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Treatment of inherited eye defects by systemic hematopoietic stem cell transplantation

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Cystinosis is caused by a deficiency in the lysosomal cystine transporter, cystinosin (CTNS gene), resulting in cystine crystal accumulation in tissues. In eyes, crystals accumulate in the cornea causing photophobia and eventually blindness. Hematopoietic stem progenitor cells (HSPCs) rescue the kidney in a mouse model of cystinosis. We investigated the potential for HSPC transplantation to treat corneal defects in cystinosis.

**METHODS.** We isolated HSPCs from transgenic DsRed mice and systemically transplanted irradiated Ctns−/− mice. A year posttransplantation, we investigated the fate and function of HSPCs by in vivo confocal and fluorescence microscopy (IVCM), quantitative RT-PCR (RT-qPCR), mass spectrometry, histology, and by measuring the IOP. To determine the mechanism by which HSPCs may rescue disease cells, we transplanted Ctns−/− mice with Ctns−/− DsRed HSPCs virally transduced to express functional CTNS-eGFP fusion protein.

**RESULTS.** We found that a single systemic transplantation of wild-type HSPCs prevented ocular pathology in the Ctns−/− mice. Engraftment-derived HSPCs were detected within the cornea, and also in the sclera, ciliary body, retina, choroid, and lens. Transplantation of HSPC led to substantial decreases in corneal cystine crystals, restoration of normal corneal thickness, and lowered IOP in mice with high levels of donor-derived cell engraftment. Finally, we found that HSPC-derived progeny differentiated into macrophages, which displayed tunneling nanotubes capable of transferring cystinosin-bearing lysosomes to diseased cells.

**CONCLUSIONS.** To our knowledge, this is the first demonstration that HSPCs can rescue hereditary corneal defects, and supports a new potential therapeutic strategy for treating ocular pathologies.

Keywords: cystinosis, hematopoietic stem cell, corneal cystine crystals, tunneling nanotubes
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associated ocular morbidity, suggesting the need for more potent and durable therapeutics.24

Hematopoietic stem and progenitor cells (HSPCs) are multipotent cells capable of self-renewal and are easily mobilized from bone marrow into the circulatory system. These cells enhance reepithelialization of corneal wounds after alkali injuries in mice;25 but beyond this finding limited data exist to indicate the potential of HSPCs for reversing ocular pathology.

We demonstrated previously that systemic transplantation of wild-type HSPCs resulted in a significant decrease of cystine levels in tissues and the long-term preservation of the kidney in Ctns−/− mice.26–28 The objective of the present study was to test whether systemic HSPC transplantation also could treat eye pathology in Ctns−/− mice. We showed that HSPCs differentiate into macrophage progeny into ocular tissues, deliver cystinosin product locally, and improve corneal defects in Ctns−/− mice.

**METHODS**

**Mice**

The C57BL/6 Ctns+/− mice were provided by C. Antignac (Inserm U1163, Paris, France) and bred continuously at University of California, San Diego (UCSD). Transgenic mice constitutively expressing GFP (C57BL/6-Tg[ACTB-EGFP]1Osby/J) or DsRed (B6.Cg-Tg[CAG-DsRed*MST]1Nagy/J) were purchased from Jackson Laboratory (Bar Harbor, ME, USA) and both were cross-bred with the C57BL/6 Ctns−/− mice to produce transgenic GFP Ctns+/− and DsRed Ctns+/− mice. All protocols were approved by the UCSD Institutional Animal Care and Use Committee (IACUC) and adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

**Bone Marrow Cell (BMC) Isolation, Transduction, and Transplantation**

Bone marrow cells were flushed from the long bones of 6- to 80-week-old GFP-transgenic mice or DsRed Ctns−/− mice. Sca1-HSCs were isolated as described previously.29 One million GFP cells were directly transplanted in lethally irradiated (8 Gy) DsRed Ctns−/− mice by tail vein injection while DsRed Ctns−/− cells were first transduced with SIN lentivirus expressing the fused protein CTNS-GFP before transplantation in lethally irradiated (8 Gy) Ctns−/− mice as described previously.29 To analyze bone marrow engraftment of the transplanted cells, fresh blood was treated with red blood cell lysis buffer (cBioScience, San Diego, CA, USA) and subsequently analyzed by flow cytometry to the GFP-expressing cells.

**Fluorescence Microscopy**

Eyes were fixed in 5% formaldehyde for 2 to 3 hours and equilibrated in 20% sucrose overnight. Eyes were frozen in Tissue-Tek Optimal Cutting Temperature buffer at −80°C (Sakura Finetek USA, Inc., Torrance, CA, USA) and 20-µm sections were cut. Sections were stained with 4',6-diamidino-2-phenylendole (DAPI). The Keyence BZ-X700 microscope (Keyence, Osaka, Japan) was used to capture the pictures and the Keyence software was used to perform image stitching specifically the cystine crystals and pixels were quantified.

**Cystine Content Measurement**

Explanted eyes were ground in 500 µL of N-ethylmaleimide (Fluka Biochemika, Bushis, Switzerland) at 650 µg/mL using the Precells 24 homogenizer (Bertin Technologies, Montigny-le-Bretonneux, France). Proteins were precipitated using 15% 5-sulfosalicylic acid dihydrate (Fluka Biochemika), resuspended in NaOH 0.1 N and measured using the Pierce BCA protein assay kit (Pierce, Rockford, IL, USA). The cystine-containing supernatants were sent to the UCSD Biochemical Genetics laboratory for measurement by mass spectrometry as described previously.28

**Reverse Transcription and Quantitative PCR (RT-qPCR)**

The RNA from explanted eyes was isolated using the RNeasy kit (Qiagen, Venlo, The Netherlands) according to manufacturer’s instructions. One microgram of RNA was reverse transcribed using iScript cDNA Synthesis kit (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The Ctns-specific qPCR was performed and probe were obtained from Applied Biosystems (Carlsbad, CA, USA): Ctns oligo 1: 5'-TTGTGGTGCAGTCGGTATC-3', Ctns oligo 2: 5'-AGCTGATGAGGAAGAAGGACAGA-3', and Ctns probe: 5'-CAGATGCTCGTGTTTGTTG-3' and 18S primer mix on a CFX96 (Bio-Rad Laboratories, Inc.), and primers and probe were obtained from Applied Biosystems (Carlsbad, CA, USA): Ctns oligo 1: 5'-TTGTGGTGCAGTCGGTATC-3', Ctns oligo 2: 5'-AGCTGATGAGGAAGAAGGACAGA-3', and Ctns probe: 5'-CAGATGCTCGTGTTTGTTG-3' and 18S primer mix on a CFX96 (Bio-Rad Laboratories, Inc.). The expression levels of Ctns gene, expressed as fold change, was calculated using the
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Histology and Central Cornea Thickness (CCT) Measurement

Explanted eyes were fixed in formalin and embedded in paraffin. Sections (10 μm) were stained with hematoxylin and eosin. Using a light microscope (Keyence), pictures of the central cornea were acquired (×20) and CCT as well as the epithelial layer thickness were measured using Keyence software at 3 different parts of the cornea and averaged.

Immunofluorescence Analysis

Eyes were fixed in 5% formaldehyde for 2 to 3 hours and equilibrated in 20% sucrose overnight. Eyes were frozen in Tissue-Tek Optimal Cutting Temperature buffer at −80°C (Sakura Finetek USA) and 20-μm sections were cut. Sections were stained with DAPI and with Rat-anti-F4/80 (14-4801-82, eBioscience), Rat-anti-MHC Class II [NIMR-4] (ab25333, Abcam) and Mouse-anti-CD11c [3.9] (ab11029; Abcam, Cambridge, UK) antibodies. To quantify HSPCs-derived cells (DsRed and F4/80+ cells), three sections per animal were used and cells were counted manually in the entire cornea. To visualize the nanotubes, all pictures were acquired using a ×65 objective on an Olympus Fluoview1000 (Olympus, Center Valley, PA, USA) or Leica TCS SP5 II confocal microscope (Leica Microsystems, Buffalo Grove, IL, USA). Images were processed with ImageJ or Volocity software (Perkin Elmer, Waltham, MA, available in the public domain at www.perkinelmer.com) to generate 3D reconstructions from optical slices (Z-stacks).

Statistics

We summarized continuous data as arithmetic means ± SEM. Group comparisons were made using t-test. Associations between blood engraftment and Ctns expression or cystine content was investigated with linear regressions. The χ² test was used to identify whether IOP above or below 20 mm Hg was dependent on cell engraftment. All analyses were performed using Prism 6 (GraphPad Software, Inc., La Jolla, CA, USA). P < 0.05 was considered as statistically significant.

Results

HSPCs Migrate to Different Regions of the Eye in Ctns+/− Mice

Lethally irradiated Ctns+/− mice between 1.5 and 3.5 months of age were transplanted with wild-type DsRed-expressing bone marrow stem cells to study the fate and impact of HSPCs on the corneal defects in cystinosis. Nontreated Ctns+/− mice and lethally irradiated Ctns+/− irradiated mice transplanted with DsRed+ Ctns−/− HSPCs were used as controls. At 1 year after transplantation, bone marrow-derived cells were present through various parts of the eyes of Ctns+/− mice, including the cornea, retina, lens, sclera, and ciliary margin (Fig. 1a), demonstrating that systemically injected HSPCs migrate to and integrate in the diseased eyes.

To determine the level of donor bone marrow stem cell engraftment, DsRed-positive cells were quantified in peripheral blood samples by flow cytometry; engraftment ranged from 16 to 95.4% (Low, n = 7, mean = 27.91% ± 12.24%; High, n = 7, mean = 85.46% ± 6.09%). Mice with high levels of engraftment (>50%; High) exhibited more abundant bone marrow-derived cells within the eyes compared to mice with a low level of engraftment (<50%; Low; Fig. 1a). This histologic observation was confirmed by RTqPCR quantitative analysis of Ctns expression (derived from the donor cells), demonstrating that Ctns expression in the eyes of the treated Ctns+/− mice increased with increasing engraftment levels (F1,5 = 9.635, P = 0.0146; Fig. 1b). Since we previously showed that a correlation exists between level of donor-derived blood cell engraftment and long-term preservation of the kidney,28 we assessed the subsequent structural, biochemical, and functional effect of HSPCs on corneas in the Ctns+/− mice by differentiating between the High and Low engraftment groups, compared to age-matched wild-type and nongrafted Ctns+/− controls.

Prevention of Corneal Cystine Crystal Accumulation in Ctns+/− Mice With High HPSC Engraftment

First, tissue cystine content was measured by mass spectrometry in whole enucleated eye. A significant decrease in cystine levels was observed in high (n = 6) and low (n = 3) treated mice compared to Ctns−/− controls (n = 3, reductions of 85% and 73%, respectively; Fig. 2a). To evaluate corneal cystine crystals, we performed IVCM in live mice, scanning through the entire cornea, differentiating the epithelium, Bowman's membrane, stroma, Descemet's membrane, and endothelium (Fig. 2b). Crystals in each layer then were quantified using ImageJ software (Fig. 2c). Because there is asymmetry of crystal deposition between the two eyes of the same animal,18 we analyzed each eye individually. At 1 year of age, all Ctns+/− mouse controls (nontreated or transplanted with Ctns−/− HSPCs, n = 4) exhibited abundant cystine crystals within all layers of the cornea, with the majority being present in the anterior stroma and endothelium layers. The Ctns−/− mice with low level of engraftment (Low; n = 5) showed a partial reduction of crystal counts, and mice with high engraftment levels (High; n = 5) exhibited almost a complete resolution of crystals from the epithelial layer to the mid-stroma (100% and 72% clearance, respectively; Fig. 2c). These data showed, for the first time to our knowledge, that systemic HSPC transplantation has a significant beneficial impact on corneal cystine crystals and also suggested that some minimum level of Ctns-expressing bone marrow-derived cells must be present in the cornea to resolve the corneal cystine crystals in the stroma and epithelium.

Cystine crystal levels in the posterior stroma also were significantly reduced in the High engraftment mice, by 28% (Fig. 2c), a less dramatic reduction than with the anterior cornea. No reduction in the posterior stroma was found in Low engraftment animals. These data might be explained by the kinetics of the formation of the corneal cystine crystals that were shown to first appear in the peripheral posterior stroma/corneal endothelium.18 Thus, crystal formation may occur within the endothelium before HSPC transplantation, and our data suggested that, although HSPCs prevent progression to stromal layers, they do not remove crystals already present in the endothelium at 2 months.

Rescue of Corneal Atrophy in HSPC-Treated Ctns−/− Eyes

Corneas, and in some cases the entire eye, degenerate over time in Ctns−/− mice.11,13,14,18 To evaluate whether HSPCs prevent corneal atrophy in Ctns−/− mice, we measured the CCT in hematoxylin-stained eye sections, as described previously.31 We found that the cornea of old Ctns−/− mice (approximately 13 months of age; n = 4) was significantly thinner than in wild-type animals (n = 4), an effect most...
apparent in the epithelial layer (Fig. 3a). Interestingly, HSPC transplantation \((n = 6)\) prevented the reduction of CCT and allowed maintenance of normal epithelial structure in mice with a high level of engraftment (Fig. 3b). Data from Ctns/C0 mice with low levels of engraftment also showed an improvement of corneal structure, but histological sections were only available from two animals (data not shown). Thus HSPC engraftment prevents late corneal atrophy in cystinosis.

**Rescue of Elevated IOP in HSPC-Treated Ctns/C0 Eyes**

Cystinosis leads to IOP fluctuations and elevation in mice and humans.\(^{13,24}\) IOP measures in Ctns/C0 mice were significantly higher compared to wild-type mice (see Table), reaching greater than 20 mm Hg in 5 of 54 eyes. In contrast, high- and low-HSPC engraftment in HSPC-treated Ctns/C0 mice demonstrated decreased IOP compared to Ctns/C0 mice, with no observed measures above 20 mm Hg \((P = 0.006, \chi^2\) test).

**Mechanism of Corneal Rescue by HSPC Transplantation in Ctns/C0 Mice**

We recently showed that the cellular mechanism of kidney repair in HSPC-transplanted cystinosis mice involved lysosomal cross-correction via tunneling nanotubes (TNTs) after HSPC differentiation into macrophages.\(^{29}\) Here, we asked whether the same mechanism is observed in eyes. Confocal microscopy showed that most HSPC progeny in the cornea differentiated into mature macrophages, as confirmed by the colocalization of all bone marrow–derived DsRed-positive cells with F4/80

\[ \text{Ctns expression} = 0.1 \times \text{Engraftment} + 0.5 \]

\(r^2\) test, IOP > 20 mm Hg: \(P\) Value

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FIGURE 2. Transplantation of HSPC leads to ocular cystine and corneal cystine crystal clearance. (a) Cystine levels measured in the whole eye were significantly reduced in the HSPC-transplanted mice, Low and High, compared to the Ctns−/− controls. Error bars: SEM (*P < 0.05, **P < 0.005). (b) Lateral cornea IVCM representations of Ctns−/− controls and Low and High HSPC-transplanted mice. Abundant cystine crystals were observed in the whole cornea in Ctns−/− mice in contrast to Ctns−/− with high level of engraftment where residual crystals can be seen only in the posterior stroma. (c) Surface crystal quantification within each layer of the full IVCM cornea scans from both eyes of Ctns−/− controls and transplanted (Low and High) mice. Transplantation of HSPC with high level of engraftment prevents almost completely cystine crystal formation within the superior layers of the cornea (epithelium to middle stroma). The mice with low level of engraftment exhibit significant but more modest crystal reduction. Error bars: SEM (*P < 0.05, **P < 0.005).

a

b

C

chronic inflammation, which is reduced when the macrophages express a functional Ctns gene associated with tissue rescue. Further characterization of the macrophages showed that most of the macrophages expressed MHC-II (Fig. 4e). We also observed that few HSPCs (~10%) colocalized with CD11c suggesting that some of the HSPCs also differentiated into dendritic cells (Fig. 4e).

Higher magnification confocal images showed that bone marrow-derived macrophages form TNT structures. We identified TNTs generated from bone marrow-derived cells in the cornea sclera margin, ciliary margin and retina (Fig. 5a). To test if lysosomal cross-correction occurs in the cornea, we transplanted normal Ctns<sup>/−</sup> mice with DsRed Ctns<sup>/−</sup> HSPCs stably expressing cystinosin-eGFP fusion protein or with DsRed Ctns<sup>/−</sup> HSPCs as control. Eyes were analyzed 7 months posttransplantation by confocal microscopy. No GFP-positive lysosomes were identified in Ctns<sup>/−</sup> mice transplanted with DsRed Ctns<sup>/−</sup> HSPCs alone (Fig. 5b, upper). In contrast, we observed cystinosin-eGFP-bearing lysosomes within the DsRed-positive bone marrow-derived cells as expected in mice transplanted with cystinosin-eGFP-HSPCs and also within host corneal cells, directly demonstrating lysosomal transfer from the HSPC-derived cells to the adjacent diseased cells (Fig. 5b, lower). Thus protein transfer from donor to host cells is detected in the corneas of HSPC-treated Ctns<sup>/−</sup> mice, providing evidence for lysosomal cross correction in the eye.

**DISCUSSION**

Here we report for the first time that systemic HSPC transplantation can treat corneal defects in the context of a degenerative, hereditary metabolic disorder, cystinosis. This treatment led to a long-term prevention of corneal cystine crystal deposition, preservation of corneal structure, and reduction in IOP in the Ctns<sup>/−</sup> mice. These results demonstrate that HSPCs have the capacity to produce bone marrow-derived cells that home to and integrate into injured/diseased eyes, to

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**Figure 3.** Corneal structure is preserved in HSPC-transplanted Ctns<sup>/−</sup> mice. (a) Representative sagittal hematoxylin and eosin sections of the central cornea of wild-type mice, nontreated Ctns<sup>/−</sup>, and highly engrafted Ctns<sup>/−</sup> (High) mice. The Ctns<sup>/−</sup> mice exhibit markedly reduced CCT compared to wild-type animal, while treated Ctns<sup>/−</sup> mice presented with CCT comparable wild-type. Scale bars: 200 μm. (b) Thickness measurement of the central cornea and central cornea epithelium of wild-type, Ctns<sup>/−</sup>, and transplanted (High) Ctns<sup>/−</sup> mice showing that HSPC transplantation prevents corneal reduction. Error bars: SEM (*P < 0.05).
actively participate in the preservation of eye function and structure over prolonged periods of time. These results are particularly important considering that they demonstrate this correction in the avascular cornea tissue after systemic transplantation. We present in this study 1-year posttransplantation data, but there is no indication that results would not extend to the entire life span of the study animals, as previously shown for the kidney. Therefore, transplantation of normal, CTNS-expressing HSPCs in patients affected with cystinosis may represent a long-term treatment that can preserve disease-affected tissues, including the kidneys and the eyes. Based on these data, ocular exams will be included as important efficacy endpoints in an upcoming hematopoietic stem cell-based human clinical trial for cystinosis. We do not know whether transplanting HSPCs into aged Ctns−/− mice with advanced ocular deficits will rescue established tissue damage, an important future study when considering the potential for therapeutic impact when treating adults with cystinosis.

We found that long-term preservation of ocular function and structure in the Ctns−/− mice were dependent on achieving a relatively high level of donor-derived blood cell engraftment (>50%), similar to our prior studies of kidney function. Improvement in some measures was noted in treated mice with a low level of engraftment (<50%) compared to Ctns−/− controls, but for corneal crystal accumulation, an outcome comparable to wild-type was achieved only following high levels of engraftment. Thus, it seems that a critical load of properly engrafted HSPCs is required to achieve a meaningful...
a

Cornea
Ciliary margin
Retina

b

DsRed
CTNS-eGFP
Merge

FIGURE 5. HSPCs-derived macrophages transfer cystinosin-bearing lysosomes via TNTs to the adjacent disease cells. (a) Tunneling nanotubes generated from the DsRed bone marrow–derived cells were observed in the cornea (sclera margin and ciliary margin) and retina (arrows). (b) Representative confocal pictures of the cornea of Ctns−/− mice transplanted with Ctns−/− DsRed HSPCs as control (upper) or with Ctns−/− DsRed HSPCs stably expressing the fusion protein CTNS-eGFP (lower). Green vesicles are observed within the DsRed-bone marrow–derived cells in the mice treated with CTNS-eGFP-HSPCs but also in the no color host cells (white arrowheads) showing that lysosomes containing CTNS-eGFP could be transferred. *Insert* shows a TNT containing cystinosin-bearing vesicles (yellow arrowhead). Scale bars: (a) 10 μm, (b) 15 μm.

The major pathological ocular symptom described in cystinosis patients is the presence of corneal cystine crystals that result in photophobia, keratopathy, and recurrent corneal erosions. In vivo confocal analysis revealed abundant cystine crystals first in the posterior stromal-endothelium layers and later progressing anteriorly, similar to previous descriptions. Interestingly, we observed that the crystal accumulation in the middle stroma of Ctns−/− mice was lower than the anterior and posterior stroma. This differed from one study of 8 patients where the highest amount of cystine crystals was found in the anterior stroma and less in the mid- and posterior stroma, although this could be explained by breakdown of crystals and/or scarring.

Studying the time course of corneal engraftment of the HSPC-derived cells will be important to determine differences in tissue response between the anterior and posterior stroma.

Our data revealed significantly lower amounts of crystals within the anterior region of the cornea (epithelium and upper stroma) in high HSPC-engrafted Ctns−/− mice. A previous study performed on Ctns−/− mice treated with topical cysteamine applied 4 times daily for 4 weeks beginning at 5 months showed that the drug only delays the formation of cystine crystals; the cysteamine-treated group still showed a 15% increase in the crystal volume index. Similar results were observed in patients using a newer gel formulation of topical cysteamine. Our data suggested that HSPC transplantation could be a more efficient alternative, almost totally preventing crystal formation in the cystinotic eyes. However, the prevention of cystine crystal accumulation was observed to a lesser extent in the posterior than in the anterior cornea. This may be explained by prior findings that engrafted HSPC-derived cells localize initially in the anterior stroma and later migrate to the posterior stromal layers. Thus, we hypothesize that by the time the HSPCs reach the posterior stroma, crystal formation and accumulation has occurred and cannot be reversed. Note that HSPC-derived cells also were present in the sclera, ciliary margin, iris, retina, choroid, and to lesser degree the lens, all previously identified sites of crystal accumulation. Cystine clearance in these regions also may have increased in HSPC-engrafted animals, as suggested by the significant decrease of global ocular cystine content in the HSPC treated mice (Fig. 2a).

Central corneal thickness was thinner in the Ctns−/− mice, and restoration of physiological corneal thickness was found in the treated mice with high level of engraftment. Thin corneas may lead to an underestimation of IOP but increased IOP in these nontreated Ctns−/− mice may have been caused by corneal sclerosis or scarring. In contrast, physiological IOPs were observed in the HSPC-engrafted Ctns−/− mice. It also should be noted that pupillary block may elevate IOP in cystinosis patients and in the mouse model. This is caused by a deposition of cystine crystals in the iris, which can lead to a seal between the iris and lens, bulging the iris forward, and closing the flow of aqueous fluid out the trabecular meshwork. In addition, in contrast to corneal-related IOP lowering, it also is possible that lower IOP we observed in HSPC engrafted Ctns−/− mice was due to lower crystal accumulation in the iris, where HSPC-derived cells were found (Fig. 4e). We also found HSPC-derived cells in the ciliary margin (Fig. 5a) and at locations in or around the trabecular meshwork, but it is not clear whether these cells have a role in lowering IOP since most documented cystinosis cases conclude that IOP increases as a result of crystal accumulation in the iris and not decreased trabecular meshwork outflow. These data emphasized the powerful impact of HSPC transplantation on the long-term structural and functional preservation of the eye.

We showed that most of the HSPC-derived bone marrow-derived cells that migrated to the eye were F4/80+ MHC class II+ macrophages, with a few dendritic cells. Interestingly, we observed that these macrophages generated TNTs as observed previously within the kidney. These membrane extensions are heterogeneous cell-connecting tubules that have been involved in pathogen spreading and intercellular communication. In vivo existence of TNT was reported initially in MHC II-positive cells present in mouse corneal stroma. The function of these nanotubes has been attributed to the transfer of small molecules, such as MHC and/or MHC-antigen complexes, to help the propagation of immune responses. Lysosomal cross-correction by delivering cystinosin-bearing lysosomes to the adjacent diseased corneal cells, thereby providing functional cystine clearance mechanisms for Ctns−/− cells, similar to the TNT-mediated crossing of the tubular membrane that halts kidney degeneration. Therefore, TNTs generated by MHC class II+ bone marrow-derived cells, which penetrate corneal layers, are likely leading to the our presented findings of structural and functional preservation in corneas. Cystinosin-containing microvesicle
transfer from the bone marrow–derived macrophages to the host cells is an alternative route for cystinosin cross-correction. Other groups have reported that microvesicles/exosomes shed by CTNS-expressing cells could lead to significant cystine decrease in cystinotic cells in vitro. This mechanism also has been described in co-culture assays of mucopolysaccharidosis VII fibroblasts with unbilinical MSCs and as the mechanism responsible for the rescue of the corneal defects in mice after direct corneal transplantation of the MSCs. However, we previously demonstrated using coculture assays of wild-type MSCs or macrophages with Ctns-deficient fibroblasts that, while microvesicle transfer could lead to a substantial decrease of cystine in the fibroblasts (approximately 19% clearance), TNT-mediated lysosomal transfer was a more efficient route (approximately 75% clearance).

Taken together, these results suggested that, rather than replacing defective corneal tissues with transplanted or stem cell-derived tissues, the transfer mode of action between gene-corrected, HSPC-derived donor cells and diseased cells and tissues may be an efficient alternative to correct genetic deficiencies and stabilize ocular pathology. Future directions should include determining if there is a therapeutic window for disease correction, if HSPCs delivered directly within the eyes have the same therapeutic potential as systemic delivery, and if similar therapeutic efficacy can be detected in human patients.

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