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An ultra-effective method of generating extramultipotent cells from human fibroblasts by ultrasound

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ABSTRACT

Multipotent cells have similar basic features of all stem cells but limitation in ability of self-renewal and differentiation compared with pluripotent cells. Here, we have developed an ultra effective, gene- and chemical-free method of generating extra multipotent (xpotent) cells which have differentiation potential more than limited cell types, by the mechanism of ultrasound-directed permeation of environmental transition-guided cellular reprogramming (Entr). Ultrasound stimulus generated a massive number of Entr-mediated xpotent (x/Entr) spheroids from human dermal fibroblasts (HDFs) 6 days after treatment. The emergence of x/Entr was first initiated by the introduction of human embryonic stem cell (ESC) environments into the HDFs to start fast cellular reprogramming including activation of stress-related kinase signaling pathways, subsequent chromatin remodeling, and expression of pluripotent-related genes via transient membrane damage caused by ultrasound-induced cavitation. And then, pluripotent markers were transported into their adjacent HDFs via direct cell-to-cell connections in order to generate xpotent clusters. The features of x/Entr cells were intermediate between pluripotency and multipotency in terms of pluripotency with three germ layer markers, multi-lineage differentiation potential, and no teratoma formation. This physical stimulus-mediated reprogramming strategy was cost-effective, simple, quick, produced significant yields, and was safe, and can therefore provide a new paradigm for clinical application.

1. Introduction

Pluripotent stem cells are clinically important as disease models and transplants because they can give rise to any type of cell or tissue in the body. However, teratoma formation, which is associated with the tumorigenic potential of pluripotent cells, is a major concern for their clinical application [1]. Therefore, stem cells possessing the advantages of both pluripotent and multipotent cells could provide a safe source for cellular therapy. However, since global gene expression has been shown to differ more than 10% between somatic cells and their derived reprogrammed cells [2], it may be difficult to achieve safe, fast, and efficient cellular reprogramming with current molecular and chemical methods, which target only a few pluripotent or multipotent genes. Cells are composed primarily of water, inorganic molecules, and organic molecules; genetic elements are only a small constituent. Therefore, an environmental influx that triggers a polygenic trait is one alternative for efficiently generating the phenotypic features of reprogrammed cells. Ultrasound-induced cavitation, which causes cellular uptake of biomolecules from the environment, has been shown to have a positive effect on cellular development [3–8]. Here we employed ultrasound-directed permeation of **en**vironmental **t**ransition-guided cellular **r**eprogramming (Entr), which uses

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physical stimuli with environmental cues, to develop a gene-free and chemical-free method for generating extramultipotent (xpotent) cells. These xpotent cells exhibit close to pluripotent differentiation and, in contrast to pluripotent cells, do not exhibit teratoma formation. To generate Entr-directed xpotent (x/Entr) cells, we exposed human ESC medium and human dermal fibroblast (HDF) cells to ultrasound stimulation and further cultured them for 6 days to acquire xpotent spheroids (Fig. 1A).

2. Materials and methods

2.1. Ethics statement

The use of patient skin fibroblasts was approved by the Institutional Review Board at the CHA Bundang Medical Center (IRB no.: BD2014-089) with informed consent. All experimental animals were housed under specific pathogen free conditions and handled in accordance with the guidelines issued by the Institutional Animal Care and Use Committee of Catholic Kwandong University International St. Mary's Hospital (CKU 01-2014-001).

2.2. Cell culture

Human dermal fibroblasts (HDFs) isolated from adult skin were purchased from Gibco, Inc (cat. C-013-5C, Gibco, Grand Island, NY, USA). Skin fibroblast cells were collected from the posterior of a 60-year-old-stroke patient. Human lung epithelial cells (L-132) were purchased from the American Type Culture Collection (ATCC,

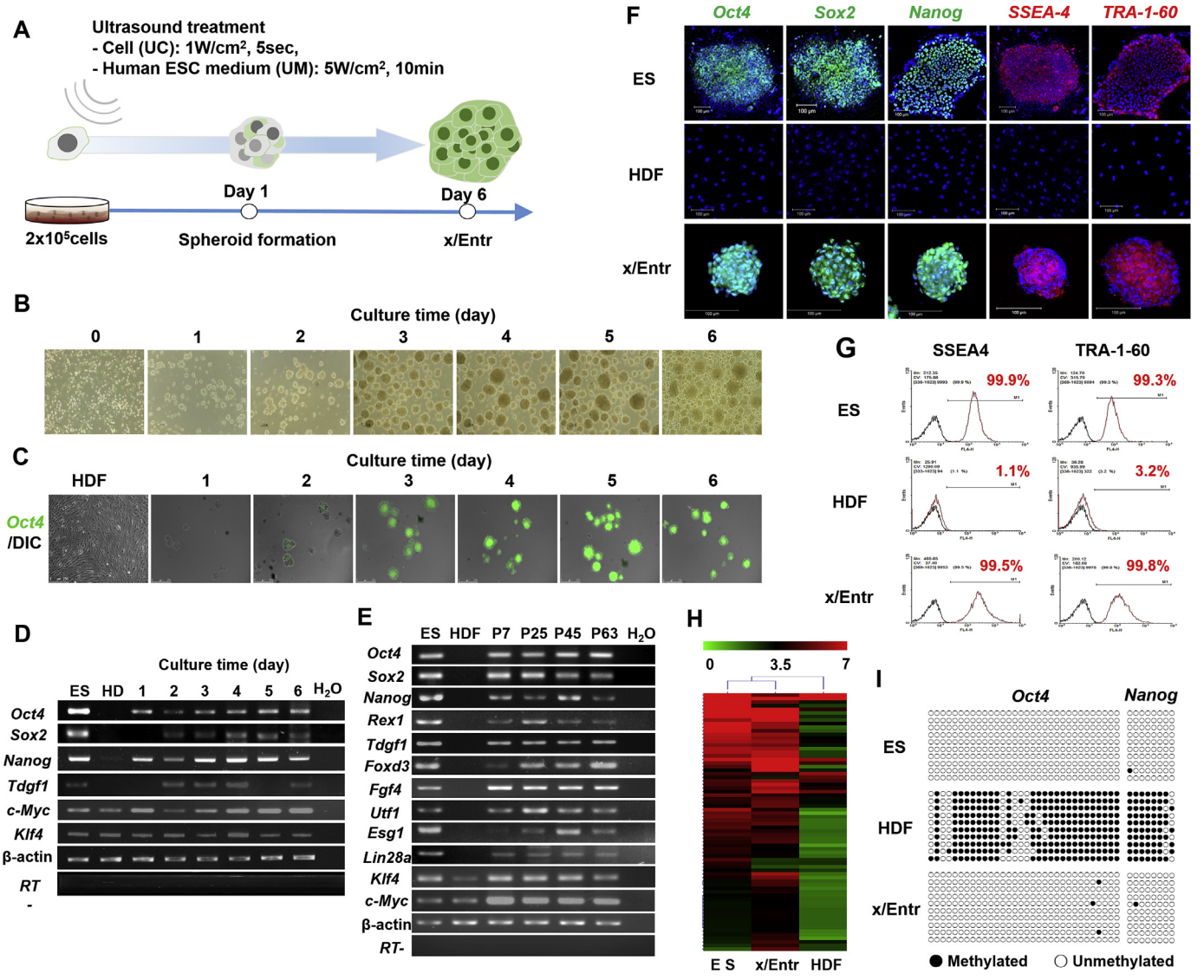

Fig. 1. Generation and characterization of x/Entr cells. **(A)**, Schematic diagram of x/Entr generation from HDFs. (**B**), Spheroid formation of UCUM-S cell for 6 days. UCUM-S cell is indicated that ultrasound treated HDF cell. HDF cells were directly ultrasonic stimulated for 5 s at an intensity 1 W/cm^2, and then suspension cultured in ultrasonically stimulated hESC medium at 5 W/cm^2 for 10 min. The cell image was captured every day for 6 days. Scale bars, 100 μm. (**C**), Change of *Oct4* expression pattern in UCUM-S cell for 6 days. Oct4 expression of UCUM-S cells was found to increase gradually during 6 days by immunocytochemistry analysis. Scale bars, 250 μm. (**D**), RT-PCR analysis of pluripotency marker genes expression pattern in UCUM-S cell during 6 days. ES: positive control about pluripotency markers, HDF: Negative control about pluripotency markers, H_2O is indicated RT-control. (**E**), Expression of pluripotency marker genes in ES, HDF and 4 random selected x/Entr cells (P7, P25, P45 and P63) by RT-PCR analysis. (**F**), Confocal image of pluripotency marker expression in ES, HDF and x/Entr cells by Immunocytochemistry analysis. Scale bars, 100 μm. (**G**), FACS analysis of pluripotency specific surface marker in ES, HDF and x/Entr cells. Black line: non stain control. Red line: antibody staining. **(H)**, Hierarchical clustering of the global expression pattern of genes related to pluripotency in ES, HDF and x/Entr cells. **(I)**, Bisulfite genomic sequencing of the *Oct4* and *Nanog* promoter regions in ES, HDF and x/Entr cells. Black symbol: methylated region, White symbol: unmethylated region. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)