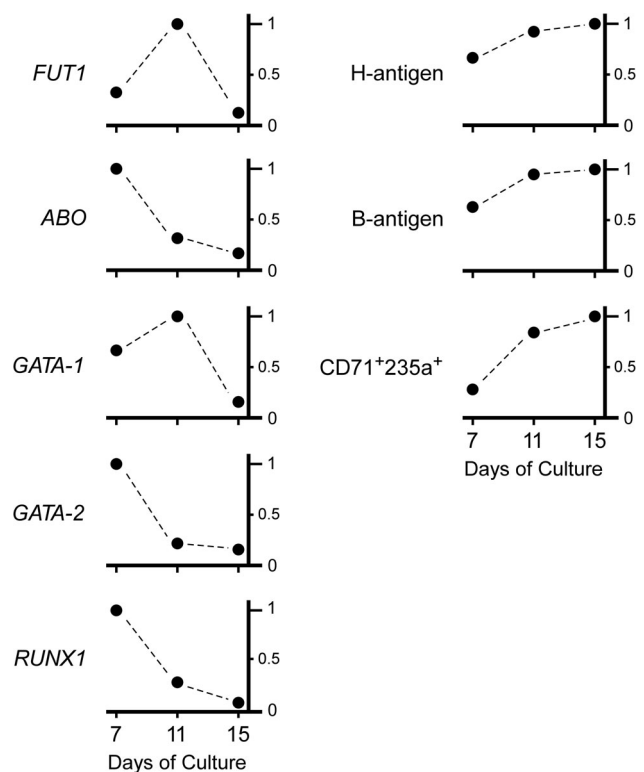

Fig. 4. Schematic illustration of the expression of cell surface antigens and genes during the course of in vitro erythroid cell differentiation. The diagrams were constructed with the expression of genes such as *FUT1, ABO, GATA-1, GATA-2,* and *RUNX1* as well as the cell surface expression of B antigen and H antigen 7 days, 11 days, and 15 days after in vitro erythroid cell differentiation from $CD34^+$ cells reported previously.[42] The time course of the relative expression of individual genes or antigens is indicated. The maximum gene expression or cell population showing antigen expression during erythroid differentiation was expressed as 1.0 on the vertical axis, whereas the relative expression ratios at other time points were calculated for individual genes and antigens. Also, changes in the proportions of cells expressing CD71 antigen and CD235 antigen that were not included in that report were added to the diagrams.

expressed at a higher level preceded that of *FUT1* expression (Fig. 4). Expression of *RUNX1* and *GATA-2* characteristically decreases during erythroid differentiation of CD34+ cells.[42] Thus, it seems likely that down regulation of *ABO* expression might be ascribed to reduction of RUNX1 in the later phase of erythroid differentiation. However, the mechanism of *ABO* expression at an early stage of erythroid differentiation remains to be explored.

Loss of ABO antigens in malignancy

ABH antigens are often absent from glycoproteins and glycolipids of malignant tissue in the gastrointestinal tract, oral cavity, uterine cervix, lung, prostate, breast, and bladder.[19,43] ABO antigen expression decreases as a result of down regulated transcription of *ABO*, which is ascribed to at least two different mechanisms: allelic loss and hypermethylation of the *ABO* promoter region in a CGI.[55–58]

There is abundant evidence that methylated CGIs at transcription start sites are associated with some silent genes. We and others have also demonstrated that hypermethylation of the *ABO* promoter could be responsible for absence of the *ABO* transcript and A-antigen in gastric and colon cancer cell lines.[56,57] Using clinical samples of oral squamous cell cancer, Gao et al.[58] showed that loss of A/B antigens was responsible for molecular events such as loss of the A/B allele or *ABO* promoter hypermethylation in two-thirds of tissue samples they examined. Thus, an additional mechanism for loss of A/B antigens other than allelic loss or promoter hypermethylation remained to be clarified in one-third of such cases. The origin of DNA hypermethylation is currently being clarified, and mutations in such genes as *Ten-eleven-translocation 2*, *DNA methylatransferase 3A*, and *isocitrate dehydrogenase 1 and 2* encoding epigenetic regulators are known to be involved in DNA methylation.[59] Besides mutated epigenetic regulators, mutations in splicing factors may also dysregulate epigenetics through splicing of epigenetic enzymes, and down regulation of transcription is associated with DNA hypermethylation of the promoter. Therefore, the causes and consequences of DNA hypermethylation need to be carefully distinguished.[59]

The association of weak A expression with acute myeloid leukemia (AML) is well documented.[19,43] Bianco-Miotto et al.[60,61] have reported that loss of ABH antigens from RBCs of patients with myeloid malignancies is a frequent phenomenon and that DNA methylation of the *ABO* promoter underlies the loss of *ABO* allelic expression in a significant proportion of leukemia patients. Such myeloid malignancies include AML, myelodysplastic syndrome (MDS) and myeloproliferative disorders including chronic myeloid leukemia. Recently, we have reported a patient with MDS in whom blood typing demonstrated mixed-field agglutination, prompting us to investigate the mechanism underlying blood group A-antigen reduction on RBCs.[62] Screening of somatic mutations using bone marrow cells demonstrated mutations in *ASXL1*, *EZH2*, *RUNX1*, and *WT1*. Experiments involving transient transfection into K562 cells showed that the expression of *ABO* was decreased by expression of the mutated *RUNX1*. Since the frame-shift mutation of *RUNX1* could encode an abnormally elongated protein without a transactivation domain that might act as a dominant negative inhibitor, it was plausible that this *RUNX1* mutation might be a genetic factor contributing to A-antigen loss on RBCs. This is another example of a factor other than DNA methylation that could be responsible for ABO antigen reduction in patients with leukemia. Therefore, there is a need for further investigation of patients with leukemia and ABO antigen reduction on RBCs to clarify which events are attributable to ABO-antigen loss on RBCs in leukemia.

The weak phenotype B_m with deletion or mutation of the +5.8-kb site

The prevalence of ABO subgroups was 0.048% among transfusion donors in Tokyo, Japan, in 2010-2011.[26] The total occurrence of the B_m and A_1B_m subgroups was 0.024% at the Kanto-Koshinetsu Blood Center, indicating that both are the most frequent ABO blood group variants in the Japanese population. A recent study demonstrated $B^m5.8$ in 1300 individuals, B^mGAGA in two, and $B^m3.0$ in one among 1303 Japanese individuals with B_m and A_1B_m with sequence-specific polymerase chain reaction (SSP) targeting $B^m5.8$ (Fig. 1A).[63] In practice, B_m is determined by serologic procedures and SSP targeting $B^m5.8$ at blood centers in Japan.[26] Genetic diagnosis of $B^m5.8$ is beneficial because B_m accounts for almost one-half of all weak phenotypes in the Japanese population. Based on the above observations, it seems likely that $B^m5.8$ might have been inherited over a long time period and spread throughout the Japanese population. Since the variant has not been reported in Korea and China, from where ancient people migrated to Japan, $B^m5.8$ could be specific to the Japanese population.[64]

Recently, the mutated GATA site in B^mGAGA has been reported in individuals with A_{el} and B_{el}.[31] Moreover, a 5.9-kb deletion of Intron 1 including the +5.8-kb site has been found in an individual with A_x.[28] It seems intriguing to explore the reason that the same mutation or a similar deletion of the +5.8-kb site could result in different phenotypes.

PROSPECT

As shown above, *ABO* transcription is regulated by the proximal promoter and cell-specific regulatory regions. However, *ABO* transcription was not completely lost in the cells with biallelic deletions of the +22.6-kb site.[48] Also, reporter assays demonstrated transcriptional activity in the DHS region +36.0 (Fig. 1A),[48] which was suggested to interact with the *ABO* transcription start site on the basis of publicly available data from GeneHancer Regulatory Elements and

Gene Interactions on the UCSC Genome Browser. In addition, recent studies have suggested coordinated activity of multiple enhancers to control a single gene, regulation of multiple genes by the same enhancer, and competition or coordination between neighboring promoters.[65,66] Therefore, it would be informative to investigate elements other than the promoter and cell-specific regulatory regions to clarify the regulatory mechanism of *ABO* transcription, and delineation of the regulatory mechanism of *ABO* transcription regulation would yield new insight into the regulatory network involving *ABO* and the genes associated with the *ABO* regulatory regions.

Histone deacetylase inhibitors are reported to reduce *ABO* expression in cultured cells, suggesting that *ABO* transcription may be regulated epigenetically.[67] This also appears to be an intriguing phenomenon in the context of cardiovascular disorders because it has been reported that the levels of von Willebrand factor (vWF) are approximately 25% higher in individuals with blood group types other than O, and this seems to be the main reason for the higher risk of venous thromboembolism and coronary heart disease in non–group O individuals.[68] Because it has been argued that addition of A/B-antigens to vWF in endothelial cells might influence circulating levels of vWF,[68] *ABO* suppression in cells might reduce the risk of developing these diseases. A similar argument could apply to organ transplantation. In cases of ABO-incompatible (ABOi) liver transplantation, acute humoral rejection directed against donor-oriented A/B antigens on endothelial cells of liver arteries or bile ducts is the most serious form of rejection, leading to graft loss.[69,70] Thus, a decrease in the amount of antigen on endothelial cells might ameliorate any adverse effects resulting from ABOi liver transplantation. Further studies may shed further light on *ABO* transcriptional regulation and provide clues for clinical applications.

CONCLUSION

Clarification of the mechanism of *ABO* transcriptional regulation has contributed to practical transfusion medicine, and delineation of the regulatory mechanism of *ABO* transcription regulation would yield new insight into the regulatory network involving *ABO* and the genes associated with the *ABO* regulatory regions on Chromosome 9.

CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

REFERENCES

1. Landsteiner K. Zur Kenntnis der antifermentativen lytischen und agglutinierenden Wirkung des Blutserums und der lymph. Zentralbl Bakteriol 1900;27:357-63.
2. Hakomori S. Antigen structure and genetic basis of histo-blood groups A, B and O. their changes associated with human cancer. Biochim Biophys Acta 1999;1473:247-66.
3. Yamamoto F. Molecular genetics of ABO. Vox Sang 2000;78:91-103.
4. Yamamoto F. Cloning and regulation of the ABO genes. Transfus Med 2001;11:281-94.
5. Yamamoto F, McNeill PD, Hakomori S. Genomic organization of human histo-blood group ABO genes. Glycobiology 1995;5:51-8.
6. Yamamoto F, Clausen H, White T, et al. Molecular genetic basis of the histo-blood group ABO system. Nature 1990;345:229-33.
7. Yamamoto F, Hakomori S. Sugar-nucleotide donor specificity of histo-blood group A and B transferases is based on amino acid substitutions. J Biol Chem 1990;265:19257-62.
8. Yamamoto F, McNeill PD. Amino acid residue at codon 268 determines both activity and nucleotide-sugar donor substrate specificity of human histo-blood group A and B transferases. in vitro mutagenesis study. J Biol Chem 1996;271:10515-20.
9. Clausen H, Hakomori S. ABH and related histo-blood group antigens; immunochemical differences in carrier isotypes and their distribution. Vox Sang 1989;56:1-20.
10. Dabelsteen E, Buschard K, Hakomori S, et al. Pattern of distribution of blood group antigens on human epidermal cells during maturation. J Invest Dermatol 1984;82:13-7.
11. Wada H, Suda T, Miura Y, et al. Expression of major blood group antigens on human erythroid cells in a two phase liquid culture systems. Blood 1990;75:505-11.
12. Bony V, Gane P, Bailly P, et al. Time-course expression of polypeptides carrying blood group antigens during human erythroid differentiation. Br J Haematol 1999;107:263-74.
13. Edvardsson L, Dykes J, Olsson ML, et al. Clonogenicity, gene expression and phenotype during neutrophil versus erythroid differentiation of cytokine-stimulated CD34+ human marrow cells *in vitro*. Br J Haematol 2004;127:451-63.
14. Sieff C, Bicknell D, Caine G, et al. Changes in cell surface antigen expression during hemopoietic differentiation. Blood 1982;60:703-13.
15. Cordón-Cardó C, Lloyd KO, Finstad CL, et al. Immuno-anatomic distribution of blood group antigens in the human urinary tract. Influence of secretor status. Lab Invest 1986;55:444-54.
16. Ørntoft TF. Expression and biosynthesis of ABH-related carbohydrate antigens in normal and pathologic human urothelium. APMIS Suppl 1990;17:1-34.
17. David L, Leitao D, Sobrinho-Simoes M, et al. Biosynthetic basis of incompatible histo-blood group A antigen expression: anti-A transferase antibodies reactive with gastric cancer tissue of type O individuals. Cancer Res 1993;53:5494-500.
18. Lee JS, Ro JY, Sahin AA, et al. Expression of blood-group antigen A- a favorable prognostic factor in non-small-cell lung cancer. N Engl J Med 1991;324:1084-90.
19. Daniels G. Human Blood Groups. 3rd ed. West Sussex, UK: Wiley-Blackwell; 2013.

20. [accessed 2020 Jan 19] Available from: https://www.isbtweb.org/workingparties/red-cell-immunogenetics-and-blood-group-terminology/.

21. Ogasawara K, Yabe R, Uchikawa M, et al. Molecular genetic analysis of variant phenotypes of the ABO blood group system. Blood 1996;88:2732-7.

22. Hansen T, Namork E, Olsson ML, et al. Different genotypes causing indiscernible patterns of A expression on A_{el} red blood cells as visualized by scanning immunogold electron microscopy. Vox Sang 1998;75:47-51.

23. Iwasaki M, Kobayashi K, Suzuki H, et al. Genetic analyses for the ABO blood group subtypes in Japanese. Jpn J Transfus Med 2000;46:532-9.

24. Vernimmen D. Uncovering enhancer functions using the α–globin locus. PLoS Genet 2014;10:e1004668.

25. Levine M, Cattoglio C, Tjian R. Looping back to leap forward: transcription enters a new era. Cell 2014;157:13-25.

26. Sano R, Kuboya E, Nakajima T, et al. A 3.0-kb deletion including an erythroid cell-specific regulatory element in intron 1 of the ABO blood group gene in an individual with the B_m phenotype. Vox Sang 2015;108:310-3.

27. Sano R, Nakajima T, Takahashi K, et al. Expression of ABO blood-group genes is dependent upon an erythroid cell-specific regulatory element that is deleted in persons with the B_m phenotype. Blood 2012;119:5301-10.

28. Wu PC, Lin YH, Tsai LF, et al. ABO genotyping with next-generation sequencing to resolve heterogeneity in donors with serology discrepancies. Transfusion 2018;58:2232-42.

29. Oda A, Isa K, Ogasawara K, et al. A novel mutation of the GATA site in the erythroid cell-specific regulatory element of the ABO gene in a blood donor with the A_mB phenotype. Vox Sang 2015;108:425-7.

30. Nakajima T, Sano R, Takahashi Y, et al. Mutation of the GATA site in the erythroid cell-specific regulatory element of the ABO gene in a B_m subgroup individual. Transfusion 2013;53:2917-27.

31. Ying Y, Hong X, Xu X, et al. A novel mutation +5904 C>T of RUNX1 site in the erythroid cell-specific regulatory element decreases the ABO antigen expression in Chinese population. Vox Sang 2018;113:594-600.

32. Takahashi Y, Isa K, Sano R, et al. Presence of nucleotide substitutions in transcriptional regulatory elements such as the erythroid cell-specific enhancer-like element and the ABO promoter in individuals with phenotypes A_3 and B_3, respectively. Vox Sang 2014;107:171-80.

33. Takahashi Y, Isa K, Sano R, et al. Deletion of the RUNX1 binding site in the erythroid cell-specific regulatory element of the ABO gene in two individuals with the A_m phenotype. Vox Sang 2014;106:167-75.

34. Tao C, Xiao J, Hu Y, et al. A novel B allele with c.28+5885C>T substitution in the erythroid cell-specific regulatory element identified in an individual with phenotype B_3. Transfusion 2017;57:1318-9.

35. Kominato Y, Hata Y, Takizawa H, et al. Alternative promoter identified between a hypermethylated upstream region of repetitive elements and a CpG Island in human ABO histo-blood group genes. J Biol Chem 2002;277:37936-48.

36. Thuresson B, Chester MA, Storry JR, et al. ABO transcript levels in peripheral blood and erythropoietic culture show different allele-related patterns independent of the CBF/NF-Y enhancer motif and multiple novel allele-specific variations in the $5'$- and $3'$-noncoding regions. Transfusion 2008;48:493-504.

37. Kominato Y, Tsuchiya T, Hata N, et al. Transcription of human ABO histo-blood group genes is dependent upon binding of transcription factor CBF/NF-Y to minisatellite sequence. J Biol Chem 1997;272:25890-8.

38. Hata Y, Kominato Y, Yamamoto F, et al. Characterization of the human ABO gene promoter in erythroid cell lineage. Vox Sang 2002;82:39-46.

39. Cai X, Jin S, Liu X, et al. Molecular genetic analysis of ABO blood group variations reveals 29 novel ABO subgroup alleles. Transfusion 2013;53:2910-6.

40. Isa K, Yamamuro Y, Ogasawara K, et al. Presence of nucleotide substitutions in the ABO promoter in individuals with phenotypes A_3 and B_3. Vox Sang 2016;110:285-7.

41. Hellberg Å, Hult AK, Moser I, et al. A novel single-nucleotide substitution in the proximal ABO promoter gives rise to the B_3 phenotype. Transfusion 2019;59:E1-3.

42. Sano R, Nogawa M, Nakajima T, et al. Blood group B gene is barely expressed in in votro erythroid culture of B_m-derived $CD34^+$ cells without an erythroid cell-specific regulatory element. Vox Sang 2015;108:302-9.

43. Schenkel-Brunner H. Human blood groups. 2nd ed. Wien: Springer; 2000. p. 54-150.

44. Kogure T, Iseki S. A family of B_m, due to a modifying gene. Proc Jpn Acad 1970;46:728-32.

45. Marsh ML, Ferrari M, Nichols E, et al. B_m^H: a weak B antigen variant. Vox Sang 1973;25:341-6.

46. Asamura H, Ota M, Takayanagi K, et al. Molecular genetic analysis of the A_m phenotype of the ABO blood group system. Vox Sang 2002;83:263-7.

47. Lin M, Hou MJ, Twu YC, et al. A novel A allele with 664G>A mutation identified in a family with the A_m phenotype. Transfusion 2005;45:63-9.

48. Sano R, Nakajima T, Takahashi Y, et al. Epithelial expression of human ABO blood-group genes is dependent upon a downstream regulatory element functioning through an epithelial cell-specific transcription factor, Elf5. J Biol Chem 2016;291:22594-606.

49. Yu LC, Chang CY, Twu YC, et al. Human histo-blood group ABO glycosyltransferase genes: different enhancer structures with different transcriptional activities. Biochem Biophys Res Commun 2000;273:459-66.

50. Shimada I, Kominato Y, Hata N, et al. DNA polymorphisms in the $5'$- flanking sequence of human ABO blood group genes and their association with the alleles for the common ABO phenotypes. Leg Med 1999;1:217-25.

51. Irshaid NM, Chester MA, Olsson ML. Allele-related variation in minisatellite repeats involved in the transcription of the blood group ABO gene. Transfus Med 1999;9:219-26.

52. Seltsam A, Wagner FF, Grüger D, et al. Weak blood group B phenotypes may be caused by variations in the CCAAT-binding factor/NF-Y enhancer region of the ABO gene. Transfusion 2007;47:2330-5.

53. Thuresson B, Hosseini-Maaf B, Hult AK, et al. A novel B^{weak} hybrid allele lacks three enhancer repeats but generates normal ABO transcript levels. Vox Sang 2012;102:55-64.

54. Kominato Y, Hata Y, Matsui K, et al. Transcriptional regulation of the human ABO histo-blood group genes is dependent on the N box upstream of the proximal promoter. Transfusion 2004;44:70-8.

55. Orlow I, Lacombe L, Pellicer I, et al. Genotypic and phenotypic characterization of the histoblood group ABO(H) in primary bladder tumors. Int J Cancer 1998;75:819-24.

56. Kominato Y, Hata Y, Takizawa T, et al. Expression of human histo-blood group ABO genes is dependent upon DNA methylation of the promoter region. J Biol Chem 1999;274: 37240-50.

57. Iwamoto S, Withers DA, Handa K, et al. Deletion of A-antigen in a human cancer cell line is associated with reduced promoter activity of CBF/NF-Y binding region, and possibly with enhanced DNA methylation of A transferase promoter. Glycoconj J 1999;16:659-66.

58. Gao S, Worm J, Guldberg P, et al. Genetic and epigenetic alterations of the blood group ABO gene in oral squamous cell carcinoma. Int J Cancer 2004;109:230-7.

59. Heuser M, Yun H, Thol F. Epigenetics in myelodysplastic syndromes. Semin Cancer Biol 2018;51:170-9.

60. Bianco-Miotto T, Farmer BJ, Sage RE, et al. Loss of red cells A, B, and H antigens is frequent in myeloid malignancies. Blood 2001;97:3633-9.

61. Bianco-Miotto T, Hussey DJ, Day TK, et al. DNA methylation of the ABO promoter underlies loss of ABO allelic expression in a significant proportion of leukemic patients. PLoS One 2009;4:e4788.

62. Hayakawa A, Sano R, Takahashi Y, et al. *RUNX1* mutation in a patient with myelodysplastic syndrome and decreased erythrocyte expression of blood group A antigen. Transfusion 2020;60:184-96.

63. Ogasawara K, Miyazaki T, Ito S, et al. The *B* allele with a 5·8 kb deletion in intron 1 of the *ABO* gene is the major allele in Japanese individuals with B_m and A_1B_m phenotypes. Vox Sang 2018;113:393-6.

64. Kominato Y, Ogasawara K. Is $B^m5.8$ specific to the Japanese population? Vox Sang 2019;114:185.

65. Fulco CP, Munschauer M, Anyoha R, et al. Systematic mapping of functional enhancer-promoter connections with CRISPR interference. Science 2016;354:769-73.

66. Engreitz JM, Haines JE, Perez EM, et al. Local regulation of gene expression by lncRNA promoters, transcription and splicing. Nature 2016;539:452-5.

67. Takahashi Y, Kubo R, Sano R, et al. Histone deacetylase inhibitors suppress *ABO* transcription *in vitro*, leading to reduced expression of the antigens. Transfusion 2017;57: 95-103.

68. Liumbruno GM, Franchini M. Beyond immunohaematology: the role of the ABO blood group in human diseases. Blood Transfus 2013;11:491-9.

69. Haga H, Egawa H, Fujimoto Y, et al. Acute humoral rejection and C4d immunostaining in ABO blood type-incompatible liver transplantation. Liver Transpl 2006;12:457-64.

70. Sakashita H, Haga H, Ashihara E, et al. Significance of C4d staining in ABO-identical/compatible liver transplantation. Mod Pathol 2007;20:676-84. 🔳