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ABO genotyping of various hematopoietic cell lines to select model cells for research purposes

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ABSTRACT

Various cells from humans and animals have been established as cell lines, and their features, characteristics, and origins have been reported. Many laboratories use cell lines as model cells, which are selected to suit research purposes.

We attempted to identify the *ABO* genotypes of 31 human leukemia and lymphoma cell lines stored in our laboratory using three methods: the PCR amplification of specific alleles (PASA), PCR-restriction fragment length polymorphism (RFLP), and the direct DNA sequencing of PCR products.

We distinguished 31 human leukemia and lymphoma cell lines examined into six major *ABO* genotypes: *A/O* (*A101/O01*: n = 1, *A101/O12*: n = 4, *A101/O26*: n = 1, *A101/O49*: n = 1, *A102/O01*: n = 3), *A/A* (*A101/A101*: n = 1, *A102/A102*: n = 2), *B/O* (*Bw29/O01*: n = 1), *B/B* (*B101/B101*: n = 2), *O/O* (*O01/O01*: n = 9, *O01/O02*: n = 1, *O01/O26*: n = 1, *O02/O03*: n = 1), and *A/B* (*A102/B101*: n = 3).

To the best of our knowledge, this is the first study to identify the *ABO* genotypes of various cell lines. The *ABO* genotypes of cell lines are important when selecting an experimental model cell for an ABO blood group study, and are essential information for cell lines. These results may be employed by research and clinical laboratories as well as in the forensic field.

1. Introduction

After extensive efforts to maintain tissues excised from individuals *in vitro* in the late 19th century, the successful cultivation of the cells of warm-blooded animals was achieved in 1910 [1], and was subsequently applied to mammalian cells [2]. Permanent cultures of animal cells have since been established [3], and Hela cells were the first cell line of human origin to be separated and established from the tumor lesion of a black woman in her thirties who died of cervical cancer in 1951 [4]. Hela cells are important as a model of human cancer cells and also of general human cells, and have been used in a large number of studies [5–9]. Various cells from humans and animals have since been established as cell lines, with their features, characteristics, and origins of cell lines being reported in detail. Cell lines are now being employed as model cells and are selected to suit research purposes.

In the present study, we attempted to identify the *ABO* genotypes of cell lines by focusing on the ABO blood group because information on the ABO blood types of cell lines is limited.

ABO blood group antigens are cell surface carbohydrates that occur in three allelic forms: the A, B, and O blood groups. After the discovery of ABO antigens on red blood cells by Karl Landsteiner in 1900 [10], the ABO blood group was classified into four antigens (A, B, O, and AB) and six genotypes (*A/A, A/O, B/B, B/O, O/O, and A/B*) in 1924 [11]. ABO blood group antigens are important in transfusions and organ transplantation, and are used as markers for personal identification in forensics [12]. Thus, *ABO* genotypes need to be considered by researchers

Abbreviations: PBPC(s), peripheral blood progenitor cell(s); PASA, PCR amplification of specific alleles; LOH, loss of heterozygosity

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when selecting an experimental model cell for an ABO blood group study, and are essential information for cell lines.

The *ABO* gene resides on chromosome 9q34.1-9q34.2 and contains 7 exons was elucidated, and has allowed ABO blood group antigens to be genetically analyzed using molecular biology techniques. In the present study, we identified the *ABO* genotypes of 31 cell lines stored in our laboratory.

2. Materials and methods

2.1. Cells

In the present study, the 31 human leukemia and lymphoma cell lines were used. These cell lines were classified into seven types based on differentiation and cell lineage. Myeloid cell lines were divided into two groups according to their differentiation stage. The PBPC types were derived from human chronic myeloid leukemia, K-562 and KOPM-28 [13,14]. Immature myeloid cell types were derived from erythroleukemia, HEL; acute monocytic leukemia, THP-1 and P31/FUJ; acute myelocytic leukemia, ML-1; acute promyelocytic leukemia, HL-60 and PL-21; eosinophilic leukemia, EoL-E5; and acute megakaryocytic leukemia, CMK [15–19]. Lymphoid cell lines were divided into five groups: common-type acute lymphocytic leukemia (ALL) (Reh, P30/OHK, HPB-ALL, KOPN-1, KM-3, and Nalm-6), T-cell-type ALL (CCRF-CEM, MOLT-4F, MOLT-3, MOLT-4B, and P12/ICH), matured peripheral T-cell types from adult T-cell leukemia (ATL-1K and MT-2) and chronic lymphocytic leukemia (SKW-3) [20–23], B-lymphoblastic types from B-ALL or Burkitt's lymphoma (Daudi, P32/ISH, P3HR-1, and BALL-1), and the matured B-cell types of B-immunoblasts to myeloma cells from immunoblastic lymphoma and myeloma (A4/Fuk, A3/KAW, and U-266) [24–28]. Cell lines were maintained in RPMI 1640 (Wako Pure Chemical Industries, Ltd., Tokyo, Japan) supplemented with 10% fetal bovine serum (Biosera, USA), 100 µg/mL penicillin G, and 100 µg/mL streptomycin at 37 °C in a humidified atmosphere of 5% CO_2. Fig. 1 shows the degrees of differentiation and cell lineages of the 31 cell lines examined.

Table 1
Allele-specific primer sets used in the PASA method.

Primer	Sequence (5′–3′)	Allele-specific band size (bp)	Allele specificity
fy-2* 526-A	CCGTTGGCCTGGTCGACCATCATGGCCTG CAGCTGTCAGTGCTGGAGGTGC	379	A or O
fy-43* 526-B2	GGATCCAGGGGTGCACGGCCGGCGGC CTGCCAGCGCTTGTAGGCGTC	224	B
fy-57* 261-O2G	GAATTCATGTGGGTGGCACCCTGCCA AGACAATGGGAGCCAGCCAAGGGGGA	104	O
AB-261N 261-AB	AGACAATGGGAGCCAGCCAAGGGGTC GAATTCAGGAAGGATGTCCTCGTGGTG	52	A or B

* Primers fy-2, fy-43, and fy-57 were quoted from Refs. [30–32].

2.2. DNA extraction

DNA from 1×10^7 cells, which had been washed with ice-cold PBS, was extracted with the ISOGEN kit (Nippon Gene, Tokyo, Japan) provided by Chomczynski and Sacchi [29], the DNA IQ System (Promega), or NucleoSpin Tissue (MACHEREY-NAGEL). The concentration and purity of extracted DNA were assessed by UV spectrophotometry and samples were stored at −20 °C until used.

2.3. ABO genotype analysis

The *ABO* genotypes of the genomic DNA of 31 cell lines were analyzed using three methods: the polymerase chain reaction (PCR)-amplification of specific alleles (PASA), PCR-restriction fragment length polymorphism (RFLP), and the direct DNA sequencing of PCR products.

The PASA method distinguished the different *ABO* genotypes of genomic DNA based on the molecular sizes of allele-specific amplification fragments that contained 261 and 526 nucleotides (the sites of amino acid substitutions) in *ABO* allelic cDNA. Table 1 shows a

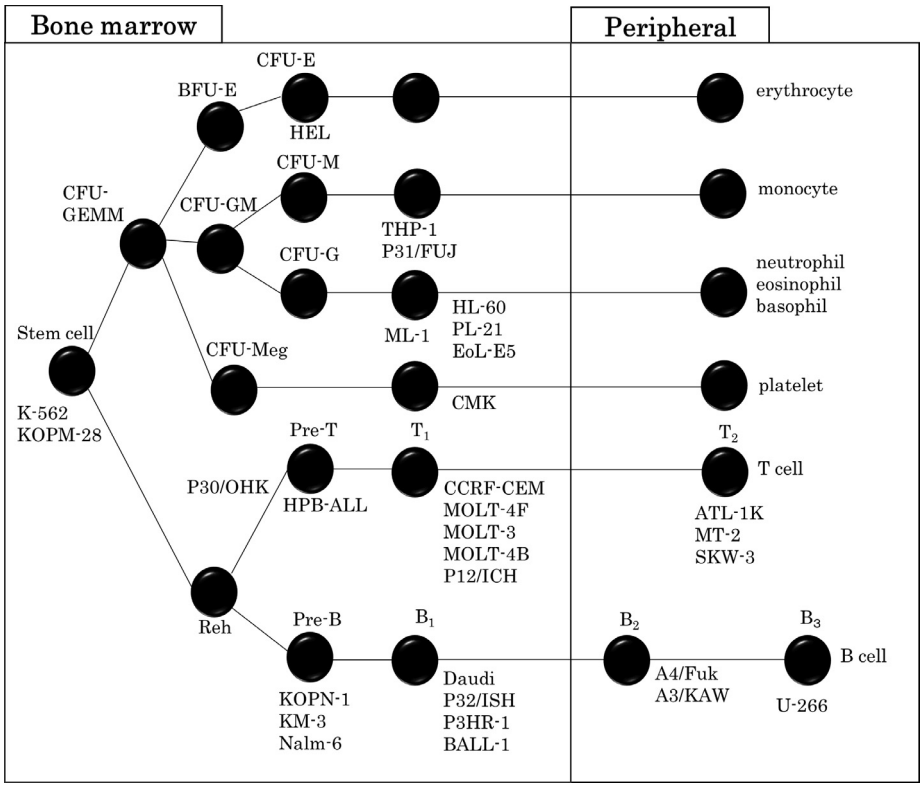

Fig. 1. Degrees of differentiation and cell lineages of various cell lines. The degrees of differentiation and cell lineages of the 31 cell lines used in the present study are shown: PBPC types (K-562 and KOPM-28), immature myeloid cell types (HEL, THP-1, P31/FUJ, ML-1, HL-60, PL-21, EoL-E5, and CMK), lymphoid cell lines: common type (Reh, P30/OHK, HPB-ALL, KOPN-1, KM-3, and Nalm-6), T-cell types (CCRF-CEM, MOLT-4F, MOLT-3, MOLT-4B, and P12/ICH), matured peripheral T-cell types (ATL-1K, MT-2, and SKW-3), B-lymphoblastic types (Daudi, P32/ISH, P3HR-1, and BALL-1), and matured B-cell types (A4/Fuk, A3/KAW, and U-266).

Table 2
ABO genotypes of 31 cell lines by PASA and PCR-RFLP methods.

Cell line		Origin*	Genotype	Cell line		Origin*	Genotype
Stem cells (n =	K-562	CML	*O/O*	T cells (n = 8)	CCRF-CEM	T-ALL T1	*A/O*
	KOPM-28	CML	*O/O*		MOLT-4F	T-ALL T1	*O/O*
Myeloid cells (n =	HEL	EL	*O/O*		MOLT-3	T-ALL T1	*O/O*
	THP-1	AMoL	*B/B*		MOLT-4B	T-ALL T1	*O/O*
	P31/FUJ	AMoL	*A/O*		P12/ICH	T-ALL T1	*A/O*
	ML-1	AML	*B/O*		ATL-1 K	ATL T2	*A/A*
	HL-60	APL	*A/O*		MT-2	ATL T2	*A/O*
	PL-21	APL	*A/O*		SKW-3	T-CLL T2	*A/O*
	EoL-E5	EoL	*O/O*	B cells (n = 7)	Daudi	Afn.BL B1	*A/A*
	CMK	AMKL	*O/O*		P32/ISH	Jpn.BL B1	*A/B*
Common ALLs (n =	Reh	cALL C/T	*A/B*		P3HR-1	Afn.BL B1	*A/A*
	P30/OHK	cALL C/T	*B/O*		BALL-1	B-ALL B1	*A/O*
	HPB-ALL	cALL C/T	*O/O*		A4/Fuk	ML.Imm B2-3	*A/O*
	KOPN-1	cALL C/B	*O/O*		A3/KAW	ML.Imm B2-3	*O/O*
	KM-3	cALL C/B	*A/B*		U-266	MM B3	*B/B*
	Nalm-6	cALL C/B	*A/B*				

* CML = chronic myelogenous leukemia; EL = erythroleukemia; AMoL = acute monocytic leukemia; AML = acute myelocytic leukemia; APL = acute promyelocytic leukemia; EoL = eosinophilic leukemia; AMKL = acute megakaryocytic leukemia; cALL = common-type acute lymphoblastic leukemia; T-ALL = T-cell-type ALL; ATL = adult T-cell leukemia; T-CLL = T-cell chronic lymphocytic leukemia; C/T = hybrid type having both antigens of CD and CD; C/B = hybrid type having both antigens of CD and CD; T1 = Thymic-T; T2 = peripheral-T; Afn. BL = African Burkitt's lymphoma; Jpn. BL = Japanese Burkitt's lymphoma; B-ALL = B-cell-type ALL; ML. Imm = malignant lymphoma Immunoblastic type; MM = multiple myeloma; B1, immature B; B2-3 = presecretory immunoblast; B3 = plasma cell.

Table 3
Comparisons of nucleotide changes (Exons 6–7) with several consensus alleles.

Allele		Nucleotide changes (Exons 6–7)																				
		261	467	503	526	547	595	646	657	681	689	703	768	771	784	796	802	803	829	871	927	930
A	*A101*	G	C	G	C	G	C	T	C	G	G	G	C	C	G	C	G	G	G	G	C	G
	A102	G	T	G	C	G	C	T	C	G	G	G	C	C	G	C	G	G	G	G	C	G
B	*B101*	G	C	G	G	G	C	T	T	G	G	A	C	C	G	A	G	C	G	G	C	A
	Bw29	G	C	A	G	G	C	T	T	G	G	A	C	C	G	A	G	C	G	G	C	A
O	*O01*	delG	C	G	C	G	C	T	C	G	G	G	C	C	G	C	G	G	G	G	C	G
	O02	delG	C	G	C	G	C	A	C	A	G	G	C	T	G	C	G	G	A	G	C	G
	O03	G	C	G	G	G	C	T	C	G	G	G	C	C	G	C	A	G	G	G	C	G
	O12	delG	C	G	C	G	T	A	C	A	G	G	C	T	G	C	G	G	A	G	C	G
	O26	delG	C	G	C	G	C	T	C	G	G	G	A	C	G	C	G	G	G	G	C	G
	O49	G	C	G	G	G	C	T	C	G	A	G	C	C	G	C	A	G	G	G	C	G

The A101 is the consensus allele of ABO.

summary of the primer combination, primer sequences, allele-specific band size, and allele specificity [30–32]. The details of the procedure and validation of the PASA method for *ABO* genotyping were reported elsewhere [33–35].

The PCR-RFLP method was used to confirm that the DNA fragments were amplified by PCR using the DNA extracted from cell lines that had an *O* allele sequence with an O_{261} single nucleotide G deletion. We used two restriction enzymes, KpnI and BstEII, to distinguish the *O* allele and *A* or *B* alleles [34,36].

The direct DNA sequencing method was used to elucidate the nucleotide sequence in detail, and distinguish the different *ABO* genotype subtypes. In the present study, we examined two exons, 6 and 7, which were previously reported to be important regions for protein activity [32].

After extracting DNA from various cell lines, amplification was performed for exons 6 and 7 by PCR. After the purification of amplification products with the FastGene Gel/PCR Extraction kit (NIPPON Genetics), they were reacted with the big dye Terminator v3.1 Cycle Sequencing kit (Thermo Fisher Scientific) and analyzed using an ABI PRISM® 310 Genetic Analyzer or ABI PRISM® 3100 Genetic Analyzer (Thermo Fisher Scientific). The direct DNA sequencing was performed with following primers; forward primer: 6-F (5′-GTGTGCCAGAGGCGC ATGTG-3′), located on intron 5 and reverse primer: 6-R (5′-GAGAGA CCTCAATGTCCACA-3′), located on exon 6, and forward primers: 7-F (5′-GGACGGGCCTCCTGCAGCCC-3′), located on intron 6, and 7-F2(5′-CAGCGAGGTGGATTACCTGG-3′) and reverse primer: 7-R (5′-TCAC GGGTTCCGGACCGCCT-3′), located on exon 7 in the present study.

3. Results

3.1. ABO genotyping using PASA and PCR-RFLP methods

The PASA reaction was used to distinguish the different *ABO* genotypes of 31 human leukemia and lymphoma cell lines based on the molecular sizes of allele-specific amplification products containing 261 and 526 nucleotides (the sites of amino substitutions) in *ABO* allelic cDNA. In this PASA method, the 379 bp, 224 bp, 104 bp, and 52 bp bands were specific bands of the *A* and *O* alleles, *B* allele, *O* allele, and the *A* and *B* alleles, respectively. The 31 cell lines were analyzed in the PASA method, and the presence or absence of the *O* allele in all cell lines was confirmed by PCR-RFLP (date not shown). The results obtained from the PASA and PCR-RFLP methods matched; therefore, we were able to identify the *ABO* genotypes of the 31 cell lines examined (type *A/O*: n = 9, type *A/A*: n = 3, type *B/O*: n = 2, type *B/B*: n = 2, type *O/O*: n = 11, and type *A/B*: n = 4). Each cell line and these *ABO* genotypes were shown in Table 2.

In the present study, *ABO* genotyping was analyzed at least twice using DNA extracted by two out of the three methods employed (ISOGEN, DNA IQ System, or NucleoSpin).

Table 4
ABO nucleotide sequences in stem cells, myeloid cell, and lymphoma cell lines in the present study.

Cell Line		Origin*	Genotype		Nucleotide changes (Exons 6–7)																				
					261	467	503	526	547	595	646	657	681	689	703	768	771	784	796	802	803	829	871	927	930
Stem cells (n = 2)	K562	CML	*O/O*	*O01/O01*	delG	C	G	C	G	C	T	C	G	G	G	C	C	G	C	G	G	G	G	C	G
	KOPM-28	CML			delG	C	G	C	G	C	T	C	G	G	G	C	C	G	C	G	G	G	G	C	G
Myeloid cells (n = 8)	P31/FUJ	AMoL	*A/O*	*A101/O01*	G/delG	C	G	C	G	C	T	C	G	G	G	C	C	G	C	G	G	G	G	C	G
	PL-21	APL		*A102/O01*	G/delG	T/C	G	C	G	C	T	C	G	G	G	C	C	G	C	G	G	G	G	C	G
	HL-60	APL		*A101/O26*	G/delG	C	G	C	G	C	T	C	G	G	G	C/A	C	G	C	G	G	G	G	C	G
	THP-1	AMoL	*B/B*	*B101/B101*	G	C	G	G	G	C	T	T	G	G	A	C	C	G	A	G	C	G	G	C	A
	HEL	EL	*O/O*	*O01/O01*	delG	C	G	C	G	C	T	C	G	G	G	C	C	G	C	G	G	G	G	C	G
	CMK	AMKL			delG	C	G	C	G	C	T	C	G	G	G	C	C	G	C	G	G	G	G	C	G
	EoL-E5	EoL		*O01/O26*	delG	C	G	C	G	C	T	C	G	G	G	C/A	C	G	C	G	G	G	G	C	G
	ML-1	AML		*O02/O03*	delG/G	C	G	C/G	G	C	A/T	C	A/G	G	G	C	C/T	G	C	G/A	G	A/G	G	C	G
Common ALL cells (n = 6)	Nalm-6	cALL C/B	*A/O*	*A101/O49*	G	C	G	C/G	G	C	T	C	G	G/A	G	C	C	G	C	G/A	G	G	G	C	G
	P30/OHK	cALL C/T	*B/O*	*Bw29/O01*	G/delG	C	A/G	G/C	G	C	T	T/C	G	G	A/G	C	C	G	A/C	G	C/G	G	G	C	A/G
	HPB-ALL	cALL C/T	*O/O*	*O01/O02*	delG	C	G	C	G	C	T/A	C	G/A	G	G	C	C/T	G	C	G	G	G/A	G	C	G
	KOPN-1	cALL C/B		*O01/O01*	delG	C	G	C	G	C	T	C	G	G	G	C	C	G	C	G	G	G	G	C	G
	Reh	cALL C/T	*A/B*	*A102/B101*	G	T/C	G	C/G	G	C	T	C/T	G	G	G/A	C	C	G	C/A	G	G/C	G	G	C	G/A
	KM-3	cALL C/B			G	T/C	G	C/G	G	C	T	C/T	G	G	G/A	C	C	G	C/A	G	G/C	G	G	C	G/A
T cells (n = 8)	CCRF-CM	T-ALL T1	*A/O*	*A101/O12*	G/delG	C	G	C	G	C/T	T/A	C	G/A	G	G	C	C/T	G	C	G	G	G/A	G	C	G
	P12/ICH	T-ALL T1			G/delG	C	G	C	G	C/T	T/A	C	G/A	G	G	C	C/T	G	C	G	G	G/A	G	C	G
	SKW-3	T-CLL T2			G/delG	C	G	C	G	C/T	T/A	C	G/A	G	G	C	C/T	G	C	G	G	G/A	G	C	G
	MT-2	ATL T2		*A102/O01*	G/delG	T/C	G	C	G	C	T	C	G	G	G	C	C	G	C	G	G	G	G	C	G
	ATL-1 K	ATL T2	*A/A*	*A102/A102*	G	T	G	C	G	C	T	C	G	G	G	C	C	G	C	G	G	G	G	C	G
	MOLT-3	T-ALL T1	*O/O*	*O01/O01*	delG	C	G	C	G	C	T	C	G	G	G	C	C	G	C	G	G	G	G	C	G
	MOLT-4F	T-ALL T1			delG	C	G	C	G	C	T	C	G	G	G	C	C	G	C	G	G	G	G	C	G
	MOLT-4B	T-ALL T1			delG	C	G	C	G	C	T	C	G	G	G	C	C	G	C	G	G	G	G	C	G
B cells (n = 7)	BALL-1	B-ALL B1	*A/O*	*A102/O01*	G/delG	T/C	G	C	G	C	T	C	G	G	G	C	C	G	C	G	G	G	G	C	G
	A4/Fuk	ML.ImmB2-3		*A101/O12*	G/delG	C	G	C	G	C/T	T/A	C	G/A	G	G	C	C/T	G	C	G	G	G/A	G	C	G
	Daudi	Afn.BL B1	*A/A*	*A101/A101*	G	C	G	C	G	C	T	C	G	G	G	C	C	G	C	G	G	G	G	C	G
	P3HR-1	Afn.BL B1		*A102/A102*	G	T	G	C	G	C	T	C	G	G	G	C	C	G	C	G	G	G	G	C	G
	U-266	MM B3	*B/B*	*B101/B101*	G	C	G	G	G	C	T	T	G	G	A	C	C	G	A	G	C	G	G	C	A
	A3/KAW	ML.ImmB2-3	*O/O*	*O01/O01*	delG	C	G	C	G	C	T	C	G	G	G	C	C	G	C	G	G	G	G	C	G
	P32/ISH	Jpn.BL B1	*A/B*	*A102/B101*	G	T/C	G	C/G	G	C	T	C/T	G	G	G/A	C	C	G	C/A	G	G/C	G	G	C	G/A

3.2. ABO genotyping by direct DNA sequencing

In this analysis, base substitutions in the exon 6 and 7 regions of the *ABO* gene of 31 cell lines were analyzed by direct DNA sequencing to identify the genotype of the *ABO* allele. Table 3 shows the base substitutions of representative *ABO* alleles revealed in the present study. Table 4 also summarize the direct DNA sequence analysis results, origins, and *ABO* genotypes of each cell line. As shown in Tables 2 and 4, the *ABO* genotypes by direct DNA sequencing differed from the results of the PASA and PCR-RFLP methods in two cell lines (ML-1 and Nalm-6). Although detailed genotyping was also performed on other cells, the results obtained were the same as those of the PASA and PCR-RFLP methods.

4. Discussion

Various cells from humans and animals have been established as cell lines, and their features, characteristics, and origins have been reported. Many laboratories use cell lines as model cells, which are selected to suit research purposes. In our laboratory, we have been performing fundamental research on analysis technologies and the expression mechanisms of ABO blood group antigens, which are important in the area of clinical laboratory examinations, particularly for transfusions and transplantation immunity. In the present study field, the *ABO* genotyping of cell lines is important when selecting an experimental model cell.

We have conducted research using many cell lines at different stages of differentiation as experimental model cells. However, information on the ABO blood types of various cell lines is limited [37]. Therefore, we herein attempted to identify the *ABO* genotypes of 31 cell lines stored in our laboratory. The degrees of differentiation, cell lineages, and origins of these cell lines differed. The *ABO* genotype of each cell line was analyzed using three methods: PASA and PCR-RFLP, which we described previously [32–35], and the direct DNA sequencing method.

ABO genotypes in the present study were identified based on the discrimination of the three major alleles by PASA and PCR-RFLP analyses of two SNPs at nucleotides 261 and 526 in *ABO* allelic cDNA. Therefore, when there are other base substitutions in *ABO* allelic cDNA, the identification of the *ABO* genotype is different. The *ABO* gene is known to have many polymorphisms and these *ABO* alleles were shown to have extensive sequence variations in the coding region of the *ABO* gene. Therefore, we attempted a direct DNA sequencing analysis of the two largest exon (6 and 7) regions of the *ABO* gene, which encode approximately 77% of the full coding region of the *ABO* gene and are important for ABO transferases activity [38]. As a result, in two cell lines (ML-1 and Nalm-6), the *ABO* genotypes identified by direct DNA sequencing differed from those using the PASA and PCR-RFLP methods.

Although the genomic DNA of the ML-1 cell line showed four amplified specific bands (379, 224, 104, and 52 bp) in the PASA analysis and the *ABO* genotype was identified as type *B/O*, the *B* allele was considered to be the *O* allele (*O03*) because of the presence of the base substitutions 526C > G and 802G > A on the *A101* allele background in direct DNA sequencing. On the other hand, although the genomic DNA of the Nalm-6 cell line showed three amplified specific bands (379, 224, and 52 bp) in the PASA analysis and the *ABO* genotype was identified as type *A/B*, the *B* allele was considered to be the *O* allele (*O49*) based on the presence of the base substitutions 526C > G, 689G > A, and 802G > A on the *A101* allele background in direct DNA sequencing.

In this analysis, we identified the rare allele *Bw29* in the P30/OHK cell line. The *Bw29* allele has the base substitution 503G > A on the *B101* allele background. The remaining cell lines were analyzed and classified. The conventional methods of checking 261 and 526 nucleotides may be used to identify *ABO* genotypes; however, it has become clear that erroneous results were obtained for two cell lines. Thus, direct DNA sequencing is necessary to obtain an accurate *ABO* genotype.

In addition, A and B antigens on the surface of each cell lines were examined using flow cytometry. The positive rate of A and B antigens was less than 0.5% in all cell lines, and A and B antigens were not detected in any cell line (data not shown). It is considered that the 31 cell lines do not express A or B antigen. Although the antigen was not confirmed, this may be affected by methylation of the *ABO* gene promoter region. This will be considered in the future.

A common issue in these types of cell lines is the risk of the loss of heterozygosity (LOH). Since 17 out of the 31 cell lines in the present study were heterozygotes, there was no LOH for these cell lines. On the other hand, the remaining 14 cell lines were homozygous. Thus, while these cell lines may be homozygous for a specific *ABO* allele (in the current version *A/A*, *B/B*, or *O/O*), they may actually be *A/-*, *B/-* or *O/-*. In bladder cancer, the loss of type A blood group antigen expression was previously reported [39]. The effects of LOH are considered to be due to many deletions on chromosome 9 being observed in bladder cancer and LOH also occurring in other cancers, which may affect the *ABO* allele similar to bladder cancer. Therefore, limited information is currently available on LOH in ABO blood types; however, the possibility of LOH needs to be considered.

Moreover, one more issue is the risk of an oversight of A^w and B^w allele that cause weak expression found earlier in the gene or in the regulatory regions. There is also a report that partial deletion of intron 1 was associated with the B (m) phenotype [40]. This risk is of course minor but needs to be considered. Further, although *FUT1* and *FUT2* gene involved in H antigen expression has a strong relationship with the *ABO* gene, it has not been analyzed in this cohort.

Many of the cell lines we possess have been passaged and stored for approximately 34 years since their provision by Dr. Masanori Shimoyama in 1985. The characteristics of cell lines may differ between different institutions, even in the same cell lines, and this possibility cannot be denied, even in the cell lines examined in the present study. However, the results obtained for the *ABO* genotypes of the cell lines examined in the present study are considered to be useful in many research fields. Accordingly, although the identity of each cell line that we passaged and stored may not be sufficient, we encourage researchers to use the present results as a reference.

In conclusion, we identified the *ABO* genotypes of the 31 human leukemia and lymphoma cell lines examined herein. To the best of our knowledge, this is the first study to identify the *ABO* genotypes of various cell lines using molecular biology techniques. The *ABO* genotypes of cell lines are important when selecting an experimental model cell for an ABO blood group study, and are essential information for cell lines. These results may be employed by research and clinical laboratories, as well as the forensic field in the future.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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