[0004] In another point, cultured hCENCs are characterized, i.e. visually, predominantly by their 'cobblestone-like' morphological appearance. Immunostaining with zonula occludins-1 (ZO-1) and sodium potassium ATPase (Na^+K^+ ATPase) have also been used frequently as markers for characterization of these cells. However, these markers are not hCENC-specific and are found ubiquitously expressed in many other cell types. Therefore, both ZO-1 and Na^+K^+ ATPase are not ideal markers for cell isolation and enrichment. Hence, there is a need for improved methods for specifically identifying and isolating hCENCs.

SUMMARY OF THE INVENTION

[0005] In a first aspect, the present invention refers to a monoclonal antibody or an antigen binding portion thereof that binds specifically to a human corneal endothelial cell (hCENC), wherein the target of the antibody, or antigen binding portion thereof, is cell surface-expressed Peroxiredoxin-6 (Prdx6). In one example, there is disclosed an antibody or an antigen binding portion thereof, that binds specifically to human corneal endothelial cells (hCENCs), wherein the target of the monoclonal antibody, or antigen binding portion thereof, is essentially cell surface-expressed Peroxiredoxin-6 (Prdx6).

[0006] In a second aspect, the present invention refers to a method for determining suitability of a cell sample for corneal transplantation, wherein the method comprises: contacting a sample of donor cells obtained from a patient with an antibody as previously described; measuring an antibody binding signal; wherein an increased antibody binding signal compared to a control signal indicates a cobblestone-like cell morphology and thus suitability of the cell sample for corneal transplantation; wherein the control signal is obtained empirically from measuring multiple cell samples with known morphologies.

[0007] In a third aspect, the present invention refers to a method for quantitative enrichment of human corneal endothelial cells from a mixture of cells, wherein the method comprises the use of an antibody, or an antigen binding portion thereof, according to any one of the preceding claims.

[0008] In a fourth aspect, the present invention refer to a method of isolating viable human corneal endothelial cells from a mixture of cell, wherein the method comprises the use of an antibody, or an antigen binding portion thereof, as previously described.

[0009] In a fifth aspect, the present invention refers to a hybridoma cell line with the deposition number of PTA-121135.

[0010] In a sixth aspect, the present invention refers to a method of generating monoclonal antibodies, as described previously, by culturing hybridoma cells with the deposition number PTA-121135.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011] The invention will be better understood with reference to the detailed description when considered in conjunction with the non-limiting examples and the accompanying drawings, in which:

culturing hybridoma cells with the deposition number PTA-121135. In a further example, the hybridoma cell line has the deposition number of PTA-121135. These cells have been deposited with the international depositary authority ATCC on 20 March 2014.

[0033] Antibodies or fragments thereof, in accordance with the invention, are prepared utilizing antibody production methods known in the art. The antibodies may be produced using methods selected from a group consisting of the hybridoma method, recombinant methods, chimeric methods, antibody isolation and antibody purification methods. Exemplary use of the hybridoma method can be found in the experimental section of the present disclosure.

[0034] As referenced herein, the methods used for the isolation and purification of antibodies may include, but are not limited to ultrafiltration, dialysis, centrifugation, ion exchange chromatography, size exclusion chromatography, affinity chromatography, precipitation, gel filtration, gel electrophoresis and capillary electrophoresis.

[0035] Described herein are monoclonal or polyclonal antibodies that bind specifically to hCENCs, more exactly monoclonal antibodies, wherein the target of the monoclonal antibody, or antigen binding portion thereof, is cell surface-expressed Peroxiredoxin-6 (Prdx6). The identity of this target antigen was determined using immunoprecipitation, as well as mass spectrometry-based approach and analysis. The antigen target of TAG-2A12 was also determined using reverse immunoprecipitation and utilizing a known antibody as a control, as shown in the experimental section of this application. As shown in the results, while both antibodies (TAG-2A12 and the known anti-Prdx6, Fig. 4) stained positive on the corneal endothelial monolayer on the corneal tissue, only mAb TAG-2A12 stained membrane bound Prdx6, whilst the known anti-Prdx6 only stained for intracellular Prdx6. Furthermore, when analysed using flow-cytometry it was observed that only TAG-2A12 was able to detect Prdx6 antigen target expressed on the surface of hCENCs.

[0036] The term "antigen" refers to a molecule or a portion of a molecule capable of being bound by a selective binding agent, such as an antibody, and additionally capable of being used in an animal to produce antibodies capable of binding to an epitope of that antigen. An antigen may have one or more epitopes.

[0037] The protein Prdx6 is a bifunctional enzyme with two distinct active sites and is encoded by the gene of the same name. It is a member of the thiol-specific antioxidant protein family and is involved in redox regulation of the cell, via its capability to reduce H_2O_2, short chain organic fatty acids, and phospholipid hydroperoxides. It plays a possible role in the regulation of phospholipid turnover, as well as in protection against oxidative injury.

[0038] In another example, the antibodies or an antigen binding portions thereof, do not bind significantly to cells selected from the group consisting of epithelial cells, stromal fibroblast cells, human lung fibroblasts, hESC-derived neural crest cells and human embryonic stem cells.

Claims

1. A monoclonal antibody or an antigen binding portion thereof that binds specifically to a human corneal endothelial cell (hCENC), wherein the target of the antibody, or antigen binding portion thereof, is cell surface-expressed Peroxiredoxin-6 (Prdx6).

2. The antibody of claim 1 or an antigen binding portion thereof, wherein the antibody or an antigen binding portion thereof is selected from a group consisting of Fab, Fab', F(ab')2, Fv, diabodies, Fd, dAb, maxibodies, single chain antibody molecules, complementarity determining region (CDR) fragments, scFv, diabodies, triabodies, tetrabodies and polypeptides that contain at least a portion of an immunoglobulin, wherein the portion of an immunoglobulin is sufficient to confer specific antigen binding to a target polypeptide.

3. The antibody of claim 2 or an antigen binding portion thereof, wherein the complementarity determining regions are selected from a group consisting of heavy chain CDR 1 (SEQ ID No. 1), heavy chain CDR 2 (SEQ ID No. 2), heavy chain CDR 3 (SEQ ID No. 3), light chain CDR 1 (SEQ ID No. 4), light chain CDR 2 (SEQ ID No. 5) and light chain CDR 3 (SEQ ID No. 6).

4. The antibody of claim 1, or an antigen binding portion thereof, wherein the antibody or antigen binding portion thereof does not bind significantly to cells selected from the group consisting of epithelial cells, stromal fibroblast cells, human lung fibroblasts, hESC-derived neural crest cells and human embryonic stem cells.

5. The antibody according to any one of the preceding claims, or antigen binding portion thereof, wherein the antibody binds to human corneal endothelial cells in a manner in which the strength of a signal derived from the binding of the antibody to the antigen is proportional to the amount of cells exhibiting a "cobblestone-like" morphology, thus enabling a grading of the human corneal endothelial cell quality.

6. The antibody according to any one of the preceding claims, or antigen binding portion thereof, wherein the antibody does not bind to immortalized human corneal endothelial cells.

7. A method for determining suitability of a cell sample for corneal transplantation, wherein the method comprises:

a. contacting a sample of donor cells obtained from a patient with an antibody according to any one of claims 1 to 6;
b. measuring an antibody binding signal;
c. wherein an increased antibody binding signal compared to a control signal indicates a cobblestone-like cell morphology and thus suitability of the cell sample for corneal transplantation; wherein the control signal is obtained empirically from measuring multiple cell samples with known morphologies.

8. A method for quantitative enrichment of human corneal endothelial cells from a mixture of cells, wherein the method comprises the use of an antibody, or an antigen binding portion thereof, according to any one of claims 1 to 6.

9. A method of isolating human corneal endothelial cells from a mixture of cell, wherein the method comprises the use of an antibody, or an antigen binding portion thereof, according to any one of claims 1 to 6.

10. The methods according to claims 8 and 9 comprising the steps of:
a. exposing a mixture of cells to an antibody according to any one of claims 1 to 6;
b. separating cells that bind to said antibody from cells that do not bind to said antibody or antigen binding fragment thereof.

11. The method according to claims 8 to 10, wherein the cell types found in the mixture are cell types associated cell samples in a clinical setting.

12. The method according to any one of claims 8 to 11, wherein the isolated human corneal endothelial cells are viable cells.

13. The method according to any one of claims 8 to 12, wherein the isolated human corneal endothelial cells are suitable for medical transplant and grafting purposes.

14. The method according to claim 10, wherein the separating is a method selected from the group consisting of column chromatography, magnetic affinity cell separation and fluorescence-activated cell sorting.

15. A hybridoma cell line with the deposition number of PTA-121135.

16. A method of generating antibodies according to claim 1 by culturing hybridoma cells with the deposition number PTA-121135.