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Regulation of Blood Group Expression: Another Layer of Complexity to Consider

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The genetic code of the DNA sequences and their polymorphisms in coding regions of blood group genes essentially explain the general variation in blood group phenotypes observed in populations, including rarer variants. However, genetic polymorphism not only leads to changes of individual amino acids in proteins and premature stop signals, the two most common causes underlying blood group antigens and null phenotypes, but may also lead to phenotypic and immunogenic variation in other ways. Many of these less conventional ways may be placed in the realm of regulation of blood group expression.

The expression of proteins in general, and thus also of the proteins carrying or synthesizing blood group molecules and their antigens, is strictly regulated by molecular mechanisms in various phases: (1) transcriptional regulation involves the action of transcription factors (TFs) that bind to specific DNA sequences, promoters, and enhancers, influencing the initiation and rate of gene transcription. There are also epigenetic modifications such as DNA methylation and histone modifications playing a fundamental role in the control of gene expression. Following transcription, (2) RNA processing mechanisms further modulate protein expression. Alternative splicing allows for the generation of multiple mRNA isoforms from a single gene, diversifying the protein products. RNA editing can further modify mRNA sequences post-transcriptionally, impacting protein composition. (3) Regulation of translation, the process of synthesizing proteins from mRNA, is regulated by factors including initiation factors and microRNAs (miRNAs). Initiation factors control the assembly of the translation machinery, while miRNAs can bind to mRNA molecules, inhibiting their translation or promoting degradation. (4) Post-translational modifications add another layer of regulation. Phosphorylation, glycosylation with its evident relevance for the expression of blood group antigens, and ubiquitination are among the modifications that can alter protein function, stability, localization, or antigenicity. Additionally, (5) protein degradation mechanisms, such as proteasomal and lysosomal degradation, ensure the timely removal of misfolded or unnecessary proteins, maintaining cellular homeostasis. Direct effects on blood group antigens have not been described for all the mechanisms and regulatory cycles described above. For some, however, there are now increasing evidence and good examples to learn from.

Interest in the regulation of blood group antigen expression has increased greatly since it was reported that the TF KLF1 has significance for Lutheran blood group expression [1]. Many years earlier, the importance of TF GATA1 in the expression of the Duffy blood group system was recognized [2]. Since then, other TFs have

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been described that play important roles in the variation of human blood group antigens. For example, the P1PK system is dependent on several TFs, including RUNX1 and EGR1 [3, 4], while erythroid expression of Xg blood group protein relies on GATA1 [5, 6]. However, miRNAs are also recognized as increasingly important for the expression of blood group traits [7]. And for the first blood group system ever described, ABO, there are also various findings on the nature of its regulation of expression with consistent phenotypic relevance, including the important finding of GATA1 sites in intron 1, crucial for erythroid ABO expression [8]. Recently, more systematic approaches, combining bioinformatics and experimental efforts, have been applied to investigate the GATA1-driven blood group regulome, i.e., how this and other TFs regulate expression of all blood groups [9] and also the dependence of epigenetic factors and how TFs cooperate with each other [10]. This special issue of *Transfusion Medicine and Hemotherapy* intends to specifically address these types of observations.

In this issue, the review article by Ogasawara et al. [11] [this TMH] focuses on the transcriptional regulation of ABO expression and correlates phenotypes such as Bm, Am, B3, and A3 with variants in the promoter and other erythrocyte-specific regulatory regions of *ABO*, correlations that many believe should be included as proven allelic *ABO* variants in the ISBT allele tables (http://www.isbtweb.org/working-parties/red-cell-immunogenetics-and-blood-group-terminology/) as soon as possible. The article by Kronstein-Wiedemann et al. [12] [this TMH] addresses regulation at a completely different functional level, but also using the ABO model. The described role of specific miRNAs in the regulation of ABH antigen expression has only been recognized in recent years and is evidenced on the one hand by their binding to the 3′ untranslated region of the *ABO*-mRNA and on the other hand by the simultaneous regulation of the expression of ABO-associated TFs, such as Sp1. Specific miRNAs have now also been discovered for many other blood group genes and show pleiotropic effects in physiological and pathophysiological processes such as hematopoiesis and tumorigenesis.

Supported by laboratory data reported in this special issue for the first time, McGowan et al. [13] [this TMH] reported the analysis of bioinformatically predicted GATA1-binding regulatory motifs in the *RHD* gene for samples with low RhD antigen expression but normal *RHD* exons. Sequencing, electrophoretic mobility shift, and luciferase assays were employed and delivered among 13 previously unexplained samples with normal coding RHD sequence and low RhD expression, a new DEL allele, *RHD* c.1-110A>C, which had a mutated GATA1 motif in the proximal promoter. In the fourth and last specific contribution to this special issue, Wipplinger et al. [14] [this TMH] focused on the expression of Lewis blood group antigens. The Lewis blood group system relies on glycan antigens adsorbed onto erythrocyte membranes, synthesized mainly in epithelial cells of the digestive tract by fucosyltransferases FUT2 and FUT3. This study screened existing literature and utilized in silico prediction tools to identify regulators for *FUT2* and *FUT3*, unveiling various potential TFs, RNA binding proteins, and miRNAs influencing their transcription and translation. Understanding this regulation is helpful for comprehending Lewis blood group phenotypes, elucidating the role of Lewis antigens in pathologies, and identifying potential diagnostic targets for associated diseases.

In summary, the manuscripts in this special issue of *Transfusion Medicine and Hemotherapy* provide current examples of how the complex mechanisms of blood group expression can be examined, emphasizing the critical role of transcriptional, post-transcriptional, translational, and post-translational regulation. With the presented articles, we hope to have given the reader an introduction to the increasingly complex world of the regulation of blood group expression and its significance for the manifestation of the blood groups themselves, but also the diseases associated with them.

Conflict of Interest Statement

C.G. acts as a consultant to Inno-Train GmbH, Kronberg im Taunus, Germany, a provider of genotyping kits for molecular blood group diagnostics since 1998. C.G. holds the European and US patents P3545102 and US20190316189 on the "Determination of the genotype underlying the S-s-U-phenotype of the MNSs blood group system." M.L.O. is an inventor on European and US patents on Vel blood group genotyping and owns 50% of the shares in BLUsang AB, an incorporated consulting firm that receives royalties for said patents.

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Author Contributions

C.G. and M.L.O. wrote, reviewed, and approved the manuscript.

References

1 Singleton BK, Burton NM, Green C, Brady RL, Anstee DJ. Mutations in EKLF/KLF1 form the molecular basis of the rare blood group In(Lu) phenotype. Blood. 2008;112(5): 2081–8. https://doi.org/10.1182/blood-2008-03-145672

2 Tournamille C, Colin Y, Cartron JP, Le Van Kim C. Disruption of a GATA motif in the Duffy gene promoter abolishes erythroid gene expression in Duffy-negative individuals. Nat Genet. 1995;10(2):224–8. https://doi.org/10.1038/ng0695-224

3 Westman JS, Stenfelt L, Vidovic K, Möller M, Hellberg Å, Kjellström S, et al. Allele-selective RUNX1 binding regulates P1 blood group status by transcriptional control of A4GALT. Blood. 2018;131(14):1611–6. https://doi.org/10.1182/blood-2017-08-803080

4 Yeh CC, Chang CJ, Twu YC, Hung ST, Tsai YJ, Liao JC, et al. The differential expression of the blood group P1 -A4GALT and P2 -A4GALT alleles is stimulated by the transcription factor early growth response 1. Transfusion. 2018;58(4):1054–64. https://doi.org/10.1111/trf.14515

5 Möller M, Lee YQ, Vidovic K, Kjellström S, Björkman L, Storry JR, et al. Disruption of a GATA1-binding motif upstream of XG/PBDX abolishes Xga expression and resolves the Xg blood group system. Blood. 2018; 132(3):334–8. https://doi.org/10.1182/blood-2018-03-842542

6 Yeh CC, Chang CJ, Twu YC, Chu CC, Liu BS, Huang JT, et al. The molecular genetic background leading to the formation of the human erythroid-specific Xga/CD99 blood groups. Blood Adv. 2018;2(15):1854–64. https://doi.org/10.1182/bloodadvances.2018018879

7 Kronstein-Wiedemann R, Nowakowska P, Milanov P, Gubbe K, Seifried E, Bugert P, et al. Regulation of ABO blood group antigen expression by miR-331-3p and miR-1908-5p during hematopoietic stem cell differentiation. Stem Cell. 2020;38(10):1348–62. https://doi.org/10.1002/stem.3251

8 Sano R, Nakajima T, Takahashi K, Kubo R, Kominato Y, Tsukada J, 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(22):5301–10. https://doi.org/10.1182/blood-2011-10-387167

9 Wu PC, Lee YQ, Möller M, Storry JR, Olsson ML. Elucidation of the low-expressing erythroid CR1 phenotype by bioinformatic mining of the GATA1-driven blood-group regulome. Nat Commun. 2023;14(1):5001. https://doi.org/10.1038/s41467-023-40708-w

10 Wu PC, McGowan EC, Lee YQ, Ghosh S, Hansson J, Olsson ML. Epigenetic dissection of human blood group genes reveals regulatory elements and detailed characteristics of KEL and four other loci. Transfusion. 2024;64(6):1083–96. https://doi.org/10.1111/trf.17840

11 Ogasawara K, Sano R, Kominato Y. Review of ABO expression and variations based on transcriptional regulation of the ABO blood group gene. Transfus Med Hemother. 2024 Mar 12. https://doi.org/10.1159/000536556

12 Kronstein-Wiedemann R, Künzel SR, Thiel J, Tonn T. Role of miRNA in the regulation of blood group expression. Transfus Med Hemother. 2024 Jun 4. https://doi.org/000538866

13 McGowan EC, Wu PC, Hellberg Å, Lopez GH, Hyland CA, Olsson ML. A bioinformatically initiated approach to evaluate GATA1 regulatory regions in samples with weak D, Del or D- phenotypes despite normal RHD exons. Transfus Med Hemother. 2024 May 10. https://doi.org/10.1159/000538469

14 Wipplinger M, Mink S, Bublitz M, Gassner C. Regulation of the Lewis blood group antigen expression: a literature review supplemented with computational analysis. Transfus Med Hemother. 2024 Jun 19. https://doi.org/000538863