Stem Cell Reports
Report
OPEN ACCESS

Gene Correction of iPSCs from a Wiskott-Aldrich Syndrome Patient Normalizes the Lymphoid Developmental and Functional Defects

Tamara J. Laskowski,[1,8] Yasmine Van Caeneghem,[2,8] Rasoul Pourebrahim,[1] Chao Ma,[3] Zhenya Ni,[3] Zita Garate,[1,4,5] Ana M. Crane,[1] Xuan Shirley Li,[1] Wei Liao,[1] Manuel Gonzalez-Garay,[6] Jose Carlos Segovia,[4,5] David E. Paschon,[7] Edward J. Rebar,[7] Michael C. Holmes,[7] Dan Kaufman,[3,9] Bart Vandekerckhove,[2] and Brian R. Davis[1,*]

[1]Center for Stem Cell and Regenerative Medicine, Brown Foundation Institute of Molecular Medicine, University of Texas Health Science Center, Houston, TX 77030, USA
[2]Laboratory for Experimental Immunology, Department of Clinical Chemistry, Microbiology and Immunology, Ghent University, Ghent 9000, Belgium
[3]Department of Medicine and Stem Cell Institute, University of Minnesota, Minneapolis, MN 55455, USA
[4]Differentiation and Cytometry Unit, Hematopoietic Innovative Therapies Division, Centro de Investigaciones Energéticas, Medioambientales y Tecnológicas (CIEMAT) - Centro de Investigaciones Biomédicas en Red de Enfermedades Raras (CIBERER), Madrid 28040, Spain
[5]Advanced Therapies Mixed Unit, Instituto de Investigación Sanitaria-Fundación Jiménez Díaz (IIS-FJD, UAM), Madrid 28040, Spain
[6]Center for Molecular Imaging, Brown Foundation Institute of Molecular Medicine, University of Texas Health Science Center, Houston, TX 77030, USA
[7]Sangamo BioSciences Inc, Richmond, CA 94804, USA
[8]Co-first author
[9]Present address: Department of Medicine, University of California, San Diego, CA 92161, USA
*Correspondence: brian.r.davis@uth.tmc.edu
http://dx.doi.org/10.1016/j.stemcr.2016.06.003

SUMMARY

Wiskott-Aldrich syndrome (WAS) is an X-linked primary immunodeficiency disease caused by mutations in the gene encoding the WAS protein (WASp). Here, induced pluripotent stem cells (iPSCs) were derived from a WAS patient (WAS-iPSC) and the endogenous chromosomal *WAS* locus was targeted with a *wtWAS-2A-eGFP* transgene using zinc finger nucleases (ZFNs) to generate corrected WAS-iPSC (cWAS-iPSC). WASp and GFP were first expressed in the earliest $CD34^+CD43^+CD45^-$ hematopoietic precursor cells and later in all hematopoietic lineages examined. Whereas differentiation to non-lymphoid lineages was readily obtained from WAS-iPSCs, in vitro T lymphopoiesis from WAS-iPSC was deficient with few $CD4^+CD8^+$ double-positive and mature $CD3^+$ T cells obtained. T cell differentiation was restored for cWAS-iPSCs. Similarly, defects in natural killer cell differentiation and function were restored on targeted correction of the *WAS* locus. These results demonstrate that the defects exhibited by WAS-iPSC-derived lymphoid cells were fully corrected and suggests the potential therapeutic use of gene-corrected WAS-iPSCs.

INTRODUCTION

Wiskott-Aldrich syndrome (WAS) is a severe X-linked primary immunodeficiency resulting from mutations in the *WAS* gene; *WAS* encodes a hematopoietic-specific and developmentally regulated cytoplasmic protein (WASp). WASp is a key regulator of the actin cytoskeleton, specifically regulating actin polymerization and formation of immunological synapses. Within the immune system, WASp deficiency results in well-documented functional defects in mature lymphocytes such as reduced antigen-specific proliferation of T cells and significantly reduced cytotoxic activity by natural killer (NK) cells when exposed to tumor cell lines (Orange et al., 2002).

Transplantation of hematopoietic stem cells (HSCs) represents a potential therapeutic approach for a variety of hematological disorders. Success in treating WAS via lentiviral-mediated gene delivery has recently been reported (Aiuti et al., 2013; Hacein-Bey Abina et al., 2015). Although no leukemogenic events were reported in up to 3 years following delivery of gene-modified $CD34^+$ cells, it remains difficult to predict whether any of the unique integration sites (e.g., ~10,000 per treated child in Aiuti et al. [2013]) will result in adverse consequences in the longer term as occurred in the original WAS retroviral gene-therapy trial (Braun et al., 2014). Thus, development of site-specific targeting strategies for treatment of WAS is warranted.

In this study, we wished to assess whether targeted gene editing of WASp-deficient induced pluripotent stem cells (iPSCs) would result in functional correction of the derived hematopoietic progeny. WAS can be caused by a diversity of mutations distributed across all 12 exons. To provide a gene correction solution potentially applicable to most, if not all, WAS patient cells, we used zinc finger nuclease (ZFN)-mediated, site-specific, homology-directed repair (HDR) to target the integration of a corrective *WAS* gene sequence into the endogenous *WAS* chromosomal locus. We hypothesized that utilizing the endogenous *WAS* promoter, the natural *WAS* chromatin environment, and transcription regulatory signals, would provide for a physiologically appropriate *WAS* transgene expression.

RESULTS

Derivation and Characterization of WAS-iPSCs

Skin fibroblasts were obtained from a WAS patient carrying the 1305 insG *WAS* mutation. This single-base-pair insertion in exon 10 of the *WAS* gene would be predicted to yield a WAS protein (WASp) frameshifted at amino acid 424, out-of-frame throughout the C-terminal VCA (verprolin homology, cofilin homology, acidic) domains critical for WASp-dependent actin polymerization and immunological synapse formation, and to conclude in a premature termination at position 493. Patients with the 1305 insG *WAS* mutation exhibit negligible WASp expression in hematopoietic cells, likely due to instability or degradation of the protein (Wada et al., 2003).

Following reprogramming, we verified the *WAS* 1305 insG mutation in WAS-iPSC clones, and confirmed characteristic pluripotent stem cell antigen expression, a normal karyotype, and pluripotency (Figures S1A–S1D). Quantitative transcriptional profiling of WAS-iPSCs revealed a gene expression pattern highly similar to human embryonic stem cells (hESCs) (line WA09) (Figure S1E).

Endogenous Targeted Integration: WAS-iPSC Gene Correction

WAS-iPSCs were corrected via ZFN-mediated HDR as shown in Figure 1A. The targeting strategy was such that successful HDR-mediated targeted integration (TI) of the WAS exon 2–12 cDNA (*WAS*$_{2–12}$) within intron 1 would result in normal transcriptional initiation at exon 1 (directed by the endogenous upstream transcriptional regulatory sequences); splicing from the splice donor at the end of exon 1 to the splice acceptor at the start of the *WAS*$_{2–12}$ cDNA to yield the *WAS-2A-GFP* mRNA; the inclusion of GFP in the *WAS*$_{2–12}$*-2A-GFP* cassette was to enable tracking of WASp-expressing cells. A loxP-flanked *pgk-puroTK*-selectable cassette was inserted just downstream of the transgene sequences in order to permit puromycin-mediated selection of initial clones as well as subsequent fialuridine (FIAU)-mediated selection of Cre-excised clones.

Successfully targeted puromycin-resistant clones were first identified by PCR amplification utilizing primers located outside the donor sequences and further confirmed by DNA sequencing (data not shown). Southern blot analysis utilizing the *pgk-puroTK* sequences as probe, confirmed the intended TI within the *WAS* locus, and the absence of off-target integrations (Figure S2A). Transient expression of Cre-recombinase, followed by FIAU selection, was utilized to excise the *pgk-puroTK*-selection cassette. The corrected WAS (cWAS) iPSCs, both prior to and following Cre-mediated excision of the selection cassette, retained a normal karyotype (Figure S2B) and pluripotency (Figure S2C). Comparative genomic hybridization (CGH) and whole-exome sequencing were performed on WAS and cWAS-iPSCs to determine whether the targeted correction methodology resulted in unanticipated changes to the chromosomal DNA. The results of these analyses are presented in Tables S1–S3. These data indicate that the cWAS-iPSCs were generated without any apparent deleterious mutation that could cloud the interpretation of further experiments.

Derivation and Characterization of Hematopoietic Progenitor Cells from WAS and cWAS-iPSCs

Since WASp is normally expressed in all hematopoietic cells including the CD34$^+$ hematopoietic progenitor cells (HPCs), we examined HPCs derived from the patient WAS- or cWAS-iPSCs; WA01 or WA09 hESCs carrying wild-type (WT) *WAS* were used interchangeably as controls. Differentiation of iPSCs progressed from CD34$^+$CD43$^-$ endothelial cells to CD34$^+$CD43$^+$ cells (CD43 is expressed early on hematopoietic cells) and finally to CD34$^+$CD45$^+$ HPCs, similar to cultures initiated by WAS- and cWAS-iPSCs, and highly similar to the patterns exhibited by control hESCs (Figure 1B). The absolute numbers of the specific cell populations (CD34$^+$CD43$^-$, CD34$^+$CD43$^+$, and CD34$^+$CD43$^+$CD45$^+$) obtained per embryoid body (EB) demonstrates that the *WAS* 1305 insG mutation does not adversely affect the emergence/development of CD34$^+$ HPCs from hemogenic endothelium when compared with cWAS (Figure 1C) ($p = 0.502$, not significant [NS]). Further differentiation of WAS-derived CD34$^+$CD43$^+$ CD45$^{+/-}$ HPC toward myeloid, erythroid, and megakaryocytic lineages revealed no major differentiation defects compared with cWAS or hESC (Figure 1D).

Restoration of WASp Expression in cWAS-iPSC-Derived Hematopoietic Cells

In order to assess restoration of WASp expression in the cWAS-iPSC-derived cells, we first assayed GFP expression in the in vitro differentiation cultures. GFP expression, albeit at low levels, was clearly present in cWAS-iPSC-derived CD34$^+$CD43$^+$CD45$^-$ hematopoietic progenitors and in all hematopoietic lineages including myeloid, erythroid, and megakaryocytic lineages (Figure 2A). GFP expression was very weak to absent in cWAS-iPSC-derived CD34$^+$KDR$^+$CD43$^-$CD45$^-$ endothelial cells (Figure 2A). There was no indication of differential GFP expression in CD34$^+$KDR$^+$CD43$^-$CD73$^-$ hemogenic versus CD34$^+$KDR$^+$CD43$^-$CD73$^+$ non-hemogenic endothelial cells (Figure S3A).

RT-PCR for *WAS* sequences was performed for the WAS- and cWAS-iPSC-derived CD34$^+$CD43$^+$ HPCs. The native WASp transcript including the 3′ UTR was present in