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Opinions and Hypotheses

Lipid droplets are formed in 2-cell-like cells

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Abstract. Embryonic stem (ES) cells, derived from the inner cell mass of a blastocyst, are believed to pluripotent cells and give rise to embryonic, but not extraembryonic, tissues. In mice, totipotent 2-cell stage embryo-like (2-cell-like) cells, which are identified by reactivation of murine endogenous retrovirus with leucin transfer RNA primer (MuERV-L), arise at a very few frequencies in ES cell cultures. Here, we found that a lipid droplet forms during the transition from ES cells to 2-cell-like cells, and we propose that 2-cell-like cells utilize a unique energy storage and production pathway.

Key words: 2-cell-like cells, Lipid droplet, MuERV-L positive cells

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Introduction

Embryonic stem (ES) cells are derived from the inner cell mass of blastocyst and maintained in a naïve state they are very similar in phenotype and function to the mouse preimplantation epiblast [1]. ES cells can self-renew indefinitely and give rise to all cell types of the body including germ cells. However, a small sub-population of ES cell cultures have 2-cell stage embryo-like (2-cell-like) features, including reactivation of murine endogenous retrovirus with leucin transfer RNA primer (MuERV-L) [2], greater histone mobility and dispersed chromocenters [3]. These "2-cell-like cells" have a transcription profile and chromatin accessibility very similar to those of 2-cell stage embryos [4, 5]. In addition, previous studies revealed that 2-cell-like cells can be induced in culture by modulating the levels of chromatin assembly factor 1 (CAF-1) [3], the non-canonical polycomb repressive complex PRC1.6 [4, 6], the transcription factor Dux [7, 8], the Dppa2/4 [9, 10], and the microRNA *miR-34a* [11]. Here, we discuss the mechanism of energy storage and production in 2-cell-like cells based on our new findings.

Lipid droplets and their possible function in 2-cell-like cells

First, we generated stable ES cell lines containing a tdTomato reporter under control of the MuERV-L long terminal repeat, as previously reported [2]. Analysis of several clones revealed 2-cell-like cells, identified by expression of tdTomato, lack of chromocenters and OCT3/4 protein, and upregulation of "2-cell genes", including *Tcstv1*, *Tcstv3*, *Eif1a-like*, and *Gm6763* (data not shown). To characterize the organelle morphology of ES and 2-cell-like cells, we used electron microscopy to examine FACS-sorted tdTomato -positive and -negative cells. We found that lipid droplet (LD)-like organelles, resembling the LDs in 2-cell embryos, were formed in 2-cell-like cells, but not in MuERV-L (–) ES cells (Fig. 1). Almost all LDs in oocytes can be visualized with the fluorescent neutral lipid dye BODIPY 493/503 [12]. We also detected LDs around the nuclei of 2-cell embryos on staining with BODIPY 493/503, as previously reported (Fig. 2A). As shown in Fig. 2B and C, 90% of MuERV-L (+) cells were stained by BODIPY 493/503, indicating that the MuERV-L (+) cells indeed contained cytoplasmic LDs .

Since 2-cell-like cells show decreased glycolytic competence and respiratory activity and lower levels of reactive oxygen species compared to ES cells, it has been suggested that a distinct metabolic state arises during the transition from ES cells to 2-cell like cells [13]. Given the decreased glycolytic and respiratory activity in 2-cell-like cells, it is reasonable to assume that the ATP levels might be lower in 2-cell-like cells than ES cells. However, no significant differences in ATP levels were found between 2-cell-like and ES cells [13]. Unlike early preimplantation embryos (up to 8-cell embryos), 2-cell-like cells cannot use exogenous pyruvate and lactate as energy sources. Thus, it is unclear how 2-cell-like cells obtain a similar amount of ATP to ES cells without using glycolysis.

Mouse oocytes/preimplantation embryos stored LDs in the cytoplasm, but the roles of LDs in mouse oocytes/preimplantation was not elucidated due to the low levels of LDs relative to porcine oocyte/preimplantation embryo. Several studies revealed that LD biogenesis is physiologically important during early preimplantation development in mouse oocytes/preimplantation embryos [12, 14, 15]. In oocytes, intracellular triacylglycerol

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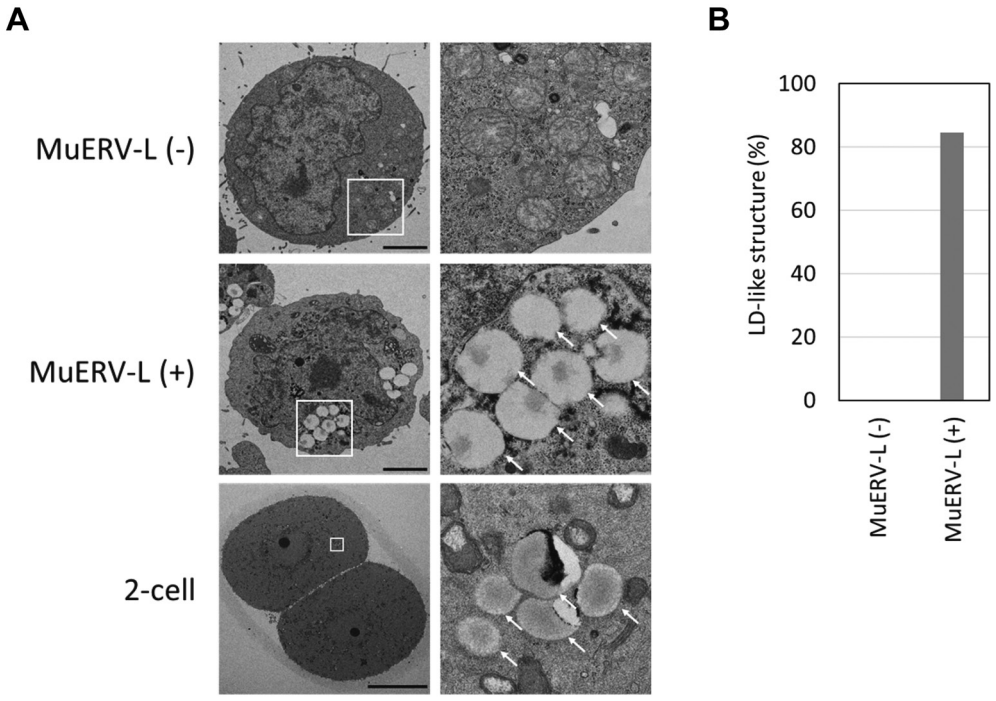

Fig. 1. The lipid droplet (LD)-like organelles in MuERV-L-positive cells. (A) Electron microscopic images of MuERV-L-positive, -negative ES cells, and 2-cell embryo. The right panel shows higher-magnification images of the boxed area. (B) Percentage of LD-like organelles in MuERV-L-positive (n = 13) and -negative (n = 13) ES cells. The scale bars of the MuERV-L(–) and (+) cells are 2.5 μm, while that of the 2-cell embryo is 20 μm.

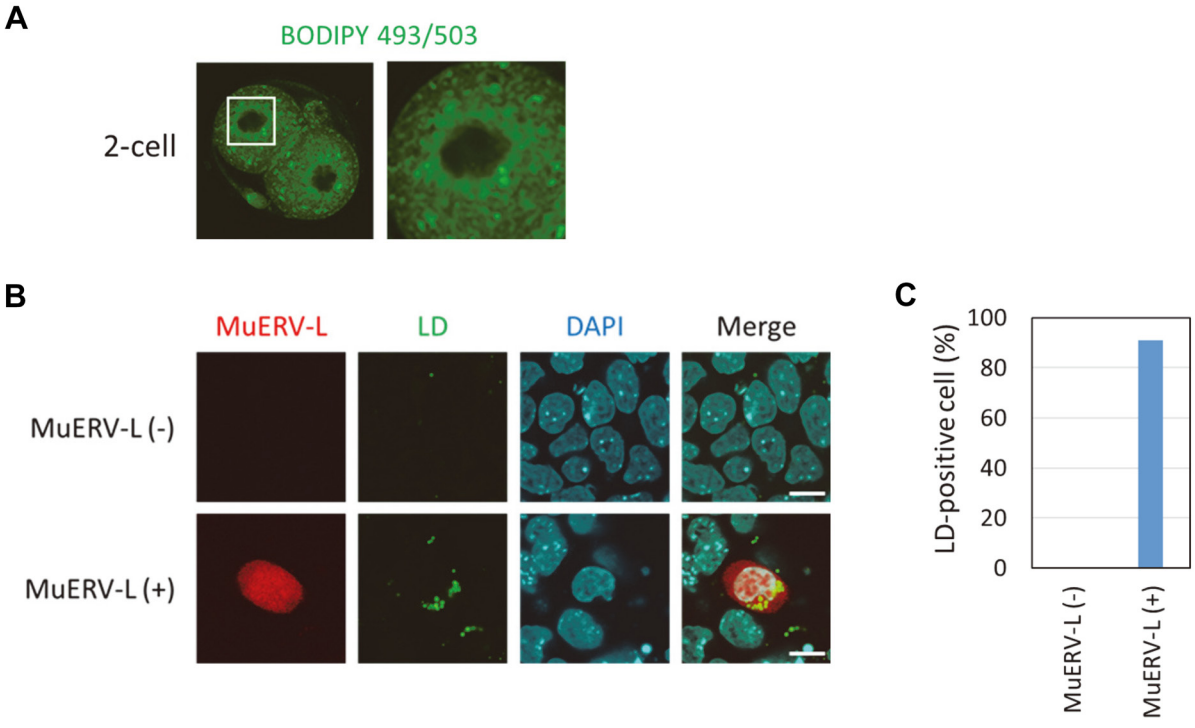

Fig. 2. Lipid droplets (LDs) in MuERV-L-positive cells. (A) Two-cell embryo was stained with BODIPY 493/503 (green) and observed by confocal fluorescence microscopy. (B) MuERV-L-positive and -negative ES cells were stained with BODIPY 493/503 and observed by confocal fluorescence microscopy. MuERV-L expression was detected by the fluorescence of tdTomato (red) and LDs stained by BODIPY 493/503 (green); nuclei were stained with DAPI (blue). (C) The percentages of BODIPY 493/503-positive LDs in MuERV-L-positive (n = 11) and -negative (n = 13) ES cells. Scale bars: 10 μm.

is stored in LDs and LD proteins facilitate the lipase-mediated hydrolysis of triacylglycerol and release of free fatty acids (FFAs) [16]. Intracellular FFAs generated via either transport or lipolysis are then catabolized to yield ATP in the mitochondrial matrix via β-oxidation [16, 17]. Given our observation of LDs in 2-cell-like cells, LDs may be used to store neutral lipids and generate ATP via β-oxidation of FFAs instead of glycolysis. Another possibility is that LDs modulate transcription; LDs have been implicated in suppressing the activity of a transcription factor by keeping it out of the nucleus [18, 19].

We hope that this hypothesis sheds light on the mechanism that regulates the transition from pluripotent cells to totipotent cells.

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