A NONINVASIVE METHOD FOR COUNTING HUMAN PLURIPOTENT STEM CELL NUMBERS BY LIVE CELL IMAGING

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Background

Cell density is a critical factor for controlling both growth and differentiation of human pluripotent stem (hPS) cells including embryonic stem (ES) cells and induced pluripotent stem (iPS) cells. Despite the fact of the evolution of hPS cell culture techniques, counting cell numbers is still problematic. Therefore, we have developed a noninvasive cell counting method for hPS cells through analyzing live cell images.

Outline

A Japanese adult human skin fibroblast (JCRB0534: TIG-114) -derived iPS-TIG114-4f1 human iPS cells (JCRB1437, JCRB Cell Bank, NIBIO, Osaka, Japan), which was established by Yamanaka's group (CiRA, Kyoto University) [1) 2)] and H9 human ES cells (WA09, WISC Bank, WiCell Research Institute, Madison, WI, USA) [3) 4)] were seeded on inactivated mouse embryonic fibroblasts (MEF) as described previously [4)], and then stained with a cell-permeant SYTO24 green fluorescent nucleic acid stain (Invitrogen). Phase contrast and fluorescent images of the cells obtained intermittently in a cell incubator observation system, **BioStation CT** (Nikon Corporation) were analyzed by automated image analysis software **CL-Quant** (Nikon Corporation). Area of ES/iPS cell colony coverage was measured from phase contrast images. Number of stained nuclei was counted from green fluorescent images. Immediately after imaging, the conventional cell counting by hemocytometer was performed to compare with the numbers of fluorescent nuclei.

Materials and Methods

Cell culture H9 and iPS-TIG114-4f1 were maintained on irradiated mouse embryo fibroblast feeder cells (MEF, Millipore Co.) in an KSR-based medium supplemented with 5 ng/ml human recombinant FGF-2 (Katayama Kagaku Kogyo LTD.). H9 were passaged with 1 mg/ml Dispase (Roche) and a plastic scraper (Sumitomo Bakelite Co. LTD.). iPS-TIG114-4f1 were mechanically passaged with EZPassage (Invitrogen). The cells were split at a ratio of 1:5–1:8 every 6 days. Human ES cells were used following the Guidelines for utilization of human embryonic stem cells of the Ministry of Education, Culture, Sports, Science and Technology of Japan. This research using hES/iPS cells was approved by the institutional ethical review board at National Institute of Biomedical Innovation.

Live cell imaging The cells seeded on a 6-well plate were cultured in the BioStation CT at 37°C/ 5% CO_2, and monitored by time lapse live imaging. Phase contrast images were captured every 12 hours automatically at a magnification of 4x. To detect nuclei of living cells, the cells were stained with 1 μM SYTO 24 green fluorescent dye (Invitrogen). Fluorescent images of the stained nuclei and phase contrast images were obtained by BioStation CT, and analyzed by CL-Quant with the custom made recipe. This recipe enables to detect individual colonies and measure the area covered by these colonies from the phase contrast image and measure the number of nuclei from the fluorescent image.

Conventional cell counting The cells were dissociated using 0.025% trypsin and 0.01% EDTA in Phosphate Buffered Saline (Gibco) and counted by disposal hemocytometer, OneCell Counter (Bio Medical Science, LTD.).

References

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Results

The fluorescent nucleus counting with images and the conventional cell counting by hemocytometer showed similar results. In the case of total cell numbers above 1 x 10^4, cell numbers by nucleus counting were similar and reproducible with those by conventional cell counting. (see Figure 1.)

There was a significant correlation between the colony coverage area and the nucleus/cell counting. The correlation curves were specific for each cell line. (see Figure 2.)

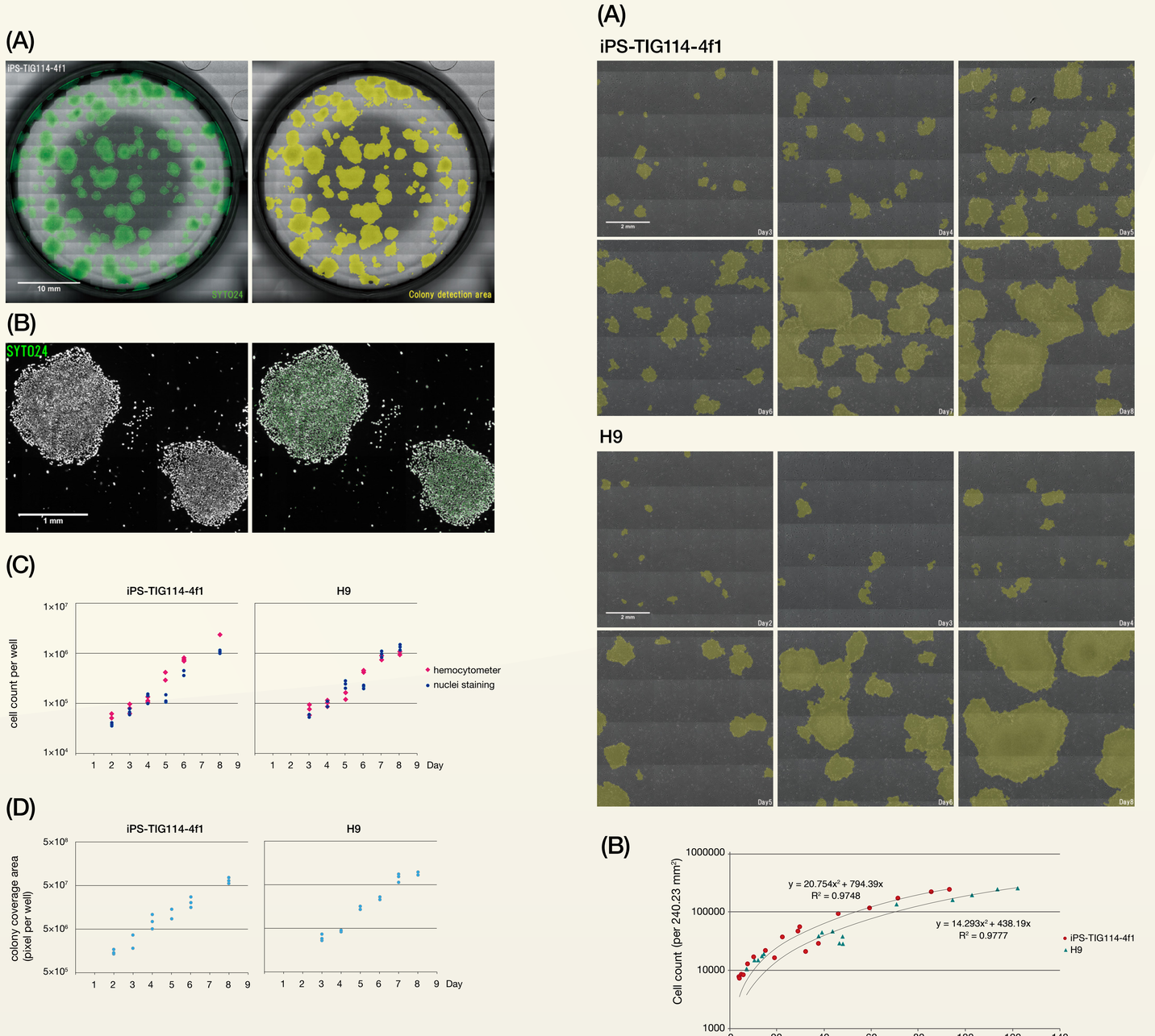

Figure 1. Daily cell growth of hPS cells was quantified by imaging.

(A) The green fluorescent and phase contrast images of living hPS cells stained with SYTO24. (Left) Merged image of SYTO24 fluorescence and phase contrast. (Right) Detection of hPS colonies from phase contrast image. Note that MEFs were separated from hPS colonies. (B) Each nucleus (right, marked as green points) was detected and segmented from the SYTO24 fluorescent image (left) using CL-Quant. All objects within the well were measured to count cell numbers. (C) Comparison of cell counting between conventional method (hemocytometer) and the image analysis (nuclei staining). (D) Daily increase of colony coverage area was quantified by image analysis.

Figure 2. Colony coverage area was analyzed from phase contrast images.

(A) Colony coverage area was analyzed from phase contrast images. Yellow region is the detected hPS colonies. MEFs were successfully removed by our hPS colony extracting procedure. (B) Correlation between cell count by nuclei staining and colony coverage area. Center area (7680 x 7820 pixel; 240.23 mm^2) of each well was analyzed. Note that the correlation curves are different between iPS-TIG114-4f1 and H9.

Conclusions

Here we showed a significant correlation between the cell numbers counted by nuclei fluorescent staining and the colony coverage area measured from phase contrast imaging. These results show that numbers of human PS cells can be estimated from the total colony coverage area through phase contrast imaging. Thus we have developed a new noninvasive cell counting method. Furthermore, obtaining time-lapse phase contrast images enables us to monitor colony morphological changes and to calculate growth rate during human PS cell culture. For accurate estimation of cell numbers, it is needed to determine the correlation curve of the cell numbers and the colony coverage area for each human PS cell lines. This correlation ratio might characterize as the property of each hPS cell lines. Our noninvasive technique could be useful for quality control or high-throughput screening analysis without wasting or damaging the cells.

Acknowledgement

This research is supported by NEDO (New Energy and Industrial Technology Development Organization) on the project "Fundamental Technology for Promoting the Industrial Application of Human Stem Cells".