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Survival and death of epiblast cells during embryonic stem cell derivation revealed by long-term live-cell imaging with an Oct4 reporter system

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ABSTRACT

Despite the broad literature on embryonic stem cells (ESCs), their derivation process remains enigmatic. This may be because of the lack of experimental systems that can monitor this prolonged cellular process. Here we applied a live-cell imaging technique to monitor the process of ESC derivation over 10 days from morula to outgrowth phase using an Oct4/eGFP reporter system. Our imaging reflects the 'natural' state of ESC derivation, as the ESCs established after the imaging were both competent in chimeric mice formation and germ-line transmission. Using this technique, ESC derivation in conventional conditions was imaged. After the blastocoel was formed, the intensity of Oct4 signals attenuated in the trophoblast cells but was maintained in the inner cell mass (ICM). Thereafter, the Oct4-positive cells scattered and their number decreased along with apoptosis of the other Oct4-nagative cells likely corresponds to trophoblast and hypoblast cells, and then only the surviving Oct4-positive cells proliferated and formed the colony. All embryos without exception passed through this cell death phase. Importantly, the addition of caspase inhibitor Z-VAD-FMK to the medium dramatically suppressed the loss of Oct4-positive cells and also other embryo-derived cells, suggesting that the cell deaths was induced by a caspase-dependent apoptotic pathway. Next we imaged the ESC derivation in 3i medium, which consists of chemical compounds that can suppress differentiation. The most significant difference between the conventional and 3i methods was that there was no obvious cell death in 3i, so that the colony formation was rapid and all of the Oct4-positive cells contributed to the formation of the outgrown colony. These data indicate that the prevention of cell death in epiblast cells is one of the important events for the successful establishment of ESCs. Thus, our imaging technique can advance the understanding of the time-dependent cellular changes during ESC derivation.

Introduction

Embryonic stem cells (ESCs) are derived from the inner cell mass (ICM) of blastocysts and retain the capacity to generate all types of cell in the body, so called pluripotency, and have the ability to self-renew in culture. Classically, ESCs have been established from purified ICM, but they can now be obtained more easily from advanced blastocyst stage embryos and also from pre-blastocyst stage mouse embryos (Brook and Gardner, 1997; Evans and Kaufman, 1981; Martin, 1981; Tesar, 2005). More recently, a novel technique has been established for ESC derivation using chemical compounds as a tool. The use of a combination of a GSK3 inhibitor and two ERK inhibitors in serum-free conditions, the so called 3i condition (now known as 2i) has markedly improved the rate of ESC establishment (Ying et al., 2008), enabling derivation of ESC even from those mouse and rat strains that were previously considered to be nonpermissive for establishment of authentic ESCs (Buehr et al., 2008; Hanna et al., 2009; Li et al., 2008; Nichols et al., 2009a). However, surprisingly little is known about the ESC derivation process. For example, it was not known why some normal-looking blastocysts disappear during ESC derivation, and why outgrown colonies are divergent both in their appearance and in marker gene expression. Moreover, even if ESCs could be established it was not known from which particular embryonic cells they originated, leading to a debate on the origin of ESCs (Brook and Gardner, 1997).

The lack of knowledge about ESC derivation may arise from the inherent nature of embryos, insufficient material being available for detailed analysis and the generally accepted notion that derivation is a 'delicate' process. Thus, the events that take place during this process were totally inside a black box. To circumvent these difficulties, long-

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term live-cell imaging techniques based on a fluorescent reporter system are necessary. However, it is widely accepted that repeated fluorescent observations cause cellular phototoxicity and affect cell viability and function (Frigault et al., 2009; Yamagata et al., 2009). This is likely to prevent such analysis under natural conditions and could result in artifacts.

We have previously developed a live-cell imaging technique that causes no obvious damage to the preimplantation embryo (Yamagata et al., 2009). In that report, we constructed the imaging devices and optimized the conditions for imaging such as intensity of excitation and time intervals for image acquisition. Importantly, live pups were usually obtained from embryos that were fluorescently imaged from

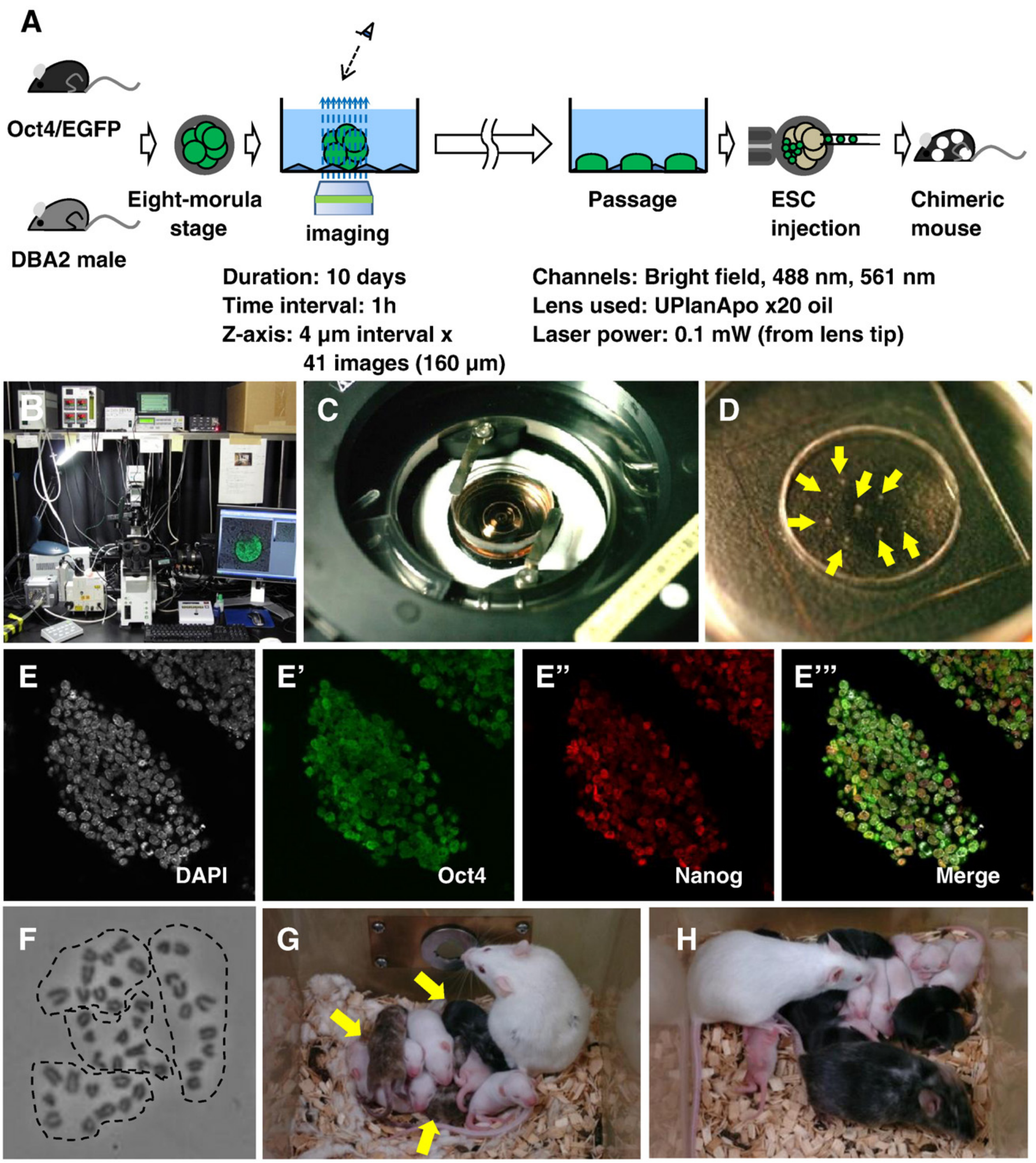

Fig. 1. Low-damage long-term live-cell imaging system for monitoring the process of ESC derivation. A) Schematic diagram of the experimental procedure to evaluate the safety of imaging. Multiple embryos recovered from Oct4/eGFP transgenic mice were placed on feeder cells in a glass-bottomed dish and immediately imaged three-dimensionally using an auto x–y stage under the indicated conditions. After the imaging, some of outgrown colonies were picked and passaged to a new dish. Some of established cell lines were used for production of chimeric mice. (B) Microscopic system for imaging. A disk-confocal unit and EM-CCD camera were attached to an inverted microscope. Sectioning in the z-axis direction was performed by the z-motor. All the devices were controlled by Metamorph software. (C) Stage incubator with glass-bottomed dish for incubation of embryos. (D) Glass-bottomed dish after 10 days imaging. The colonies are detectable by eye (yellow arrows). (E) ESCs established after imaging were immunostained with anti-Nanog antibody. The colonies are shown as DAPI (E, white), Oct4 (E′, green), Nanog (E′′, red) and merged (E′′′) images. (F) Typical image of chromosome spread of ESCs with normal karyotype (40 chromosomes). Ten chromosomes are surrounded by a circle. (G) Picture of chimeric mice (yellow arrows) with their ICR littermates and recipient. (H) Picture of pups from the chimeric mouse. Pups derived from Oct4/eGFP ESCs that were long-term imaged are shown (black-haired pups).