Cultured Human Corneal Endothelial Cell Aneuploidy Dependence on the Presence of Heterogeneous Subpopulations With Distinct Differentiation Phenotypes

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Cornea

PURPOSE. Cultured human corneal endothelial cells (cHCECs) are anticipated to become an alternative to donor corneas for the treatment of corneal endothelial dysfunction. However, cHCECs reportedly tend to exhibit chromosomal abnormality during in vitro cell division, thereby hampering their use in the clinical setting. The purpose of this study was to clarify whether a specified subpopulation (SP) of heterogeneous cHCECs would exhibit aneuploidy, whereas other SPs would not.

METHODS. The presence of SPs in cHCECs was analyzed on the basis of surface cluster of differentiation (CD) antigen CD166, CD105, CD44, CD26, and CD24 expression levels by flow cytometry. Cytogenetic examination was performed for 23 lots of cHCECs, either as whole-cell preparations (bulk) consisting of mixed SPs or as a semipurified SP by magnetic activated cell sorting (MACS). The HEC donars ranged from 9 to 69 years of age and the culture passages from primary to fifth passage.

RESULTS. Flow cytometry analysis demonstrated the presence of at least three cHCEC SPs. One SP purified by MACS, with surface expression of CD166+, CD105−, CD44−, CD24+, and CD26− did not show any aneuploidy in 50 cells. However, CD166+, CD44++, CD24−, and CD26+ cHCEC SPs showed sex chromosome loss in all cells (60 cells), whereas CD166+, CD44++, CD24+, and CD26− SPs exhibited, albeit partly, trisomy on chromosomes 6, 7, 12, and 20.

CONCLUSIONS. We found that cHCEC aneuploidy is linked to specified SPs present in cHCECs and that the SP sharing the surface phenotype with mature HCECs in corneal tissues was devoid of the karyotype abnormality.

Keywords: aneuploidy, cell therapy, cultured cell subpopulation, cultured corneal endothelial cells, HCECs, regenerative medicine

Corneal endothelium is composed of a monolayer of hexagonal cobble stone-shaped cells and is indispensable for the maintenance of corneal transparency. Thus, corneal endothelium dysfunction results in corneal turbidity and a marked deterioration of visual acuity. Human corneal endothelium functions as a barrier between the aqueous humor and corneal stroma, characterized by focal tight junctions and the Na+/K+-ATPase functions that regulate corneal hydration to maintain transparency.

Because the proliferative potential of human corneal endothelial cells (HCECs) is extremely limited, severe damage to the corneal endothelium resulting from pathologic conditions leads to corneal endothelial dysfunction and, ultimately, to the loss of corneal transparency. To restore the injured tissue, the residual HCECs are prone to migrate and spread over the damaged area. Corneal endothelial dysfunction is caused by various pathologic conditions and by surgeries such as laser iridotomy and those for the treatment of cataract and glaucoma.

Endothelial keratoplasty involving dissection of Descemet’s membrane with the endothelium layer has been performed worldwide to restore the function of the damaged tissue. However, the global shortage of donor corneas has prompted researchers to establish new treatment protocols, such as the use of cultured HCECs (cHCECs) as a cell source for regenerative medicine. In fact, the transplantation of cHCECs in the form of a cell sheet, with or without carrier, has enabled the recovery of corneal transparency in animal models. Our group, together with others, have been investigating the possibility of cHCEC transplantation in the form of a cell suspension. However, the trials involving the in vitro expansion of cHCECs without cell-state transition (CST), namely a senescence phenotype, epithelial-mesenchymal transition, and a transformed fibroblastic cell morphology, have been hampered due to restricted insights on cHCECs, as well as the limited availability of cells.

HCECs are well known to have poor proliferative ability under in vitro culture conditions. cHCECs have a tendency...
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mature HCECs that are present in corneal tissues. We have now prepared according to published protocols. Briefly, basal
membranes with the CECs were stripped from donor

Karyotyping

as described above and suspended at a concentration

Flow Cytometry Analysis of cHCECs
cHCECs were collected from the culture dish by TrypLE Select
treatment as described above and suspended at a concentration

Isolation of HCEC Subpopulations by MACS

Karyotyping

Cytogenetic examination was performed at several

Materials and Methods

HCEC Donors

The HCEC donor tissue used in this study was handled in accordance with the tenets set forth in the Declaration of Helsinki. HCECs were obtained from 23 human donor corneas obtained from SightLife (Seattle, WA, USA) eye bank and were cultured before performing karyotyping analysis. Informed written consent for eye donation for research was obtained from the next of kin of all deceased donors. All tissues were recovered under the tenets of the Uniform Anatomical Gift Act of the particular state in which the donor consent was obtained and the tissue was recovered.

The 14 male and 7 female donors ranged from 9 to 69 years of age. All donor corneas were preserved in Optisol-GS (Chiron Vision, Irvine, CA, USA) and imported through international air transport for research purposes. Donor information accompanying the donor corneas showed that they were all considered healthy and absent of any corneal disease and that all donors had no history of chromosomal abnormality.

Cell Culture of HCECs

The HCECs were cultured according to published protocols, with some modifications. A total of 30 human donor corneas were used for the experiments. Briefly, Descemet’s membranes with the CECs were stripped from donor corneas and digested with 1 mg/mL collagenase A (Roche Applied Science, Penzberg, Germany) at 37°C for 2 hours. The HCECs obtained from a single donor cornea were seeded in one well of a Type I collagen-coated 6-well plate (Corning, Inc., Corning, NY, USA). The culture medium was prepared according to published protocols. Briefly, basal

medium was prepared with Opti-MEM-I (Life Technologies Corp., Carlsbad, CA, USA), 8% fetal bovine serum (FBS), 5 ng/mL epidermal growth factor (EGF; Life Technologies), 20 μg/mL ascorbic acid (Sigma-Aldrich Corp., St. Louis, MO, USA), 200 mg/L calcium chloride (Sigma-Aldrich Corp.), 0.08% chondroitin sulfate (Wako Pure Chemical Industries, Ltd., Osaka, Japan), and 50 μg/mL gentamicin. Mesenchymal stem cell-conditioned medium was prepared. The HCECs were cultured using mesenchymal stem cell-conditioned medium at 37°C in a humidified atmosphere containing 5% CO2, and the culture medium was changed twice a week. The HCECs were passaged at ratios of 1:3 using 10X TrypLE Select (Life Technologies) cell dissociation enzyme at 37°C for 12 minutes when they reached confluency. The HCECs at passages 2 through 5 were used for all experiments.

Flow Cytometry

Screening of the cell surface markers was conducted by assessing the expression of markers through the human cell surface marker screening panel (BD Biosciences, San Jose, CA, USA) according to the manufacturer’s protocol. Briefly, cultured HCECs were incubated with primary 242 antibodies and isotype immunoglobulin G (IgG; BD Biosciences) at the dilution indicated by the manufacturer’s protocol at 4°C for 30 minutes. The cells were washed with phosphate-buffered saline (PBS) containing 1% bovine serum albumin (BSA) and 5 mM EDTA and then incubated with Alexa Fluor 647-conjugated secondary antibodies (1:200 dilution) and 5 mM EDTA at 4°C for 30 minutes. The cells were washed again with PBS containing 1% BSA and 5 mM EDTA and then analyzed by flow cytometry using a FACScant II instrument (BD Biosciences) and CellQuest Pro software (BD Biosciences).

Flow Cytometry Analysis of cHCECs
cHCECs were collected from the culture dish by TrypLE Select treatment as described above and suspended at a concentration of 4 × 10^6 cells/mL in fluorescence-activated cell sorting (FACS) buffer (PBS containing 1% BSA and 0.05% NaN3). An equal volume of antibody solution was added and incubated at 4°C for 2 hours. The antibody solutions were fluorescein isothiocyanate-conjugated anti-human CD26 mAb, phycoerythrin (PE)-conjugated anti-human CD166 mAb, PerCP-Cy5.5 conjugated anti-human CD24 mAb, PE-Cy7-conjugated anti-human CD44 (all from BD Biosciences), and APC-conjugated anti-human CD105 (eBioscience, San Diego, CA, USA) and 5 mM EDTA and then incubated with Alexa Fluor 647-conjugated secondary antibodies (1:200 dilution) at 4°C for 30 minutes. The cells were washed again with PBS containing 1% BSA and 5 mM EDTA and then analyzed by flow cytometry using a FACScant II instrument (BD Biosciences) and CellQuest Pro software (BD Biosciences).

Isolation of HCEC Subpopulations by MACS

The HCECs were detached using TrypLE Select treatment as described above, and the CD44+ HCEC subpopulation (the effector subpopulation) was isolated using anti-human CD44 MicroBeads (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany) and the depl05 software of the auto-magnetic activated cell sorting (MACS) Pro Separator (Miltenyi Biotec). Flow cytometry demonstrated that the purity of the isolated effector subpopulation was higher than 95% in all cases.

Karyotyping

Cytogenetic examination was performed at several passages of the cells obtained from the 21 donors as shown in Table 1. In the early stage of this study, cytogenetic examination was performed only for the bulk culture without cell sorting. Standard cytogenetic harvesting, fixation, and G-
bands after trypsin and Giemsa staining (GTG banding) techniques were used for the HCECs. After incubation with 0.06 μg/mL colcemid for 16 hours to arrest cell mitosis, HCECs were detached using 0.05% trypsin/EDTA. HCECs were treated with 0.075 M KCl and then fixed with Carnoy’s fixative (a 3:1 mixture of methanol and glacial acetic acid). HCEC solution was dropped onto a glass slide and then air dried. Next, HCECs were treated with 0.005% trypsin for 7 minutes and stained with 6% Giemsa stain solution for 3.5 minutes. The number of chromosomes was analyzed in 50 cells for each HCEC preparation, and a detailed karyotype was analyzed in 20 cells for each HCEC preparation. The standard International System for Human Cytogenetic Nomenclature (ISCN) 1995 and definitions were followed, and the frequency of loss or gain of individual chromosomes was examined. In addition, the frequency of aneuploidy per case (the number of abnormal cells divided by the total number of cells examined at metaphase) was tested. All analyses were carried out at Nippon Gene Research Laboratories, Inc., Sendai, Japan.

**RESULTS**

**General Features of the cHCEC Karyotypes**

The karyotypes of cHCECs from donors between 9 and 69 years of age were analyzed. Not only the morphologic variations of cultured cells but also the composition of cHCECs varied greatly in size and morphology from culture to culture, even under identical culture protocols. The reason for that variation might be related to the donor age or to the differences of culture passages. The cornea donor ages and the frequency of the CD44+/CD0 SP showed an apparent inverse association, in contrast to the positive correlation in karyotype aneuploidy.17

At the early stage of this study, cytogenetic examination was performed only for the bulk culture without cell sorting. The number of chromosomes was analyzed in 50 cells for each HCEC preparation, and a detailed karyotype was analyzed in 20 cells for each HCEC preparation. The standard International System for Human Cytogenetic Nomenclature (ISCN) 1995 and definitions were followed, and the frequency of loss or gain of individual chromosomes was examined. In addition, the frequency of aneuploidy per case (the number of abnormal cells divided by the total number of cells examined at metaphase) was tested. All analyses were carried out at Nippon Gene Research Laboratories, Inc., Sendai, Japan.

### TABLE 1. Donor Information and Karyotyping of Cultured HCECs

<table>
<thead>
<tr>
<th>Lot no.</th>
<th>Age, y</th>
<th>Sex</th>
<th>Passage</th>
<th>Donor ECD, Cells/mm²</th>
<th>Abnormality</th>
<th>No. of Chromosomes</th>
<th>ECD at Time of Analysis, Cells/mm²</th>
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<td>49</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>68</td>
<td>Male</td>
<td>1</td>
<td>2830</td>
<td>Deletion of Y chromosome (98%)</td>
<td>49</td>
<td>1</td>
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<td>4</td>
<td>67</td>
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<td>0</td>
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<td>3309</td>
<td>Trisomy on chromosome 12 (4%)</td>
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<td>48</td>
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ECD, endothelial cell density; HCEC, human corneal endothelial cells.

**FIGURE 1.** Examples of karyotypes of cHCECs. (A) Normal karyotypes (46, XX) were observed in 20 of 20 cells by karyotyping, and 50 of 50 cells had 46 chromosomes (mode analysis; 16-year-old male donor, passage 2). (B) Deficiency of sex chromosome Y (45, X, -Y) was observed in 20 of 20 cells, and 50 of 50 cells had 45 chromosomes (mode analysis; 63-year-old male donor, passage 4). (C) Chromosome 20 trisomy (47, XX, +20) was observed in 8 of 20 cells, and 17 of 50 cells had 47 chromosomes (mode analysis; 67-year-old female donor, passage 0).
Karyotyping of 21 cHCEC bulk cultures from donors between 9 and 69 years of age were analyzed in regard to culture passages and donor variations, and the donor information are summarized, together with the results of the karyotype analysis, in Table 1. No description means an insufficient proliferation to gain metaphase cHCECs before cytogenetic examination. As mentioned above, cHCECs showed frequent karyotype abnormality, such as sex chromosome loss and trisomy, in accordance with the previous study.17 However, only 1 case (donor 19) of the 21 cases showed translocation, which was not observed in the previous study. In the current study, bulk cultured HCECs demonstrated aneuploidy in most cases, regardless of the passages, from primary culture to fifth passage. This finding does not exclude the conclusions of previous works, because in our present study the number of cell culture passages was limited only up to five or fewer. Although statistical analyses were not carried out, donor corneal endothelial cell density (ECD) did not show any direct influence on the frequency of aneuploidy. In contrast to the ECD of the donors, the lowered cell density of cHCECs at the time of analysis might have had some association with the frequency of aneuploidy. Among 8 lots of cHCECs which showed a cell density below 1000 cells/mm², 7 lots showed evident aneuploidy (nearly 90%). Conversely, none of the four lots of cHCECs with a cell density greater than 1000 cells/mm² showed abnormality. This indirectly indicates that the quality or efficiency of the HCEC cultures or the presence of CST may have an association with the observed aneuploidy. Donor Age and Karyotype Aneuploidy Consistent with the findings in the previous report,10 the frequency of aneuploidy showed a clear inverse association with the age of the HCEC donors. In 10 of 14 young donors under 29 years of age (71%), the HCECs showed normal karyotype, whereas those from the remaining four donors showed either sex chromosome loss or trisomy. In the case of donors between 30 and 69 years of age, the HCECs of 6 of 8 (75%) showed aneuploidy with only 25% normal karyotypes. When taking into consideration the generally accepted concept of ageing and genetic instability and decreased expression of DNA repair genes, the increase of the aneuploidy may be conceivable.

However, the most relevant issue is the presence of karyotype abnormality in 29% of the cHCECs from young donors under 29 years of age. Typical examples of karyotyping are shown in Figure 2A through 2D (i.e., absence of aneuploidy in cHCECs from elder donor 10 [58-year-old female, culture passage P3] and from young donors 19 [23-year-old male, culture passage P2], 9 [23-year-old male, culture passage P2], and 14 [15-year-old female, culture passage P5]). The presence of karyotype abnormality in 29% of cHCECs from young donors below 29 years of age indicates that some thus-far-unknown intrinsic elements latent in HCEC cultures may facilitate the karyotype aneuploidy, either irrespective of or in addition to, donor age, hence forcing us to detail the SP composition in cHCECs.

Analysis of SPs in cHCECs by Flow Cytometry cHCECs were heterogeneous in size and morphology and composed of distinct SPs depending on the culture conditions.26 To explore the thus-far-unknown intrinsic elements latent in HCEC cultures, cHCECs were analyzed by flow cytometry from the context of surface CD antigen markers. The cHCECs with a high content of CD44+/CD0/CD26+ SP exhibited hexagonal morphology and no sign of CST, whereas the cultures containing SPs with either CD44++/CD24-, or CD26+ expression exhibited irregular CST-like morphology (Fig. 3). Thus, the findings of this analysis clearly demonstrate that the phenotypic features of cHCECs varied greatly from culture to culture, accompanied by the variation in composite SPs in cHCECs. These variations may override the influence of the factors described above, such donor age, passage number, or donor ECD, in regard to the frequency of aneuploidy in cHCECs.
SP Composition in cHCECs Is Critical for Aneuploidy

The specific cHCEC SP with CD44\(^+\), CD166\(^+\), CD105\(^+\), CD24\(^+\), and CD26\(^-\) was purified by MACS. Depending on the heterogeneous expression of CD44 in the SPs shown in Figure 3, CD44 magnetic beads were used mainly to separate SPs. CD44\(^+\), CD166\(^+\), CD105\(^+\), CD24\(^+\), and CD26\(^-\) SPs separated by CD44 magnetic beads were semipurified to over 90% (Fig. 4C). The SPs with either CD44\(^+++\), CD166\(^+\), CD24\(^+\), and CD26\(^-\) or CD44\(^++\), CD166\(^+\), CD24\(^+\), and CD26\(^-\) were also semipurified to over 70% (Figs. 4A, 4B).

As shown in Figure 4, the first SP with CD44\(^+\), CD166\(^+\), CD105\(^+\), CD24\(^+\), and CD26\(^-\) did not show any sign of aneuploidy in 50 cells. To the contrary, the SP with CD44\(^++\), CD166\(^+\), CD24\(^+\), and CD26\(^-\) elicited the loss of sex chromosomes in 100% of cells (60 cells), whereas the SP with CD44\(^++\), CD166\(^+\), CD24\(^+\), and CD26\(^-\) exhibited frequent trisomy on chromosomes 6, 7, 12, and 20.

**Figure 3.** Phase-contrast images and FACS analysis of the cHCECs. (A) HCECs with hexagonal morphology were detached from the culture dish, and the expression levels of CD166, CD24, CD44, CD105, and CD26 were analyzed using FACS flow cytometry (see Materials and Methods). After gating for CD166\(^+\)CD24\(^-\) (R1) or CD166\(^+\)CD24\(^+\) (R2), the following 5 SPs were defined: CD166\(^+\)CD24\(^-\)CD44\(^+\)CD105\(^+\) SP (gate G1); CD166\(^+\)CD24\(^-\)CD44\(^++\)CD105\(^+\) SP (G2); CD166\(^+\)CD24\(^+\)CD44\(^++\)CD105\(^+\) SP (G3); CD166\(^+\)CD24\(^+\)CD44\(^+\)CD105\(^+\) (G4); and CD166\(^+\)CD24\(^+\)CD44\(^++\)CD105\(^+\) SP (G5). CD44\(^++\)CD26\(^+\) cells were also detected. (B, C) Cell-surface markers of HCECs with cell-state-transition-like morphology were analyzed as in A. P, passage. Scale bar: 200 \(\mu\)m.
Cornea donor age and the frequency of CD44⁺, CD166⁺, CD105⁻, CD24⁻, and CD26⁻ SP elicited an inverse association,26 in contrast to the positive association in karyotype aneuploidy.17

**DISCUSSION**

Recent advances have raised the possibility of novel therapies based on tissue engineering techniques for the treatment of various diseases.27,28 cHCECs expanded in vitro culture can be mixtures of cHCEC SPs with distinct CST, which has been causing an obstacle to definitive refinement of the features of cHCECs.29–34 Because HCECs can be grown in culture,29–34 a cHCEC injection therapy to treat corneal endothelial dysfunctions has been extensively explored.35–40 Cultured cells generally tend to include a potential risk of karyotype changes.41,42 Thus, cHCECs need to be strictly monitored in regard to quality, as it is vital that both safety and stability of the cells be ensured in the clinical setting. The possibility exists of a leakage from anterior chamber-injected cHCECs into a vein and reaching other organs such as the liver. Also, there is the possibility that the pharmacodynamics are distinct among SPs involved in cHCECs.44 CD200, as reported by Cheong et al.,43 was not differentiated from among the HCECs. However, we found that it is expressed on the SPs of cHCECs with a certain CST (Toda M, unpublished data, 2016). The group of CD antigens reported recently by Okumura et al.45 might actually not be practical for the clinical setting because in that study the authors tried to discriminate between only nonfibroblastic phenotypes retaining normal functions and those undergoing fibroblastic changes. The findings of the present study clearly demonstrate the presence of SPs in nonfibroblastic cells with karyotype abnormality. To identify the quality of cHCECs from the aspect of their use in the clinical setting, a much more detailed analysis is needed to distinguish SPs with CST other than fibroblastic changes in cHCEC. Hence, the aim of this study was to specify the cell surface CD antigens expressed on cHCEC SPs, with or without aneuploidy, in order to clarify whether the aneuploidy thus far observed in cHCECs is dependent on the presence of specific SPs with distinct differentiation phenotypes.

In the current study, cultured HCECs demonstrated aneuploidy in most cases, regardless of the passages from the primary culture to fifth passage. The aneuploidy in cHCECs observed in this study may have been induced during cell division in culture. Consistent with the observation of Miyai et al.,25 most of the abnormal karyotypes observed in the cHCECs might have been induced at a very early stage during culture due to the presence of abnormalities even at the primary culture (Table 1, lots 4, 5, and 6). Although statistical analysis

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**TABLE 2.** SP Contents of Cultured HCECs Used in Figure 4

<table>
<thead>
<tr>
<th>Lot no.</th>
<th>Age, y</th>
<th>Sex</th>
<th>Passage</th>
<th>Donor ECD, Cells/mm²</th>
<th>% Content of SP</th>
<th>% CD26⁺CD44+++</th>
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<tr>
<td>A</td>
<td>52</td>
<td>Male</td>
<td>1</td>
<td>2615</td>
<td>0.1 16.8 48.1 6.2 30.2</td>
<td>74.3</td>
</tr>
<tr>
<td>B</td>
<td>25</td>
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<td>2</td>
<td>3114</td>
<td>0.1 20.1 80.2 0.1 1.6</td>
<td>16.2</td>
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<td>C</td>
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<td>2</td>
<td>3280</td>
<td>92.8 3.0 0.1 3.1 0.0</td>
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Bold numbers indicate main SPs in each lot. ECD, endothelial cell density; G, gate; HCEC, human corneal endothelial cells; SP, subpopulation.
was not carried out, there may be a significantly positive correlation between donor age and frequency of aneuploidy, as pointed out by Miyai et al.25

In addition, although we did not analyze the change of telomere length in this study, chronic cellular stress driven by low levels of DNA damage or telomere erosion is often involved in the prolonged culture process. It may be of interest to elucidate the presence or absence of polarized changes of telomere lengths among distinctive SPs of cHCECs.

In this study, cHCECs tended to have a chromosome mosaic of sex chromosome monosomy and trisomy on chromosomes 6, 7, 12, and 20. Previous studies reported an age-dependent loss in sex chromosomes in peripheral lymphocytes,46–49 bone marrow cells,49 corneal keratocytes,50,51 and cHCECs.17 Thus, the loss of sex chromosomes is a frequent phenomenon associated with age-dependent chromosome abnormality, regardless of cell type. The findings in this study coincided well with those of Miyai et al.,25 at least in terms of the loss of sex chromosomes but not in regard to the presence of trisomy on chromosomes 6, 7, 12, and 20, because their report described trisomy only on chromosome 8. It has been reported that chromosome 8 trisomy mosaic syndrome is associated with corneal opacity.25 Thus, further detailed study is necessary to elucidate this discrepancy regarding the location of trisomy. Our results also indicated that cHCECs for clinical therapies should be efficiently obtained from young donors.

Moreover, careful examination of the karyotype in cHCECs is crucial before their use should be considered in the clinical setting.

Flow cytometry analysis demonstrated that the cHCEC SP, semipurified by MACS, with the surface expression of CD166+, CD105−, CD44+, CD24+, and CD26− did not show any kind of aneuploidy in 50 cells. Even in this SP, the presence of CD90+ and CD90− SPs were indicated (Hamuro J, unpublished observations, 2016) but that finding might not have any effect on the karyotyping results described here.

The findings in the present study are the first to directly indicate the presence of a cHCEC SP devoid of karyotype aneuploidy, thus opening the door for its use in the clinical setting. In contrast, the CD166+, CD44+, CD24+, and CD26− cHCEC SP showed sex chromosome loss in 100% of the cells, whereas the CD166+, CD44+, CD24+, and CD26− SP exhibited, albeit partly, trisomy on chromosomes 6, 7, 12, and 20. In our preliminary yet extensive experiments, the fresh human corneal endothelial cells in situ showed no sign of the presence of CD24, CD26, CD44, and CD90, but it uniformly displayed the expression of CD166 (Toda M, unpublished observations, 2016). This indicates that the phenotypes of cHCEC SP specified here (i.e., with complete absence of aneuploidy) may well coincide with those of HCECs in fresh corneal endothelium tissues.

It should be noted that in vivo data supporting the claim that the specified SP without aneuploidy has a direct impact on the outcome of endothelial repair is critical. Our findings of the good correlation between the in vivo endothelial density measured by specular microscopy and the improvement of central corneal thickness and VA post cHCEC injection therapy with the SP without aneuploidy confirmed here will be published elsewhere (manuscript in preparation).

In conclusion, the new findings presented in this study show that the thus-far reported presence of aneuploidy in cHCEC culture is closely restricted to limited cHCEC SPs.

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