The Journal of Veterinary Medical Science

NOTE

Anatomy

Paraffin-embedded vertical sections of mouse embryonic stem cells

Hiroyuki IMAI[1,2)]*, Wataru FUJII[3)], Ken Takeshi KUSAKABE[1)], Yasuo KISO[1)], Etsuro ONO[2)] and Kiyoshi KANO[1)]

[1)]Laboratory of Veterinary Anatomy and Developmental Biology, The United Graduate School of Veterinary Science, Yamaguchi University, Yamaguchi 753-0841, Japan
[2)]Department of Biomedicine, Graduate School of Medical Science, Kyushu University, Fukuoka 812-8582, Japan
[3)]Laboratory of Applied Genetics, Graduate School of Agricultural and Life Science, The University of Tokyo, Tokyo 113-8657, Japan

J. Vet. Med. Sci.
80(10): 1479–1481, 2018
doi: 10.1292/jvms.18-0352

Received: 25 June 2018
Accepted: 27 July 2018
Published online in J-STAGE: 9 August 2018

ABSTRACT. Cultured cells are generally observed through the bottom of dishes or flasks using an inverted microscope. Two-dimensional and horizontal observation is insufficient for histological analysis of several cell lines, such as embryonic stem cells or cancer cells, because they form three-dimensional colonies. In the present study, we aimed to establish a more informative method for analysis of such stereoscopic cultured cells. We cultured mouse embryonic stem cells using a temperature-sensitive culture dish, embedded these cells in paraffin, and successfully observed vertical sections of embryonic stem cells. This vertical analysis of the stereoscopic colony emphasized structural features such as the dome shape of naïve pluripotent stem cells. This method could have the potential for analysis of three-dimensional structures and histological preservation in cultured cells.

KEY WORDS: embryonic stem cell, histological preservation, mouse, vertical section

In general, flat or two-dimensional cultured cells such as fibroblasts are observed horizontally through the bottom of culture dishes using an inverted microscope. However, two-dimensional observation is insufficient to analyze colonizing cells, such as pluripotent stem cells and cancer cells, that go through three-dimensional proliferation. In the present study, we aimed to establish a novel method for the observation of such stereoscopic cultured cells. We focused on a temperature-sensitive culture dish, UpCell (Cellseed Inc., Tokyo, Japan), for culturing vessels and mouse embryonic stem cells (ESCs) for colonizing cells. The UpCell culture dish could control the strength of cellular adhesion to the dish by managing the temperature and therefore enable the easy collection of cultured cells as sheets, while maintaining the colony structure. This temperature-dependent property of the culture dish has been used for various applications, such as regenerative medicine [6, 8] and three-dimensional culture [7, 9]. Pluripotent stem cells have two types of colony morphology, naïve and primed, with the former having dome-like colonies [5]. In previous studies, naïve embryonic stem cells have been observed through inverted microscopes [1–3, 10]; however, there have been no reports on the observation of vertical sections of naïve pluripotent stem cells. Although previous studies reported that colonies were similar between diploid and tetraploid ESCs, detailed morphological observation was not performed for each colony. The purpose in the present study is to establish a novel method for vertical observation of cultured cells. Using the method, the structure of colonies of diploid and tetraploid ESCs were comparatively analyzed. To this end, we successfully produced and observed vertical sections of mouse embryonic stem cells by paraffin-embedding and sectioning the whole cell sheet using UpCell culture dishes (Fig. 1A).

In the present study, mouse diploid and tetraploid ESCs we previously established [4] were used. Both mouse diploid and tetraploid ESCs were cultured with ESGRO Complete plus clonal grade medium (Merck, NJ, U.S.A.) supplemented with 20% Knockout Serum Replacement (Thermo Fisher Scientific Inc., MA, U.S.A.) on MEF (mouse embryonic fibroblast) feeder cells. The feeder cells obtained were mitomycin C-inactivated fibroblasts established from ICR mouse fetus 12.5 dpc of age. The medium was routinely changed using pre-warmed PBS and ESGRO medium kept on a hotplate at 37°C to prevent cell detachment. After culture, mouse diploid and tetraploid ESCs on feeder cells were peeled off as cell sheets by leaving the culture dishes at room temperature for 10 min. After removing the medium, 10% formalin was added for fixation. The fixed cell sheets were then embedded in paraffin and processed according to the standard method, using sliced sections of 4.0 μm thickness and staining by hematoxylin-eosin (H&E).

*Correspondence to: Imai, H.: himai@anim.med.kyushu-u.ac.jp

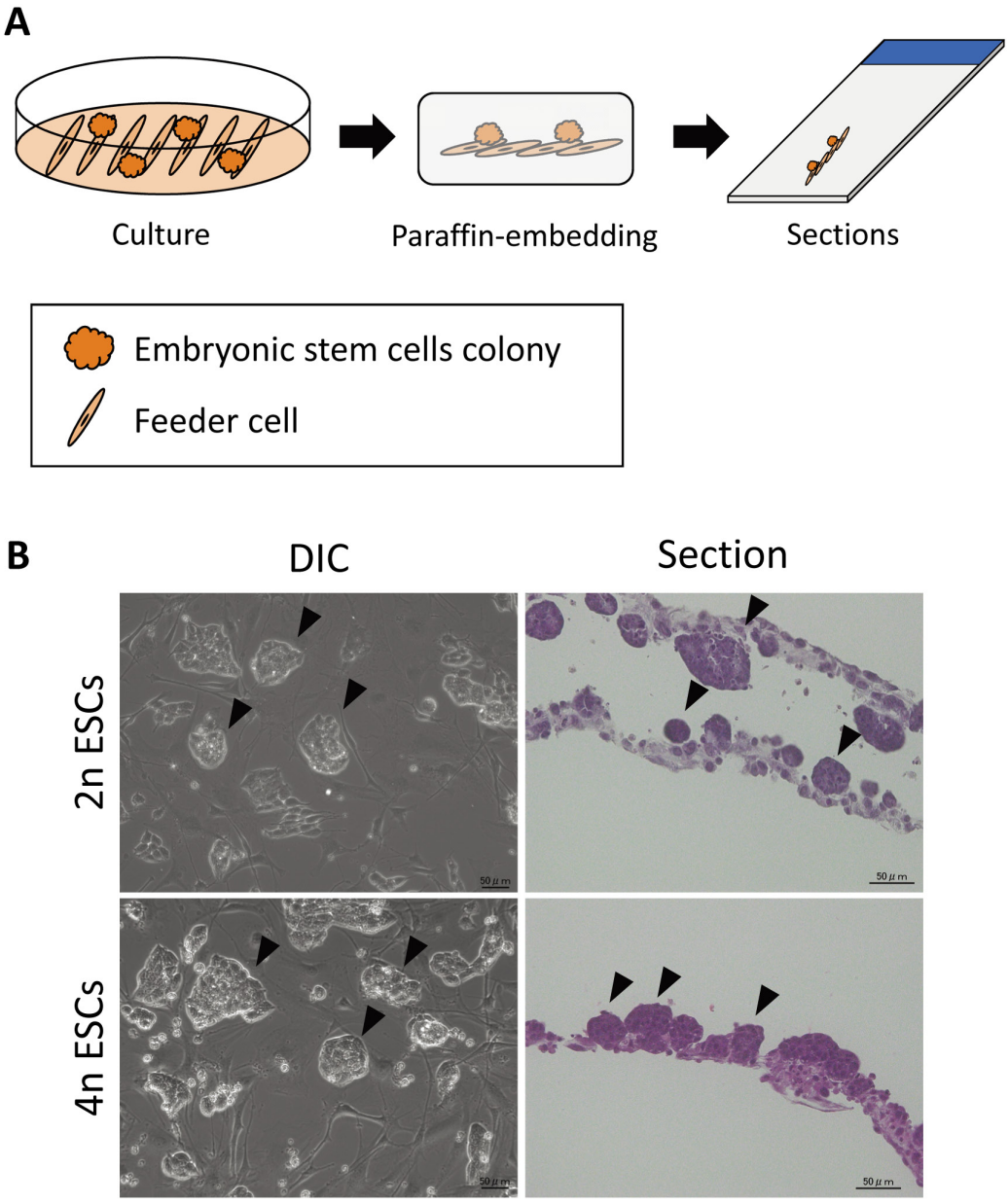

Fig. 1. Colony morphology of embryonic stem cells using temperature-sensitive culture dishes. A) Schematic drawing of observed direction for embryonic stem cells. B) Vertical colony morphology of diploid (2n) and tetraploid (4n) embryonic stem cells (ESCs). Left column shows bright field images from the general direction of observation and right sectional observation. Arrowheads indicate 2n ESCs or 4n ESCs colonies.

We successfully observed the domed-shaped cell colonies of naïve pluripotent stem cells in both diploid and tetraploid ESCs using inverted microscopes (Fig. 1B; left column, arrowheads). Tetraploid ESCs adhering to the feeder cells were also observed in the paraffin embedded section stained with H&E (Fig. 1B right column arrowheads). Previous studies reported that horizontal morphology of colonies was similar between diploid and tetraploid ESCs as assessed using inverted microscopes [2, 4, 11], and this vertical analysis strongly supported the similarity. Here, we showed the histological utility of the new method of vertically sectioning stereoscopic cell colonies, such as mouse embryonic stem cells, for the first time. Another merit of this method is that the embedded ESCs can be histologically preserved for a long time, which allows the same samples to be analyzed by various staining methods. In the future, it can be used for basic research on adhesion factors such as those in the extracellular matrix of ESCs.

In summary, we successfully analyzed, for the first time, vertical sections of stereoscopic cell colonies in mouse embryonic stem cells using temperature-sensitive cell culture dishes. Our novel method could be useful for the three-dimensional analysis of pluripotent stem cells or cancer cells. Furthermore, multiple analyses including immunostaining could be performed using the same sample, by histological preservation of cultured cells as embedded sections.

ACKNOWLEDGMENTS. This work was supported by the JSPS KAKENHI, Grant Numbers JP24658237, JP25660254, JP15K14880, and 17J07902, and the upgrade challenge project for JSPS KAKENHI Grant, Yamaguchi University.

REFERENCES

1. Evans, M. J. and Kaufman, M. H. 1981. Establishment in culture of pluripotential cells from mouse embryos. *Nature* **292**: 154–156. [Medline] [CrossRef]
2. Horii, T., Yamamoto, M., Morita, S., Kimura, M., Nagao, Y. and Hatada, I. 2015. p53 suppresses tetraploid development in mice. *Sci. Rep.* **5**: 8907. [Medline] [CrossRef]
3. Imai, H., Fujii, W., Kusakabe, K. T., Kiso, Y. and Kano, K. 2016. Effects of whole genome duplication on cell size and gene expression in mouse embryonic stem cells. *J. Reprod. Dev.* **62**: 571–576. [Medline] [CrossRef]
4. Imai, H., Kano, K., Fujii, W., Takasawa, K., Wakitani, S., Hiyama, M., Nishino, K., Kusakabe, K. T. and Kiso, Y. 2015. Tetraploid embryonic stem cells maintain pluripotency and differentiation potency into three germ layers. *PLoS One* **10**: e0130585. [Medline] [CrossRef]
5. Nichols, J. and Smith, A. 2009. Naive and primed pluripotent states. *Cell Stem Cell* **4**: 487–492. [Medline] [CrossRef]
6. Nishida, K., Yamato, M., Hayashida, Y., Watanabe, K., Maeda, N., Watanabe, H., Yamamoto, K., Nagai, S., Kikuchi, A., Tano, Y. and Okano, T. 2004. Functional bioengineered corneal epithelial sheet grafts from corneal stem cells expanded ex vivo on a temperature-responsive cell culture surface. *Transplantation* **77**: 379–385. [Medline] [CrossRef]
7. Ohashi, K., Yokoyama, T., Yamato, M., Kuge, H., Kanehiro, H., Tsutsumi, M., Amanuma, T., Iwata, H., Yang, J., Okano, T. and Nakajima, Y. 2007. Engineering functional two- and three-dimensional liver systems in vivo using hepatic tissue sheets. *Nat. Med.* **13**: 880–885. [Medline] [CrossRef]
8. Ohki, T., Yamato, M., Murakami, D., Takagi, R., Yang, J., Namiki, H., Okano, T. and Takasaki, K. 2006. Treatment of oesophageal ulcerations using endoscopic transplantation of tissue-engineered autologous oral mucosal epithelial cell sheets in a canine model. *Gut* **55**: 1704–1710. [Medline] [CrossRef]
9. Shimizu, T., Yamato, M., Isoi, Y., Akutsu, T., Setomaru, T., Abe, K., Kikuchi, A., Umezu, M. and Okano, T. 2002. Fabrication of pulsatile cardiac tissue grafts using a novel 3-dimensional cell sheet manipulation technique and temperature-responsive cell culture surfaces. *Circ. Res.* **90**: e40–e48. [Medline] [CrossRef]
10. Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Ichisaka, T., Tomoda, K. and Yamanaka, S. 2007. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* **131**: 861–872. [Medline] [CrossRef]
11. Wen, B., Li, R., Cheng, K., Li, E., Zhang, S., Xiang, J., Wang, Y. and Han, J. 2017. Tetraploid embryonic stem cells can contribute to the development of chimeric fetuses and chimeric extraembryonic tissues. *Sci. Rep.* **7**: 3030. [Medline] [CrossRef]