FEBS Letters 565 (2004) 6–10 FEBS 28299

Human limbal epithelium contains side population cells expressing the ATP-binding cassette transporter ABCG2

Katsuhiko Watanabe[a], Kohji Nishida[a,*], Masayuki Yamato[b], Terumasa Umemoto[b], Taizo Sumide[a], Kazuaki Yamamoto[a], Naoyuki Maeda[a], Hitoshi Watanabe[a], Teruo Okano[b], Yasuo Tano[a]

[a]*Department of Ophthalmology, Osaka University Medical School, Room E7, Yamadaoka 2-2, Suita, Osaka 565-0871, Japan*
[b]*Institute of Advanced Biomedical Engineering and Science, Tokyo Women's Medical University, 8-1 Kawada-cho, Shinjuku-ku, Tokyo 162-8666, Japan*

Received 9 January 2004; revised 9 March 2004; accepted 9 March 2004

First published online 7 April 2004

Edited by Veli-Pekka Lehto

Abstract Many types of organ-specific stem cells have been recently shown to exhibit a side population (SP) phenotype based on their ability to efflux Hoechst 33342 dye. Because stem cells from corneal epithelium reside in the basal layer of the limbal epithelium, the purpose of this study was to examine whether the limbal epithelium contains SP cells. The ATP-binding cassette transporter Bcrp1/ABCG2 is reported to contribute to the SP phenotype in cells from several diverse sources. Here we show data from fluorescence-activated cell sorting and real-time quantitative RT-PCR analysis showing that harvested limbal epithelial cells contain SP cells expressing ABCG2. Immunofluorescence revealed that a portion of limbal epithelial basal cells expressed ABCG2. Data indicate that ABCG2 positive limbal epithelial cells are putative corneal epithelial stem cells.

Keywords: Corneal epithelium; Limbal epithelium; Stem cell; Side population; ABCG2

1. Introduction

The cornea – the transparent outer anterior tissue layer of the eye – provides the eye with protection and refractive properties essential for vision. These functions depend, in part, on the corneal epithelium, a highly specialized cell layer comprising both basal and stratified squamous cells. Corneal epithelial stem cells reside in the basal layer of the limbus [1,2], the transitional zone between the cornea and the more peripheral bulbar conjunctiva. These cells allow the renewal of the corneal epithelium by generating transient amplifying cells that migrate, proliferate and differentiate to replace lost corneal epithelial cells [3–5]. However, because of the absence of the definite biological markers, unequivocal corneal epithelial stem cell identification remains elusive.

In 1996, Goodell et al. [6] demonstrated that mouse hematopoietic stem cells with long-term multi-lineage reconstituting ability can be isolated as side population (SP) cells based on their ability to efflux the Hoechst 33342 dye. Using dual wavelength flow cytometric analysis, SP cells were identified as a distinct population with low Hoechst 33342 blue/red fluorescence representing approximately 0.1% of total bone marrow cells [6]. SP cells have also been identified in hematopoietic compartments in a number of animals [7–10]. In addition, SP cells have been isolated from various types of adult tissue where they demonstrate stem cell activity [6,10–16]. These findings suggest that the SP phenotype represents a common feature of stem cells.

Recently, Zhou et al. [13,17] reported that the ATP-binding cassette transporter Bcrp1/ABCG2 is a molecular determinant of the SP phenotype. A number of other studies in a wide variety of organs have also indicated that the SP phenotype is largely determined by the expression of Bcrp1/ABCG2 [13,15,18–20]. More recently, Mogi et al. [21] have shown by targeted gene ablation studies in mice that serine/threonine kinase Akt signaling modulates the SP phenotype by regulating the expression of Bcrp1/ABCG2.

At present, it remains unclear if corneal epithelial stem cells might exhibit the SP phenotype. We report our recent studies on human limbal epithelium, providing evidence that these cells contain SP cell subsets expressing ABCG2, implicating possible relationships between these SP cells and limbal stem cells.

2. Materials and methods

2.1. Cell preparation

Human corneoscleral rims from USA eye bank eyes were used. Limbal tissues were obtained by using scissors, and 8.0-mm diameter central portions of corneas were obtained by trephination. Limbal tissues and central corneas were incubated separately at 37 °C in Dulbecco's modified Eagle's medium (DMEM; Nikken Biomedical Laboratory, Kyoto, Japan) containing 2.4 units/ml dispase (Invitrogen, Carlsbad, CA) for 1 h. Epithelial cells were separated under a dissecting microscope and treated with 0.25% trypsin-1 mM EDTA solution (Invitrogen) for 15 min at 37 °C to achieve each single cell suspensions from the limbal epithelium and the corneal epithelium. Enzymatic activity was then stopped by adding an equal volume of DMEM containing 10% fetal calf serum (FCS; Morgate Biotech, Qld., Australia).

2.2. Hoechst 33342 dye exclusion assay

Separate populations of epithelial cells from the limbus and from the cornea were resuspended at 1.0×10^6 cells/ml in incubation medium

* Corresponding author. Fax: +81-6-6879-3458.
E-mail address: knishida@ophthal.med.osaka-u.ac.jp (K. Nishida).

0014-5793/$22.00
doi:10.1016/j.febslet.2004.03.064

(DMEM containing 2% FCS and 10 mM HEPES (Sigma)) and subsequently divided into two portions. After incubation in incubation medium for 90 min at 37 °C, (*R*)+-verapamil (Sigma, St. Louis, MO) or tryprostatin A (TPS-A; provided by Dr. H. Osada (RIKEN Institute)) was added to one portion (final concentration: 50 μM), after which both portions were incubated for a further 30 min at 37 °C. Antibody-mediated inhibition assay using anti-ABCG2 monoclonal antibody (5D3; eBioscience, San Diego, CA) or isotype control (mouse IgG_{2b}; eBioscience) was performed as previously described [22]. Hoechst 33342 dye (Sigma) was then added to both portions (final concentration: 3 μg/ml), and incubation continued for another 90 min at 37 °C. After the final incubation, cells were kept on ice and analyzed for Hoechst 33342 dye efflux by EPICS Altra FACS analysis (Beckman Coulter, Fullerton, CA). Prior to analysis, propidium iodide (Sigma) was added (final concentration: 2 μg/ml) to distinguish live from dead cells. Hoechst 33342 dye was excited at 350 nm using a UV laser. Fluorescence emission was detected through 450 nm BP (Hoechst blue) and 675 nm LP (Hoechst red) filters, respectively. Propidium iodide in cells was excited at 488 nm (argon ion laser) and fluorescence emission was detected through a 610 nm BP filter.

2.3. Real-time quantitative RT-PCR analysis

SP and non-SP cells were isolated from limbal epithelial cells using EPICS Altra flow cytometric sorting. Total RNA was obtained from equal numbers (2000–3000) of SP and non-SP cells according to the manufacturer's instructions (Isogen; Nippongene, Tokyo, Japan). After DNase I (Invitrogen) treatment, total cellular RNA was divided into two portions. One portion was reverse transcribed with oligo$(dT)_{12-18}$, according to the manufacturer's instructions (SuperScript First-Strand Synthesis System for RT-PCR; Invitrogen), and a 1/10 volume (2 μl) of synthesized cDNA was used as a template for PCR. The other portion was treated in the same manner except for the omission of SuperScript II reverse transcriptase (Invitrogen). The oligonucleotide primers (Invitrogen) used for ABCG2 amplification were 5′-GGTTTCCAAGCGTTCATTCAAA-3′ (forward) and 5′-TAGCCCAAAGTAAATGGCACCTA-3′ (reverse), with an expected product length of 111 bp. The TaqMan probe (Applied Biosystems, Foster City, CA) used for ABCG2 detection was 5′-CCCAGGCCTCTATAGCTCAGATCATTGTCA-3′, labeled with 6-carboxyfluorescein (FAM) at the 5′-end, and with 6-carboxytetraethylrodamine (TAMRA) at the 3′-end. Quantitative PCR was performed using ABI Prism 7900HT Sequence Detection System (Applied Biosystems), according to the manufacturer's instructions. The reaction mixture (30 μl) contained 15 μl of TaqMan Universal PCR Mastermix (Applied Biosystems), 15 pmol of forward and reverse primers, 7.5 pmol of TaqMan probe and 2 μl of the investigated sample. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression was measured using TaqMan GAPDH Control Reagents (Applied Biosystems), according to the manufacturer's instructions. Thermocycling used 50 cycles at 95 °C for 15 s and 60 °C for 1 min with an initial cycle at 50 °C for 2 min and 95 °C for 10 min. A negative control with non-reverse transcribed total RNA as a template was included in every experiment. All assays were run in duplicate. To represent the ABCG2 mRNA expression level, we used the ABCG2 mRNA expression index as the value of the ABCG2 gene copies divided by the value of the housekeeping GAPDH gene copies.

2.4. Statistical analysis

Statistics were calculated using SigmaStat 2.0 (SPSS, Chicago, IL). To compare ABCG2 expression between SP and non-SP cells, the Mann–Whitney rank sum test was applied.

2.5. Immunofluorescence

Cryostat sections (20 μm) were cut from the limbus and from the cornea, dried for 1 h at room temperature, and fixed in acetone for 10 min also at room temperature. Immunofluorescence was performed using DakoCytomation CSA II (DakoCytomation, Kyoto, Japan). Briefly, endogenous peroxidase activity was blocked with 3% hydrogen peroxide in water for 15 min. The slides were first incubated with serum-free protein in buffer for 15 min to block non-specific binding of antibodies, then incubated at 4 °C overnight with a 1:2500 dilution of BXP-21 monoclonal antibody (MBL, Aichi, Japan). BXP-21 was diluted in tris-buffered saline containing 1% bovine serum albumin and 0.1% Tween 20. After incubation, slides were incubated for 15 min with anti-mouse immunoglobulins-HRP, followed by 15 min in fluorescyl-tyramide hydrogen peroxide in buffer. After counterstaining with propidium iodide (Sigma), slides were mounted and observed on a confocal laser scanning microscope (LSM 510 META; Carl Zeiss, Jena, Germany). For each type of tissue, identically treated negative controls were included using normal non-specific IgG_{2a} (DakoCytomation).

3. Results

3.1. SP cells are present in human limbal epithelium

In the Hoechst 33342 dye exclusion assay, a distinct population of cells with a low Hoechst 33342 blue/red fluorescence was detected in epithelial cells from the limbus (Fig. 1A; 0.20% gated cells) but not in cells from the cornea (Fig. 1B, 1D; 0.02% gated cells). Generation of this subpopulation was not seen in the presence of (*R*)+-verapamil, the specific inhibitor of Hoechst 33342 dye transport (Fig. 1C; 0.01% gated cells). These observations indicate that the limbal epithelium contains SP cells.

3.2. Effect of 5D3 and TPS-A on Hoechst 33342 dye efflux

When SP cells were incubated with an anti-ABCG2 monoclonal antibody, 5D3, recognizing an external plasma membrane epitope on living cells [13,22], prior to Hoechst 33342 dye exclusion assay, a marked decrease in SP cell dye efflux was observed (Fig. 2A). Hoechst 33342 dye efflux activity was also inhibited by a novel ABCG2 inhibitor, TPS-A (Fig. 2B), that is known not to inhibit another important ATP-binding cassette transporter, P-glycoprotein [23]. These data strongly suggest that ABCG2 is a major contributor to the SP phenotype of limbal epithelial cells.

3.3. ABCG2 mRNA expression in SP and non-SP cells

To investigate the link between the SP phenotype and ABCG2 expression, SP and non-SP cells from limbal epithelial tissues were analyzed for ABCG2 gene expression by TaqMan real-time

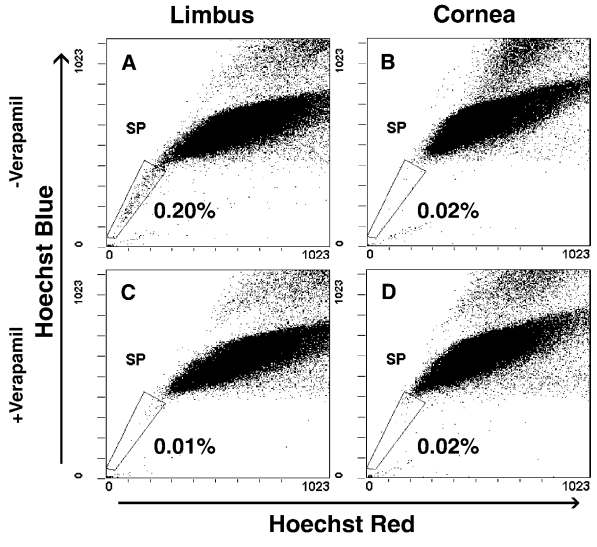

Fig. 1. Flow cytometric analysis of epithelial cells harvested from the limbus and from the cornea. Epithelial cells were isolated from eye bank limbus and cornea tissues, and analyzed for Hoechst 33342 dye efflux by FACS. (A) SP cells were detected in limbal epithelial cells after Hoechst 33342 dye staining. (B) SP cells were absent in corneal epithelial cells. (C) Dye efflux from SP cells was inhibited by verapamil. (D) Verapamil had no effect on dye efflux.

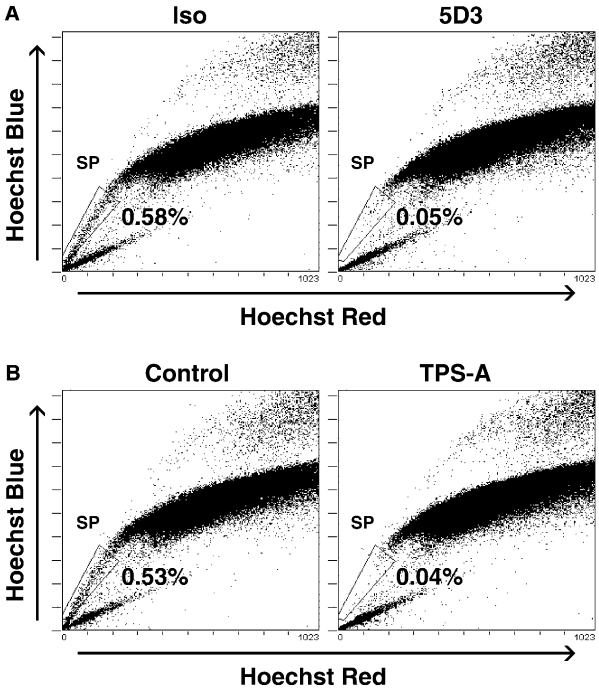

Fig. 2. Influence of ABCG2 inhibitors on the SP phenotype of limbal epithelial cells. Limbal epithelial cells were analyzed for Hoechst 33342 dye efflux in the presence of ABCG2 inhibitors. Dye efflux from SP cells was effectively inhibited by 5D3 monoclonal antibody (A) and by TPS-A (B).

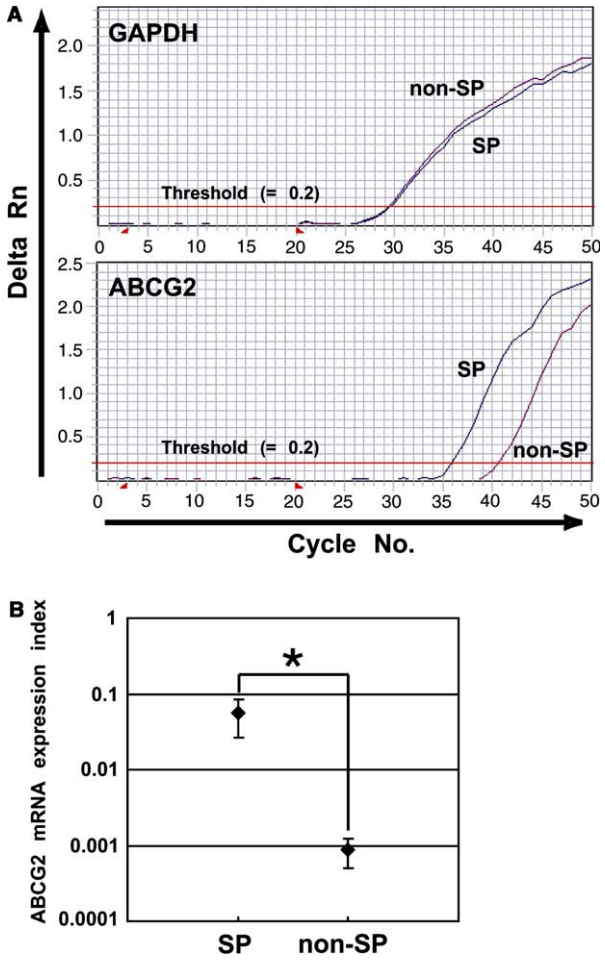

Fig. 3. Quantification of ABCG2 mRNA in SP and non-SP cells. (A) Amplification plots from real-time RT-PCR analysis. Delta Rn is plotted against cycle number. Horizontal red lines show the threshold used for calculation of ABCG2 expression indices. Real-time quantitative RT-PCR procedures were as described in Section 2. (B) ABCG2 mRNA expression indices in SP and non-SP cells. The relative expression of ABCG2 gene was normalized to that of GAPDH in each sample. Data represent the mean value from four samples. Error bars indicate the S.E. ABCG2 mRNA expression in SP cells was significantly higher than in non-SP cells ($P = 0.029$).

RT-PCR (Fig. 3A). ABCG2 mRNA expression indices were $5.61 \times 10^{-2} \pm 2.92 \times 10^{-2}$ (SP) and $8.66 \times 10^{-4} \pm 3.56 \times 10^{-4}$ (non-SP) ($n = 4$, mean ± S.E.) (Fig. 3B). Statistical analysis showed that the ABCG2 mRNA expression level in SP cells was significantly higher than in non-SP cells ($P = 0.029$).

3.4. Distribution of ABCG2-expressing cells

Our immunofluorescence data using BXP-21 monoclonal antibody specific for human ABCG2 revealed that ABCG2 is expressed in the limbal epithelial basal cells (Fig. 4A, B), but not in corneal epithelial cells (Fig. 4C). Observed ABCG2 expression in basal cells is inconsistent and discontinuous in the epithelium, and does not persist throughout the entire limbus (Fig. 4A). Fig. 4B shows that positive staining cells are present not only in the basal but also in the suprabasal cells.

4. Discussion

Corneal epithelial stem cell deficiency caused by ocular trauma or diseases causes corneal opacification and visual loss. Patients with corneal epithelial stem cell deficiency can be treated with autologous or allogenic limbal transplantation [24,25]. However, autologous limbal transplantation requires a large limbal withdrawal from the patient's eye, and allogenic limbal transplantation has a significant risk of rejection. Moreover, shortage of donor tissues is a serious current challenge. To avoid these problems, transplantations of cultivated corneal limbal epithelial cells have recently been reported [26–31]. In such tissue engineering approaches, development of new, effective methods for rapid identification and enrichment of corneal epithelial stem cells would be a considerable advantage since long-term, functional recovery of corneal epithelium is linked to graft constructs that retain viable stem cell populations [26,28,32].

In this study, we examined whether human limbal epithelium contains cells with SP phenotype, similar to stem cells in other organs [6,10–16]. Hoechst 33342 dye exclusion assays revealed that a number of SP cells are present in the limbal epithelium but not in the corneal epithelium (Fig. 1). The mean percentage of SP cells in limbal epithelial cells obtained from six independent experiments was calculated to be 0.29% (data not shown). This is a reasonable abundance value compared with that previously reported in other organs [6,10–16]. Though verapamil is generally used to identify the SP phenotype in the Hoechst 33342 dye exclusion assay, it is insufficient in specificity to demonstrate the contribution of ABCG2 to the SP phenotype of limbal epithelial cells. As ABCG2-specific inhibitors, fumitremorgin C [33], 5D3 monoclonal antibody

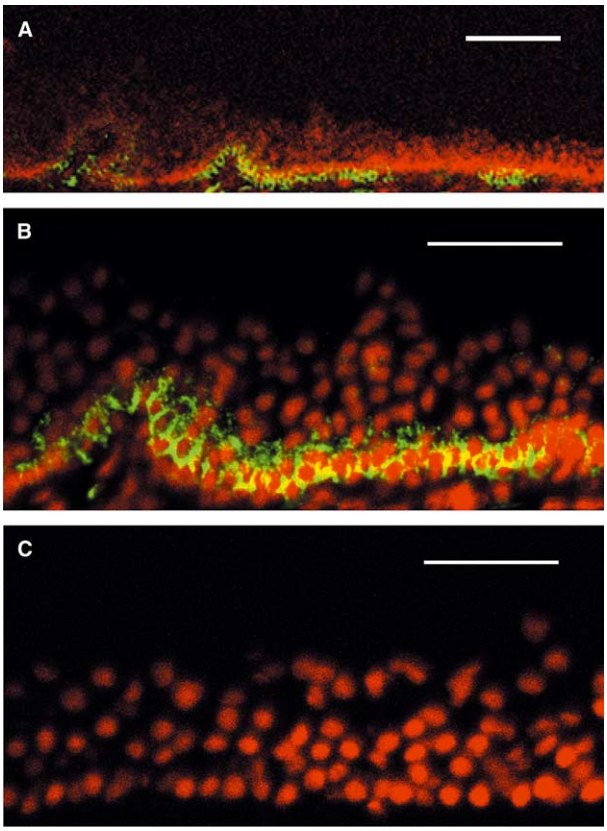

Fig. 4. Expression patterns of the ABCG2 marker using immunomarkers. Distribution of cells expressing ABCG2 protein was examined by immunofluorescence staining using the BXP-21 monoclonal antibody. (A) The basal layer of the limbal epithelium was inconsistently and discontinuously stained with anti-ABCG2 antibody (green). (B) A higher-magnification view of the limbal region showed that positively stained cells are present not only in the basal but also in the suprabasal cells. (C) ABCG2 was not expressed in the corneal epithelium. Nuclei were labeled with propidium iodide (red). Scale bar: 100 μm (A), 50 μm (B, C).

[13,22], and TPS-A [23] are reported. Our SP analysis of limbal epithelial cells using 5D3 and TPS-A showed that both 5D3 and TPS-A effectively inhibit Hoechst 33342 dye efflux from these cells (Fig. 2A, B). These data strongly suggest a correlation between SP phenotype and ABCG2 expression, but the possibility of partial contribution of other transporters to SP phenotype cannot be excluded. Real-time quantitative RT-PCR analysis showed a strong correlation between ABCG2 expression and the SP phenotype. The ABCG2 mRNA expression indices in SP and non-SP cells varied considerably between samples, but SP cells consistently expressed a significantly higher level of ABCG2 mRNA over non-SP cells (Fig. 3B). The variation observed in these experiments may be due to variable conditions of eye bank tissue samples affected by many uncontrolled factors including donor age, death-to-preservation time and tissue storage time.

Immunofluorescence work revealed that ABCG2 was inconsistently expressed within the basal layer of the limbal epithelium (Fig. 4A), but not in the corneal epithelium (Fig. 4C). These observations also indicated that ABCG2 positive cells are present not only in the basal but also in the suprabasal cells in some areas (Fig. 4B). This localization pattern for ABCG2-positive cells coincides almost identically with that for corneal epithelial stem cells demonstrated previously by several investigators [1,2,34–37]. These findings imply that ABCG2 positive cells in the limbal epithelium are putative corneal epithelial stem cells, consistent with the observation that SP cells are present in the limbal epithelium but not in the corneal epithelium (Fig. 1). Flow cytometry revealed that SP cells are only 0.20% of total limbal epithelial cells (Fig. 1A), but immunofluorescence showed that many more cells express ABCG2 (Fig. 4A, B). Because limbal epithelial basal cells have a small cytoplasmic volume, it is not clear whether ABCG2 molecules localize in the plasma membrane or in the cytoplasm (Fig. 4A, B). Since it has been reported that active ABCG2 is expressed on the surface of SP cells, and cytoplasmic ABCG2 cannot efflux Hoechst 33342 dye outside these cells [19,21,38,39], the smaller number of SP cells observed here can be explained by possible cytoplasmic localization of ABCG2. In addition, it is possible that our methods actually underestimate functional SP populations in limbal epithelial cells: cells were collected from eye bank eyes with varying death-to-preservation times and storage conditions, with the likelihood that viability, including dye efflux capability, might be reduced.

One possible biological function of ABCG2 expressed in the limbal epithelium is a protective function. Because Bcrp1/ABCG2 is known to transport a variety of toxic lipophilic compounds [40–42], ABCG2 may protect corneal epithelial stem cells from cytotoxic agents. Recently, Jonker et al. [43] found that Bcrp1/ABCG2-null mice become extremely sensitive to the dietary chlorophyll-breakdown product, pheophorbide a, resulting in severe, sometimes lethal, phototoxic lesions on light-exposed skin. This finding suggests that ABCG2 may protect corneal epithelial stem cells from solar damage. This is also consistent with the assertion that corneal epithelial stem cells are protected from solar damage by melanin pigmentation of the limbus in non-Caucasian people [2]. Another possibility is that ABCG2 may regulate the development of corneal epithelial stem cells by functioning as transporters of small hydrophobic regulatory molecules involved in proliferation, differentiation, or apoptotic pathways. However, the function of ABCG2 expressed in limbal epithelial basal cells remains a subject for further investigations.

In summary, our results suggest that the SP cell phenotype and cellular surface expression of ABCG2 may characterize putative corneal epithelial stem cells. Thus, ABCG2 may serve as a useful cell surface marker in the enrichment of viable corneal epithelial stem cells, and could be readily exploited for new tissue engineering approaches that attempt to reconstruct damaged ocular surfaces using cell transplantation methods.

Acknowledgements: We appreciate useful comments and technical criticism from Prof. D.W. Grainger (Colorado State University, USA) and Dr. A.J. Quantock (Cardiff University, UK). Dr. H. Osada (RIKEN Institute, Japan) provided TPS-A. The present work was supported in part by Grant-in Aid for Scientific Research (15390530) from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

References

[1] Schermer, A., Galvin, S. and Sun, T.T. (1986) J. Cell Biol. 103, 49–62.
[2] Cotsarelis, G., Cheng, S.Z., Dong, G., Sun, T.T. and Lavker, R.M. (1989) Cell 57, 201–209.

[3] Kinoshita, S., Friend, J. and Thoft, R.A. (1981) Invest. Ophthalmol. Vis. Sci. 21, 434–441.
[4] Thoft, R.A. and Friend, J. (1983) Invest. Ophthalmol. Vis. Sci. 24, 1442–1443.
[5] Buck, R.C. (1985) Invest. Ophthalmol. Vis. Sci. 26, 1296–1299.
[6] Goodell, M.A., Brose, K., Paradis, G., Conner, A.S. and Mulligan, R.C. (1996) J. Exp. Med. 183, 1797–1806.
[7] Goodell, M.A., Rosenzweig, M., Kim, H., Marks, D.F., DeMaria, M., Paradis, G., Grupp, S.A., Sieff, C.A., Mulligan, R.C. and Johnson, R.P. (1997) Nat. Med. 3, 1337–1345.
[8] Storms, R.W., Goodell, M.A., Fisher, A., Mulligan, R.C. and Smith, C. (2000) Blood 96, 2125–2133.
[9] Uchida, N., Leung, F.Y. and Eaves, C.J. (2002) Exp. Hematol. 30, 862–869.
[10] Bhattacharya, S., Jackson, J.D., Das, A.V., Thoreson, W.B., Kuszynski, C., James, J., Joshi, S. and Ahmad, I. (2003) Invest. Ophthalmol. Vis. Sci. 44, 2764–2773.
[11] Jackson, K.A., Mi, T. and Goodell, M.A. (1999) Proc. Natl. Acad. Sci. USA 96, 14482–14486.
[12] Hulspas, R. and Quesenberry, P.J. (2000) Cytometry 40, 245–250.
[13] Zhou, S., Schuetz, J.D., Bunting, K.D., Colapietro, A.M., Sampath, J., Morris, J.J., Lagutina, I., Grosveld, G.C., Osawa, M., Nakauchi, H. and Sorrentino, B.P. (2001) Nat. Med. 7, 1028–1034.
[14] Asakura, A. and Rudnicki, M.A. (2002) Exp. Hematol. 30, 1339–1345.
[15] Lechner, A., Leech, C.A., Abraham, E.J., Nolan, A.L. and Habener, J.F. (2002) Biochem. Biophys. Res. Commun. 293, 670–674.
[16] Alvi, A.J., Clayton, H., Joshi, C., Enver, T., Ashworth, A., Vivanco, M.M., Dale, T.C. and Smalley, M.J. (2003) Breast Cancer Res. 5, R1–R8.
[17] Zhou, S., Morris, J.J., Barnes, Y., Lan, L., Schuetz, J.D. and Sorrentino, B.P. (2002) Proc. Natl. Acad. Sci. USA 99, 12339–12344.
[18] Kim, M., Turnquist, H., Jackson, J., Sgagias, M., Yan, Y., Gong, M., Dean, M., Sharp, J.G. and Cowan, K. (2002) Clin. Cancer Res. 8, 22–28.
[19] Scharenberg, C.W., Harkey, M.A. and Torok-Storb, B. (2002) Blood 99, 507–512.
[20] Shimano, K., Satake, M., Okaya, A., Kitanaka, J., Kitanaka, N., Takemura, M., Sakagami, M., Terada, N. and Tsujimura, T. (2003) Am. J. Pathol. 163, 3–9.
[21] Mogi, M., Yang, J., Lambert, J.F., Colvin, G.A., Shiojima, I., Skurk, C., Summer, R., Fine, A., Quesenberry, P.J. and Walsh, K. (2003) J. Biol. Chem. 278, 39068–39075.
[22] Abbott, B.L., Colapietro, A.M., Barnes, Y., Marini, F., Andreeff, M. and Sorrentino, B.P. (2002) Blood 100, 4594–4601.
[23] Woehlecke, H., Osada, H., Herrmann, A. and Lage, H. (2003) Int. J. Cancer 107, 721–728.
[24] Kenyon, K.R. and Tseng, S.C. (1989) Ophthalmology 96, 709–723.
[25] Tsubota, K., Satake, Y., Kaido, M., Shinozaki, N., Shimmura, S., Bissen-Miyajima, H. and Shimazaki, J. (1999) N. Engl. J. Med. 340, 1697–1703.
[26] Pellegrini, G., Traverso, C.E., Franzi, A.T., Zingirian, M., Cancedda, R. and De Luca, M. (1997) Lancet 349, 990–993.
[27] Tsai, R.J., Li, L.M. and Chen, J.K. (2000) N. Engl. J. Med. 343, 86–93.
[28] Rama, P., Bonini, S., Lambiase, A., Golisano, O., Paterna, P., De Luca, M. and Pellegrini, G. (2001) Transplantation 72, 1478–1485.
[29] Tseng, S.C., Meller, D., Anderson, D.F., Touhami, A., Pires, R.T., Gruterich, M., Solomon, A., Espana, E., Sandoval, H., Ti, S.E. and Goto, E. (2002) Adv. Exp. Med. Biol. 506, 1323–1334.
[30] Nishida, K. (2003) Cornea 22, S28–S34.
[31] Nishida, K., Yamato, M., Hayashida, Y., Watanabe, K., Maeda, N., Watanabe, H., Yamamoto, K., Nagai, S., Kikuchi, A., Tano, Y. and Okano, T. (2004) Transplantation 77, 379–385.
[32] Ti, S.E., Anderson, D., Touhami, A., Kim, C. and Tseng, S.C. (2002) Invest. Ophthalmol. Vis. Sci. 43, 2584–2592.
[33] Allen, J.D., van Loevezijn, A., Lakhai, J.M., van der Valk, M., van Tellingen, O., Reid, G., Schellens, J.H., Koomen, G.J. and Schinkel, A.H. (2002) Mol. Cancer Ther. 1, 417–425.
[34] Kurpakus, M.A., Stock, E.L. and Jones, J.C. (1990) Invest. Ophthalmol. Vis. Sci. 31, 448–456.
[35] Matic, M., Petrov, I.N., Chen, S., Wang, C., Dimitrijevich, S.D. and Wolosin, J.M. (1997) Differentiation 61, 251–260.
[36] Pellegrini, G., Dellambra, E., Golisano, O., Martinelli, E., Fantozzi, I., Bondanza, S., Ponzin, D., McKeon, F. and De Luca, M. (2001) Proc. Natl. Acad. Sci. USA 98, 3156–3161.
[37] Espana, E.M., Romano, A.C., Kawakita, T., Di Pascuale, M., Smiddy, R. and Tseng, S.C. (2003) Invest. Ophthalmol. Vis. Sci. 44, 4275–4281.
[38] Rocchi, E., Khodjakov, A., Volk, E.L., Yang, C.H., Litman, T., Bates, S.E. and Schneider, E. (2000) Biochem. Biophys. Res. Commun. 271, 42–46.
[39] Summer, R., Kotton, D.N., Sun, X., Ma, B., Fitzsimmons, K. and Fine, A. (2003) Am. J. Physiol. Lung. Cell. Mol. Physiol. 285, L97–L104.
[40] Allen, J.D., Brinkhuis, R.F., Wijnholds, J. and Schinkel, A.H. (1999) Cancer Res. 59, 4237–4241.
[41] Jonker, J.W., Smit, J.W., Brinkhuis, R.F., Maliepaard, M., Beijnen, J.H., Schellens, J.H. and Schinkel, A.H. (2000) J. Natl. Cancer Inst. 92, 1651–1656.
[42] Robey, R.W., Medina-Perez, W.Y., Nishiyama, K., Lahusen, T., Miyake, K., Litman, T., Senderowicz, A.M., Ross, D.D. and Bates, S.E. (2001) Clin. Cancer Res. 7, 145–152.
[43] Jonker, J.W., Buitelaar, M., Wagenaar, E., Van Der Valk, M.A., Scheffer, G.L., Scheper, R.J., Plosch, T., Kuipers, F., Elferink, R.P., Rosing, H., Beijnen, J.H. and Schinkel, A.H. (2002) Proc. Natl. Acad. Sci. USA 99, 15649–15654.