STEM CELLS AND DEVELOPMENT 17:853–856 (2008)

DOI: 10.1089/scd.2007.0226

# Letters to the Editor

## H1 and H9 Human Embryonic Stem Cell Lines Are Heterozygous for the *ABO* Locus

You-Tzung Chen,[1,3] Marion Dejosez,[2] Thomas P. Zwaka,[2] and Richard R. Behringer[1]

### To the Editor

The establishment of human Embryonic Stem (ES) cell lines holds great promise for regenerative medicine (1). This is because ES cells can be propagated indefinitely *in vitro* and they have the potential to differentiate into a variety of cell lineages (2). However, to use human ES cells and their derivatives for therapeutic cell transplantation, as it is in all other transplantation treatments, the first obstacle encountered is the histocompatibility problem. One possible way to overcome this histocompatibility barrier is to genetically modify the histocompatibility loci within the human ES cell genome (3, 4). *ABO* is the most important histocompatibility locus that determines the ABO histo-blood type. The hyperacute rejection in an ABO-incompatible transfusion or solid organ transplantation may lead to a lethal consequence. Therefore it is important to avoid ABO incompatibility when transplanting human ES cell-derived tissues. Here we describe the characterization of *ABO* alleles in H1 and H9, two of the most widely distributed and researched human ES cell lines among the 78 human ES cell lines on the NIH Human Embryonic Stem Cell Registry (http://stemcells.nih.gov/research/registry/). Our findings suggest that H1 is heterozygous for the *ABO* locus and bears two different O type alleles, *O101* and *O201*. H9 carries *ABO* alleles of 2 different types, one is A1 type (*A101*) and the other is O type (*O101*).

The ABO blood group system was first described by Karl Landsteiner more than a century ago. It is a phenomenon observed only in *Catarhinni* (human, apes, and old word monkeys)(5). The presence of A and/or B antigens on the surface of the red blood cell, endothelium and almost all the epithelia is determined by different galactosyltransferase alleles carried in a highly polymorphic locus, the *ABO* locus (6–9). So far there are at least 180 ABO alleles in the Blood Group Antigen Gene Mutation Database (http://www.ncbi.nlm.nih.gov/projects/mhc/xslcgi.fcgi). Three types *ABO* alleles, A type, B type, and O type, are commonly found in the human population. The A type alleles encodes A transferase (α1-3 N-acetylgalactosaminyltransferase), which puts N-acetylgalactosamnine (UDP-GalNAc) to the H antigens (Fuctose-α1-2-Galactose-β1-R). The most common A allele found in the human population is *A101*, which is used as the reference sequence for the *ABO* gene. *A101* also represents the A1 subtype alleles (responsible for 80% A phenotypes). There are A2 subtype alleles, observed at a lower frequency (about 20% of A phenotype), which create a phenotype with only about 1/5 A antigens on the surface of red blood cells compared to that of the A1 subtype. The O alleles are loss-of-function alleles. A frameshift mutation, 261ΔG, which results in the mistranslation of the glactosyltransferase, is found in the majority of the O alleles. The B alleles encode B transferase (α1-3 galactosyltransferase), which possesses different substrate specificity. Instead of building an A antigen, B transferases use galactose (UDP-Gal) as a substrate to build B antigens. There are seven B allele-specific single nucleotide polymorphisms (SNPs), four of them (C526G, G703A, C796A, and G803C) fall within the enzyme catalytic domain and result in 4 amino acid changes in the substrate binding pocket, Arg176Gly, Gly235Ser, Leu266Met, and Gly268Ala, thus changing the substrate specificity (10). In rare cases, *cis*-AB (AAAB), which possesses both A transferase and B transferase activity, and B(A)(BABB), which possesses a strong B weak A transferase activity, are also observed.

To examine the *ABO* genotypes of H1 and H9 human ES cells, genomic sequences of exon 6, 7, and intron 2 were amplified by polymerase chain reactions (PCR) and sequenced. Related primer sequences used in this study were listed in Table 1. PCR were performed using a high fidelity DNA polymerase kit (Expand Long Template PCR System, Roche Applied Science, Indianapolis, IN) on genomic templates of H1 cells (passage 29) and H9 cells (passage 27) (WiCell,

[1]Department of Molecular Genetics and Center for Stem Cell and Developmental Biology, The University of Texas, M.D. Anderson Cancer Center, Houston, Texas.
[2]Department of Molecular and Cellular Biology and Center for Cell and Gene Therapy, Baylor College of Medicine, Houston, Texas.
[3]Current Address: Graduate Institute of Clinical Medcine, National Taiwan University College of Medicine, Taiwan.

Table 1. ABO-Specific Primer Sequences Used in this Study

| *Description* | *Sequence (5′–3′)* |
|---|---|
| PCR amplification primers | |
| intron 2 – 5′ | GGC CAG ATC TGG ACT GGG TTT GG |
| intron 2 – 3′ | GGC CAC AGA GTT GAG CAT GTC TAC AC |
| exon 6 – 5′ | GGT TGG AGT CGC ATT TGC CTC TGG |
| exon 6 – 3′ | GAG GAC AAG GCT GGC CGC CAC |
| exon 7 – 5′ | GTG CAG GAC GGG CCT CCT GC |
| exon 7 – 3′ | GGC CTA GGC TTC AGT TAC TCA CAA CAG |
| Sequencing primers | |
| exon 6 – 5′ | CGC ATG TGG GTG GCA CCC TGC |
| exon 6 – 3′ | CTC TGT CTT GAA CAC AAG GAG AGA CCT C |
| exon 7 – 5′ | CCC GTC CGC CTG CCT TGC AG |
| exon 7 – 3′ | CTC CCA GAG CCC CTG GCA GC |

Madison, WI). PCR products were gel purified and either directly sequenced or subcloned to a pGEM-T Easy Vector (Promega, Madison, WI) and then sequenced using T7 or SP6 primers. Table 2 summarizes the SNP features in exon 6 and 7 of the two human ES cell lines. First, from sequencing the H1 exon 6 PCR product we found that H1 is homozygous for 261ΔG, a character for O type alleles, but shows heterozygeneity (A/G) at nucleotide position 297. Sequence analysis of the H1 exon 7 PCR product and plasmid clones (H1-Ex7#1 and H1-Ex7#2) further indicates that H1 carries two different O type alleles most commonly seen in the human population, *O101* and *O201*. On the other hand, we had difficulty to read through nucleotide 261 of the H9 exon 6 template by direct sequencing of the PCR product, the first indication for heterozygosity of the 261ΔG frameshift mutation. Two different sequences (with and without 261G) derived from the H9 exon 6 plasmid clones (H9-Ex6#1 and H9-Ex6#2) confirmed that there is an intact *ABO* allele in H9 cells. Lacking any of the B allele specific SNPs (297G, 529G, 657T, 703A, 796A, 803C and 930A) and A2 allele-specific SNPs (467T and 1060ΔC) from the H9 exon 7 PCR product sequence further suggests that the functional allele H9 carrying is A1 type (*A101*). Additional SNP data on plasmids containing the H9 intron 2 genomic fragments (Table 3, H9-In2#1 and H9-In2#2) confirmed that H9 bears *A101* and *O101* alleles.

Previously Carpenter et al. suggested that there was at least one O type allele in the H1 genome and H9 carried an A type allele and an O type allele (11). Our results are consistent with their findings but further suggest that there are two different O type alleles in the H1 genome and the A type allele H9 carries is the A1 type. Serological typing of

Table 2. SNP Analysis on Genomic Sequences of Exon 6 and 7 in H1 and H9 Cells

| | *Nucleotide positions of exons* | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *6* | | *7* | | | | | | | | | | | |
| *Allele (clone)* | *261* | *297* | *467* | *526* | *646* | *657* | *681* | *703* | *771* | *796* | *803* | *829* | *930* | *1060* |
| *A101*($A^1$) | G | A | C | C | T | C | G | G | C | C | G | G | G | C |
| *A201*($A^2$) | . | . | T | . | . | . | . | . | . | . | . | . | . | Δ |
| *B101*($B^1$) | . | G | . | G | . | T | . | A | . | A | C | . | A | . |
| *O101*($O^1$) | Δ | . | . | . | . | . | . | . | . | . | . | . | . | . |
| *O201*($O^{1v}$) | Δ | G | . | . | A | . | A | . | T | . | . | A | . | . |
| H1 PCR p. | Δ/Δ | ./G | . | . | ./A | . | ./A | . | ./T | . | . | ./A | . | . |
| H1–Ex7#1 | | | . | . | . | . | . | . | . | . | . | . | . | . |
| H1–Ex7#2 | | | . | . | A | . | A | . | T | . | . | A | . | . |
| H9 PCR p. | – | . | . | . | . | . | . | . | . | . | . | . | . | . |
| H9–Ex6#1 | . | . | | | | | | | | | | | | |
| H9–Ex6#2 | Δ | . | | | | | | | | | | | | |

PCR p., polymerase chain reaction product.
Dot (.), identity to the reference sequence *A101*.
Triangle (Δ), deletion compared with *A101*.
Dash (–), cannot read through.

Table 3. SNP Analysis on Intron 2 Genomic Sequences of H9 Cells

| *Allele (clone)* | *Nucleotide positions in intron 2* | | | | | |
|---|---|---|---|---|---|---|
| | *127* | *362* | *369* | *396* | *437* | *539* |
| *A101*($A^1$) | T | C | C | T | C | C |
| *A201*($A^2$) | . | . | . | . | . | . |
| *B101*($B^1$) | . | . | . | . | . | . |
| *O101*($O^1$) | . | T | G | C | T | A |
| *O201*($O^{1v}$) | . | . | . | C | T | . |
| H9–In2#1 | . | . | . | . | . | . |
| H9–In2#2 | . | T | G | C | T | A |

Dot(.), identity to the reference sequence A101.

differentiated human ES cells to confirm the phenotypes of these *ABO* genotypes is crucial for their clinical applications. The SNP data presented here should provide the knowledge for future experiments using these two lines in ABO compatibility studies.

## Acknowledgments

We are grateful to Ms. Cordelia P. Conley for her administrative support. Sequences used in this study were generated by the M.D. Anderson Cancer Center DNA Analysis Core Facility supported by the National Institutes of Health (NIH) Cancer Center Support Grant CA16672. This study is supported by NIH grants HD30284, GM81627 and the Ben F. Love endowment to R.R.B. T.P.Z. is supported by the Diana Helis Henry Medical Research Foundation, the Huffington Foundation and by the NIH (grant R01 EB005173-01 and P20 EB007076). M.D. is supported by the Cell and Gene Therapy Training Grant (T32 DK064717).

## References

1. Thomson JA, J Itskovitz-Eldor, SS Shapiro, MA Waknitz, JJ Swiergiel, VS Marshall and JM Jones. (1998). Embryonic stem cell lines derived from human blastocysts. Science 282:1145–1147.
2. Reubinoff BE, MF Pera, CY Fong, A Trounson and A Bongso. (2000). Embryonic stemcell lines from human blastocysts: somatic differentiation in vitro. Nat Biotechnol 18:399–404.
3. Bradley JA, EM Bolton and RA Pedersen. (2002). Stem cell medicine encounters theimmune system. Nat Rev Immunol 2:859–871.
4. Zwaka TP and JA Thomson. (2003). Homologous recombination in human embryonic stem cells. Nat Biotechnol 21:319–321.
5. Galili U, SB Shohet, E Kobrin, CL Stults and BA Macher. (1988). Man, apes, and Old World monkeys differ from other mammals in the expression of alpha-galactosyl epitopes on nucleated cells. J Biol Chem 263:17755–17762.
6. Clausen H and S Hakomori. (1989). ABH and related histo-blood group antigens; immunochemical differences in carrier isotypes and their distribution. Vox Sang 56:1–20.
7. Yamamoto F, H Clausen, T White, J Marken and S Hakomori. (1990). Molecular genetic basis of the histo-blood group ABO system. Nature 345:229–233.
8. Yip SP. (2002). Sequence variation at the human ABO locus. Ann Hum Genet 66:1–27.
9. Seltsam A, M Hallensleben, A Kollmann and R Blasczyk. (2003). The nature of diversity and diversification at the ABO locus. Blood 102:3035–3042.
10. Patenaude SI, NO Seto, SN Borisova, A Szpacenko, SL Marcus, MM Palcic and SV Evans. (2002). The structural basis for specificity in human ABO(H) blood group biosynthesis. Nat Struct Biol 9:685–690.
11. Carpenter MK, ES Rosler, GJ Fisk, R Brandenberger, X Ares, T Miura, M Lucero and MS Rao. (2004). Properties of four human embryonic stem cell lines maintained in a feeder-free culture system. Dev Dyn 229:243–258.

Address reprint requests to:
*Richard R. Behringer*
*Department of Molecular Genetics*
*The University of Texas M.D. Anderson Cancer Center*
*1515 Holcombe Blvd.*
*Houston, Texas 77030*

*Email:* rrb@mdanderson.org

Received for publication October 9, 2007; accepted after revision November 26, 2007.