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Rapid Isolation of Mitochondrial DNA-Depleted Mammalian Cells by Ethidium Bromide and Dideoxycytidine Treatments

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Abstract Mitochondrial DNA (mtDNA)-depleted (ρ^0) cells are often used as mtDNA recipients to study the interaction between the nucleus and mitochondria in mammalian cells. Therefore, it is crucial to obtain mtDNA-depleted cells with many different nuclear backgrounds for the study. Here, we demonstrate a rapid and reliable method to isolate mammalian mtDNA-depleted cells involving treatment with the antimitochondrial agents ethidium bromide (EtBr) and 2',3'-dideoxycytidine (ddC). After a short exposure to EtBr or ddC, followed by rapid clonal isolation, we were able to generate viable mtDNA-depleted cells from mouse and human cells and were able to successfully repopulate them with exogenous mitochondria from platelets isolated from mouse and human blood samples. These mtDNA-depleted cells can be used to characterize the nuclear mitochondrial interactions and to study mtDNA-associated defects in mammalian cells. Our method of isolating mtDNA-depleted cells is practical and applicable to a variety of cell types.

Keywords cybrid · dideoxycytidine · ethidium bromide · mitochondria · mtDNA-depleted

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Introduction

Most mitochondria in mammalian cells contain small double-stranded circular mitochondrial DNAs (mtDNA), which are approximately 16 to 17 kb in size. These mtDNAs encode only 13 polypeptide subunits of the ATP-generating pathway, two rRNAs and 22 tRNAs for the mitochondrial gene expression machinery (Yoon et al., 2010). Nevertheless, the functions of mitochondria are regulated by their own genomes and the nuclear genome as the majority of the mitochondrial proteins are encoded in the nucleus and targeted to mitochondria (Dolezal et al., 2006). The nuclear and mitochondrial genomes have been coevolved to enhance the nuclear and mitochondrial interactions, and this coevolution has led to species-specific compatibility between the nuclear and mitochondrial genomes (Kenyon and Moraes, 1997; Gray et al., 1999). Thus, mitochondria from closely related species are capable of repopulating mtDNA-depleted (ρ^0) cells derived from the related species, while the mitochondrial genomes from distantly related or distinctively different species cannot provide functional mitochondrial activities in these cells.

The ρ^0 cells, which are devoid of mtDNA, are useful tools for studying the interaction between the nuclear and mitochondrial genomes and the phenotypic expression of various mtDNA-associated defects in mammalian cells. The phenotypic expression of the mitochondrial genes can be separated from the nuclear genome effects using the ρ^0 cell culture system (Nelson et al., 1997). The most widely used *in vitro* model is produced by the fusion of enucleated cells carrying mtDNA mutations with ρ^0 cells, and this system allows us to test the effects of mitochondrial genome mutations in different nuclear backgrounds (Chomyn et al., 1996). For this reason, there is a great need for the establishment of a reliable method to generate mtDNA-depleted ρ^0 cells in a variety of cell types. Despite the high level of interest in ρ^0 cells, no practical and reliable isolation methods have been described in detail until now. Relatively ambiguous descriptions using several antimitochondrial agents and restriction endonucleases have been presented (Inoue et al., 1997; Kukat et al., 2008). Some antimitochondrial agents are very toxic to cells at low concentrations, and

therefore, it is difficult to treat these cells to obtain the ρ^0 cells (Dupont et al., 1990). Although mitochondrially targeted restriction enzymes have also been employed to generate human ρ^0 cells (Kukat et al., 2008), the procedure involves transfection and antibiotic selection steps, which might affect and modify the nuclear gene expression patterns in these cells.

We have now established a reliable method to isolate ρ^0 cells from mouse and human cells using the antimitochondrial agents ethidium bromide (EtBr) and 2',3'-dideoxycytidine (ddC). EtBr has been reported to rapidly inhibit mtDNA synthesis in cultured mouse and human cells and is widely used for the isolation of ρ^0 cell lines (Smith et al., 1971). The DNA in these EtBr-treated cells maintains its integrity even at high concentration (5 μg/mL) and during long treatment times. Thus, EtBr is believed to have no detrimental or negligible effects on the genomic DNA in the treated cells (Smith, 1977). DdC is an antiviral pyrimidine nucleoside analogue that is effective against HIV replication (Mitsuya and Broder, 1986; Yarchoan and Broder, 1987). It was later found that ddC interacts with the mitochondrial DNA polymerase γ, thereby inhibiting mtDNA synthesis and eventually depleting mtDNA in mammalian cells (Chen and Cheng, 1989; Nelson et al., 1997). In the present paper, we introduce a user-oriented experimental scheme to screen and isolate ρ^0 cells from mouse and human tissue culture cells using the well-known antimitochondrial agents EtBr and ddC. The isolated ρ^0 cells were found to be completely depleted of their mtDNA; however, these cells were able to maintain the mtDNA provided by exogenous mitochondria, and thus, their mitochondrial function was restored to normal.

Materials and Methods

Cell lines and culture media. The human cell line HeLa229 (ATCC CCL-2.1) was grown in Minimum Essential Medium (MEM)-α in the presence of heat-inactivated 10% fetal bovine serum (FBS) at 37°C in a humidified incubator with 5% CO_2. The HeLa229 ρ^0 cells generated in this study were grown in MEM-α supplemented with 10% FBS, 50 μg/mL uridine and 0.1 mg/mL pyruvate. The mouse cell line LL/2 (ATCC CRL-1642) was grown in Dulbecco's Modified Eagle Medium (DMEM) in the presence of heat-inactivated 10% FBS at 37°C in a humidified incubator with 10% CO_2. The LL/2 ρ^0 cell lines were grown in DMEM supplemented with 10% FBS, 50 μg/mL uridine and 0.1 mg/mL pyruvate.

Polymerase chain reaction (PCR) analysis of mtDNA. For the detection of mtDNA in the mouse and human cells, total cellular DNA (0.2 mg) was used as a template for PCR analysis. The nucleotide sequences from the mitochondrial D-loop regions of human and mouse mtDNA were synthesized as oligonucleotide primers (Table 1). Human mitochondrial D-loop PCRs were amplified using the following cycling parameters: 2 min of denaturation at 95°C, followed by 30 cycles of 30 s of denaturation at 95°C, 45 s of annealing at 55°C and 30 s of extension at 72°C. Mouse mitochondrial D-loop PCRs were amplified using the following cycling parameters: 2 min of denaturation at 95°C, followed by 30 cycles of 30 s of denaturation at 95°C, 1 min of annealing at 60°C and 2 min of extension at 72°C. The PCR products were separated on a 1% agarose gel.

Cloning of ρ^0 cells from mouse and human tissue culture cells. To generate the mtDNA-free ρ^0 cell lines, mouse LL/2 and human HeLa229 cells were grown in the presence of the antimitochondrial agents EtBr or ddC for 3 weeks in medium supplemented with 50 mg/mL uridine and 0.1 mg/mL pyruvate. Clonal LL/2 ρ^0 and HeLa229 ρ^0 cell lines were then obtained by the limited dilution of the treated cells into 96-well plates in the presence of EtBr and ddC. The cloned cells were then cultured in normal medium supplemented with 50 μg/mL uridine and 0.1 mg/mL pyruvate. The ρ^0 state of the cloned cells was verified using a PCR assay with the primers described in Table 1 and by their inability to grow in medium lacking pyruvate and uridine (King and Attardi, 1996).

Platelet-mediated mitochondrial transformation. The isolation of platelets from blood samples was performed using a published method (Chomyn, 1996). Venous blood was collected in heparin tubes and was kept at room temperature until processing. Each sample was mixed by inversion with $1/10^{th}$ volume of 0.15 M NaCl and 0.1 M sodium citrate, and the sample was centrifuged for 20 min at 200 g in a tabletop centrifuge at 4°C. The top $3/4^{th}$ of the platelet-rich plasma was removed and centrifuged for 30 min at 2,300 g and 4°C. The pellet was resuspended in 10 mL of physiological saline (0.15 M NaCl, 0.015 M Tris-HCl pH 7.4). The platelet suspension (~2×10^7) was then transferred to a fresh sterile 15-mL tube and centrifuged at 2,300 g for 15 min at 15°C. Each platelet pellet was overlaid with 1×10^6 ρ^0 cells in 2 mL of DMEM without Ca^{2+}. After centrifugation in the tabletop centrifuge at 180 g for 10 min, all of the supernatant was aspirated, and the

Table 1 Oligonucleotide primers for PCR assay

Primer	5'-3' Sequence	Nucleotide No.
human mtDNA specific primers		
h-L strand	CAGGTCTATCACCCTATTAA	Light chain 6-25[a]
h-H strand	CGCCTGTAATATTGAACGTA	Heavy chain 168-187[a]
mouse mtDNA specific primers		
m-L strand	ACCCAACGCGGCAAACTAACC	Light chain 11406-11426[b]
m-H-strand	TCTTGTTCGTCTGCCAGGCT	Heavy chain 12786-12805[b]

[a]Nucleotide numbering according to accession no. AC_000021.
[b]Nucleotide numbering according to accession no. AY172335.

pellet was resuspended thoroughly in 0.1 mL of 42% polyethylene glycol (PEG) solution (8.4 g of PEG 1500 (ICN, USA), 2 mL of dimethyl sulfoxide, 9.6 mL of DMEM without Ca^{2+}). After 1 min at room temperature, the suspension was diluted with 10 mL of DMEM supplemented with 10% FBS, 50 μg/mL uridine and 0.1 mg/mL pyruvate, and the suspension was distributed into five 10-cm petri dishes (2×10^5 ρ^0 cells/dish). Two to three days later, the pyruvate- and uridine-containing medium was replaced with selective medium (DMEM or MEM-α without pyruvate and uridine).

Results and Discussion

The underlying principle for the isolation of ρ^0 cells is based on the use of antimitochondrial agents to inhibit mtDNA replication, thus depleting the mtDNA from the cells. EtBr has been shown to have inhibitory effects on mammalian mtDNA replication (Smith et al., 1971), and ddC, which has been used as a prescription anti-retroviral drug and causes mitochondrial toxicity, reduces the level of mtDNA by inhibiting mitochondrial DNA polymerase γ (Martin et al., 1994; Rossi et al., 1999). In the following sections, we introduce a practical and reliable technique to isolate ρ^0 cells from mouse and human cells using the antimitochondrial agents EtBr and ddC.

Experimental design. We have designed a user-oriented experimental scheme for the screening and isolation of ρ^0 cells after treatment with the antimitochondrial agents EtBr and ddC (Fig. 1). Although several ρ^0 cell lines have been isolated using EtBr treatments, ρ^0 derivatives from various sources of mammalian cell lines have not been isolated, possibly due to the lack of standard methods for generating ρ^0 cells. As shown in Fig. 1, we used a fixed number of cells (10^4–10^5 cells per plate) and treated the cells with EtBr or ddC for a relatively small amount of time (3 weeks). After short exposure to the antimitochondrial agents, the treated cells were quickly spread onto the 96-well plates to isolate the pure cell clones derived from a single colony. The ρ^0 status of these clonally isolated colonies was then verified by mtDNA-specific PCR, and their growth phenotypes in media without pyruvate and uridine and their ability to be repopulated with exogenous mitochondria were examined. The growth phenotype test in media lacking pyruvate and uridine was used to analyze the unique growth requirements of the ρ^0 cells, and this is the most reliable method to determine if any cells in the population possess mtDNA (King and Attardi, 1996).

Treatment with EtBr and ddC decrease the mtDNA content. To analyze the presence of mtDNA, we performed mtDNA-specific PCR after preparing total cellular DNA from the cell clones isolated from the 3-week EtBr or ddC treatment (Table 1 and Fig. 2). In the experiments, high concentrations of EtBr (5 μg/mL) and ddC (20 μM) were used for the mouse LL/2 cells and relatively low concentrations of EtBr (50 ng/mL) and ddC (2 μM) were used for the human HeLa229 cells; additionally, two different numbers of cells were used (2×10^4 and 2×10^5 cells). Interestingly,

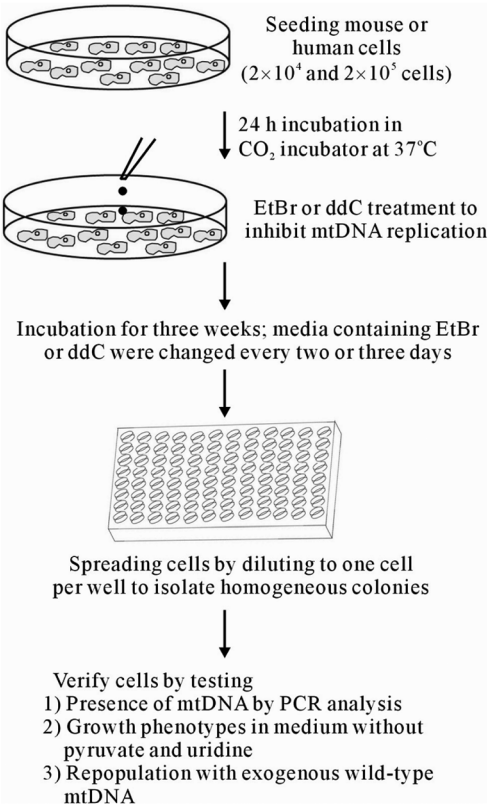

Fig. 1 Schematic representation of the protocol used to screen and isolate mtDNA-depleted ρ^0 cells. The mouse or human cells were spread on a 35-mm dish at a cell density of 2×10^4 or 2×10^5 cells per plate and were incubated in a humid CO_2 incubator for 24 h. EtBr or ddC was then added to each plate to inhibit mtDNA replication. The level of mtDNA in the cells was assayed using an mtDNA-specific PCR. When the mtDNA levels decreased, the antimitochondrial agent-treated cells were diluted and inoculated into 96-well plates at a density of one cell per well to separate the pure ρ^0 cells from the heterogeneous colonies. The state of the ρ^0 cells was verified using a mtDNA-specific PCR assay, by growth phenotype analysis in medium lacking uridine and pyruvate, and by their ability to be repopulated with exogenous wild-type mtDNA.

as shown in lanes 3 and 4 in Fig. 2A and lanes 1 and 2 in Fig. 2B, the ddC treatment (20 μM) of the mouse LL/2 cells and the EtBr treatment (50 ng/mL) of the human HeLa229 cells did not decrease the levels of mtDNA. In contrast, the EtBr treatment (5 μg/mL) of the LL/2 cells and the ddC treatment (2 μM) of the HeLa229 cells significantly reduced the levels of mtDNA (lanes 1 and 2 in Fig. 2A and lanes 3 and 4 in Fig. 2B). From these results, we found that the short exposures of the mouse and human cells to EtBr and ddC, respectively, at the proper concentrations could decrease the quantities of mtDNA to some extent. Thus, we hypothesized that some of the treated cells lost their mtDNA at this point, and as a result, the mtDNA-free ρ^0 cells might be mixed with cells still containing their mtDNA in the cultures. We therefore tried to isolate the pure ρ^0 cells from the mixed cultures using the limited dilution method (see Figs. 1 and 3).

The reason that EtBr did not influence the level of mtDNA in the HeLa229 cells might be due to the low concentration of EtBr

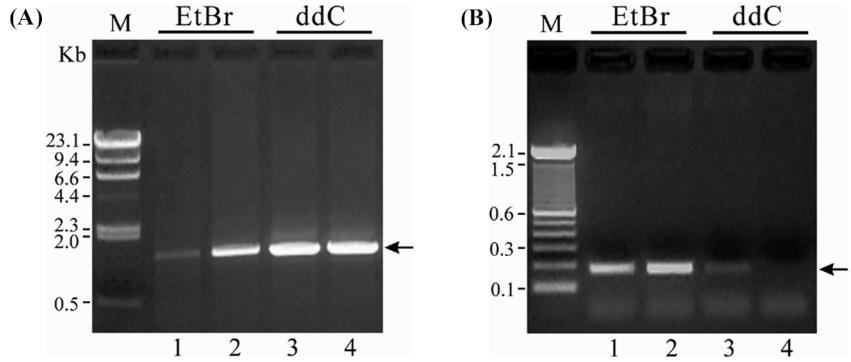

Fig. 2 PCR analysis of mtDNA levels following treatment with antimitochondrial agents. (A) Effects of EtBr and ddC on the mtDNA content in the mouse lung carcinoma cell line LL/2. The cells were treated with EtBr (5 μg/mL) and ddC (20 μM) for 3 weeks. Two different cell numbers (2×10^4 cells in lanes 1 and 3; 2×10^5 cells in lanes 2 and 4) were used in this experiment. The EtBr treatment (5 μg/mL) significantly reduced the levels of mtDNA. (B) Effects of EtBr and ddC on the mtDNA content in the human cervix adenocarcinoma cell line HeLa229. The cells were treated with EtBr (50 ng/mL) and ddC (2 μM) for 3 weeks. Two different cell numbers (2×10^4 cells in lanes 1 and 3; 2×10^5 cells in lanes 2 and 4) were used in this experiment. The ddC treatment (2 μM) significantly reduced the levels of mtDNA.

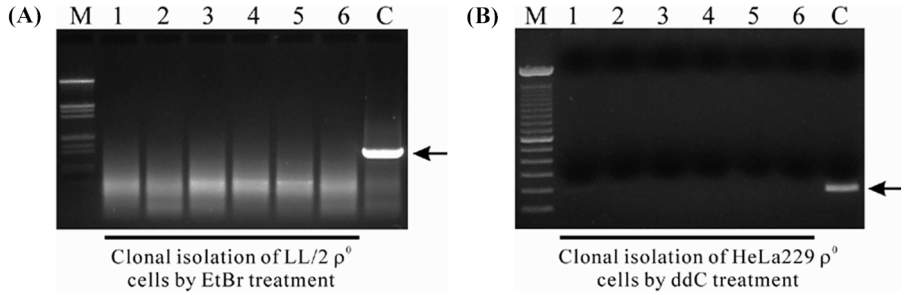

Fig. 3 Detection of mtDNA from the clonally isolated mtDNA-depleted ρ^0 cells. (A) PCR amplification of mouse mtDNA in the DNA samples prepared from LL/2 and LL/2 ρ^0 cells. PCR was carried out using the mouse mtDNA-specific primers shown in Table 1, resulting in a 1.4 kb fragment when mouse mtDNA was present in the cells. C, wild-type LL/2 cells. (B) PCR amplification of human mtDNA in the DNA samples prepared from HeLa229 and HeLa229 ρ^0 cells. PCR was carried out using the human mtDNA-specific primers shown in Table 1, resulting in a 182 bp fragment when human mtDNA was present in the cells. C, wild-type HeLa229 cells.

(50 ng/mL). However, when we used a high level of EtBr (5 μg/mL) in the human cells, no viable or culturable cells were obtained. From this result, we knew that treatment with low concentrations of EtBr for a short period of time (3 weeks) had no effect on the level of mtDNA in the HeLa229 cells, and tedious experiments and optimizations may be needed to find the correct concentration of EtBr. Thus, we used ddC instead of EtBr to deplete the mtDNA as ddC has previously been used to deplete mtDNA in human cells (Nelson et al., 1997). Indeed, treatment with ddC dramatically decreased the level of mtDNA in the HeLa229 cells. ddC has been known to exert its biological effects through its metabolite dideoxyCTP (ddCTP), which inhibits HIV-1 reverse transcriptase. Mitochondrial DNA polymerase γ, a key enzyme in mitochondrial DNA replication, is also highly susceptible to inhibition by ddCTP (Rossi et al., 1999). Cytoplasmic deoxycytidine kinase (dCK) can phosphorylate ddC to form ddCMP, and subsequently, the phosphorylated ddC appears to be transported into the mitochondria to exert its inhibitory effects on mtDNA synthesis (Rossi et al., 1999). The reason for the ineffectiveness of ddC treatment on the mtDNA depletion in mouse cells is complicated. It was reported that ddC phosphorylating enzymes (dCK) in human and mouse cells have significantly different substrate specificity (Habteyesus et al., 1991). The differences in ddC substrate specificity may be responsible for the inability of 100 μM ddC to inhibit cell growth in mouse myeloma cells (Rossi et al., 1992) and for its inability to deplete the mtDNA of the mouse cells in our experiment (lanes 3 and 4 in Fig. 2).

Isolation of mouse and human mtDNA-less ρ^0 cell lines. To obtain pure unmixed ρ^0 cell lines, the cells were treated with ddC and EtBr for 3 weeks and were counted, diluted and inoculated into 96-well plates with a single cell per well. During the culture, the same concentrations of ddC and EtBr, along with 50 μg/mL uridine and 0.1 mg/mL pyruvate, were also applied to prevent the recovery of mtDNA from the cells that maintained low amounts of the DNA. As shown in Fig. 3, many ρ^0 cell clones from the LL/2 and HeLa229 cells were obtained using this clonal isolation method. Products of the mtDNA-specific PCR were not detected in six LL/2 and HeLa229 clones (lanes 1–6 in Figs. 3A and B), meaning that these clones have no mtDNA or contain negligible amounts of the DNA.

Growth phenotypes. To verify the ρ^0 state of the cloned cells, we assayed the unique growth requirements of the ρ^0 cells in medium

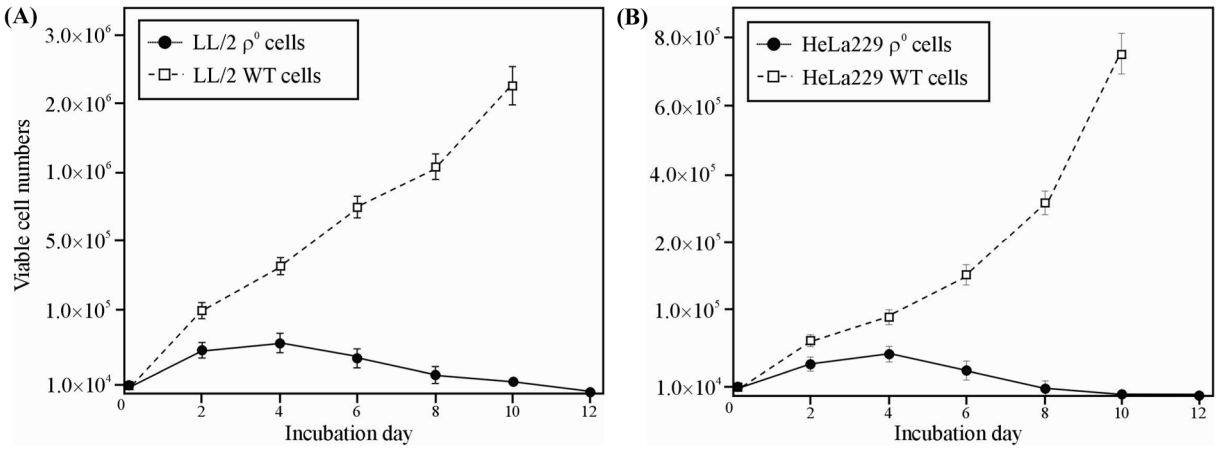

Fig. 4 Growth of ρ^0 cells. The ρ^0 state of the cloned cells was verified by assaying the growth requirements of the cells in medium without uridine and pyruvate. (A) Growth curves of the LL/2 wild-type and LL/2 ρ^0 cells. The cells were cultured in DMEM selective media lacking pyruvate and uridine for 12 days to compare cell viability and proliferation. (B) Growth curves of the HeLa229 wild-type and HeLa229 ρ^0 cells. The cells were cultured in MEM-α selective media lacking pyruvate and uridine for 12 days to compare cell viability and proliferation. The error bars were presented as means and standard deviations obtained from three independent experiments.

without uridine and pyruvate. The ρ^0 cells are thought to require pyruvate and pyrimidines for growth. Because the ρ^0 cells contain no mtDNA, they cannot produce the mitochondrial proteins that are incorporated into complexes I, III, IV, and V in the inner mitochondrial membranes. Dihydrooratate dehydrogenase, which is involved in the pyrimidine biosynthetic pathway, is located in the inner mitochondrial membrane, and this enzyme requires functional electron transport in the mitochondrial membrane for its activity (King and Attardi, 1996). Due to the defective electron transport in the inner mitochondrial membrane, this enzyme involved in pyrimidine biosynthesis cannot be activated; thus, the ρ^0 cells that have no electron transport become pyrimidine auxotrophs. Therefore, the ρ^0 cells are dependent on pyrimidine and require the addition of uridine to the media.

The cell's dependence on pyruvate can be explained by the fact that cytoplasmic NADH produced by the breakdown of glucose would not be properly oxidized via the cytosol-mitochondrial shuttle system (King and Attardi, 1996). Because the cytoplasmically generated NADH itself cannot cross the mitochondrial membranes, the electrons derived from NADH instead are transferred from the cytosol across the mitochondrial membrane through the cytosol-mitochondrial shuttle system for use in the mitochondria. Because the ρ^0 cells do not contain a functional mitochondrial membrane system, it is thought that the cytosol-mitochondrial shuttle system cannot be activated to consume the electrons produced from NADH. The excess amount of cytoplasmic NADH is instead oxidized through the activity of the NAD-linked lactate dehydrogenase in the cytosol, which rapidly reduces the amount of pyruvate and quickly generates lactate. Thus, the ρ^0 cells cannot grow without pyruvate and uridine in their culture media.

When we cultured the LL/2 and HeLa229 ρ^0 cells and their parental wild-type cells in media without pyruvate and uridine, the ρ^0 cells were unable to grow well, whereas the wild-type cells robustly and quickly grew in this selective media (Figs. 4A and B). The ρ^0 cells can grow for 2 days in the selective media because the starting cells that were seeded in the plates were selected from rich media containing pyruvate and uridine. Because the starting ρ^0 cells were adapted in this rich media, these cells may have small amounts of these nutrients present, which might sustain the growth of the ρ^0 cells for a while. After day 4, the number of ρ^0 cells began to decrease, and eventually, the cells could not survive in this selective media. From these results, we found that the ρ^0 cells that were isolated using the method described here could not grow in media lacking pyruvate and uridine, which indicated that these cells had defective respiratory chain machinery. These cells definitely had no mtDNA as they could not grew back in a long period (4–6 weeks) of culture time in the selective media without EtBr or ddC.

Repopulation with exogenous mouse mitochondria by platelet fusion. Because the clonal ρ^0 cells were isolated using chemicals with mutagenic effects, it may be necessary to verify that no mutations have occurred in genes involved in mitochondrial biogenesis and function during the ρ^0 cell isolation procedure. To verify the ability of the ρ^0 cells to recover mitochondrial function, repopulation experiments were performed using the clonal mouse LL/2 and human HeLa229 ρ^0 cells with mouse and human platelets as exogenous mitochondrial donors, respectively. As shown in Fig. 5A (see arrow), a repopulated colony was formed when the clonal mouse LL/2 ρ^0 cells were fused with mouse platelets and grown in selective media without pyruvate and uridine. The ρ^0 cells cannot survive in this selective media due to the absence of mitochondrial function, whereas the wild-type cells grow quickly because they maintain active mitochondrial biogenesis and function.

After platelet-mediated ρ^0 cell transformation, we observed the growth of several colonies in the selective media, and we assayed these colonies for the presence of mtDNA using the mtDNA-specific PCR. As shown in Fig. 5B and C, positive PCR bands

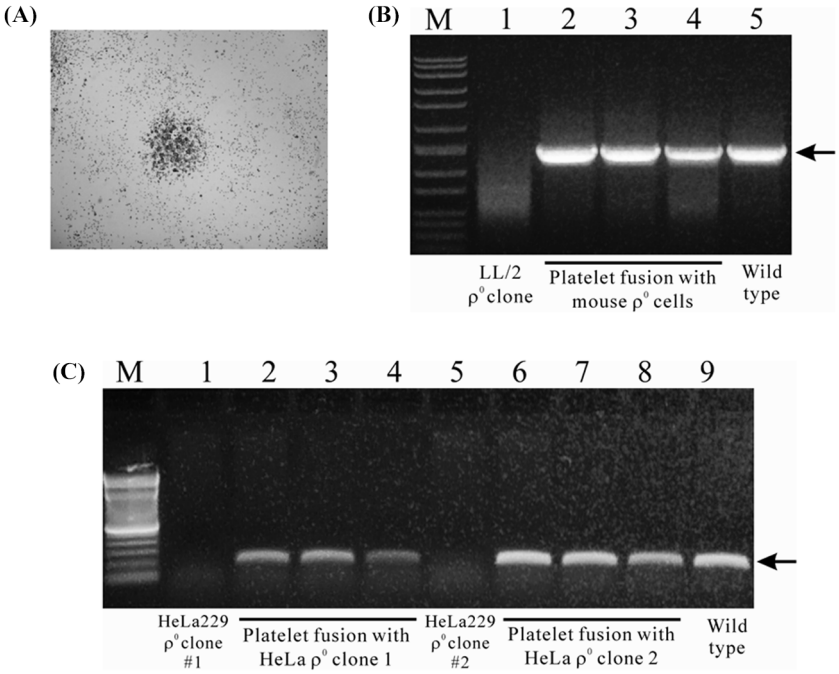

Fig. 5 Characterization of mtDNA-depleted ρ^0 cells by platelet-mediated mitochondrial transformation. (A) Mouse LL/2 cybrid cells derived from the fusion of LL/2 ρ^0 cells and platelets isolated from mouse blood samples. The mouse cybrid colony (arrow) was formed after incubating the fused cells for 3 weeks. (B) PCR amplification of mouse mtDNA in the DNA samples prepared from LL/2 wild-type, LL/2 ρ^0, and platelet-fused LL/2 cybrid cells. M, DNA molecular marker. (C) PCR amplification of human mtDNA in the DNA samples prepared from HeLa229 wild-type, HeLa229 ρ^0, and platelet-fused HeLa229 cybrid cells. M, DNA molecular marker. In these cybrid cells, positive PCR bands were observed, which meant that the mouse and human ρ^0 cells were repopulated with exogenous wild-type mtDNA and could maintain the mtDNA, resulting in functional mitochondrial activity.

appeared from the mouse and human cybrid cells that were fused with the platelets and were selected using media lacking pyruvate and uridine (lanes 2–4 in Fig. 5B and lanes 2–4 and 6–8 in Fig. 5C). These results mean that the ρ^0 cells were repopulated using exogenous wild-type mtDNA, and these cells can maintain the mtDNA for mitochondrial function. When we tested the growth of the repopulated cybrid cells compared to the wild-type mouse LL/2 and human HeLa229 cells, the growth phenotypes of both the cybrids and the wild-type cells were essentially identical (data not shown). From these results, we concluded that the ρ^0 cells obtained using our simple isolation method were able to recover mitochondrial functions and that no mutational changes in the genes involved in mitochondrial biogenesis and function occurred during the ρ^0 cell isolation procedure.

In this paper, we described a rapid and practical technique for screening and isolating ρ^0 cells from mammalian tissue culture systems. Reliable methods for the isolation of ρ^0 cells offer great promise for the study and analysis of mutated mitochondrial genomes in different nuclear backgrounds. As shown in this paper, we practically and successfully isolated ρ^0 cells from mouse and human cells following short exposure to antimitochondrial agents and a rapid clonal isolation using 96-well plates in a cost and time efficient manner. We found that the isolated ρ^0 cells could not grow in the media lacking pyruvate and uridine, which means that the cells had defective mitochondria due to the absence of mtDNA. However, we found that the mouse and human ρ^0 cells have the ability to maintain mitochondrial genomes provided from exogenous mitochondria, and these cells could be successfully repopulated when grown in media lacking pyruvate and uridine, meaning that the cells were able to recover mitochondrial function and biogenesis. These mtDNA-depleted cells can be used to characterize the nuclear-mitochondrial interactions and to study mtDNA-associated mutations in mammalian cells. Our method of isolating the ρ^0 cells can be applied to a variety of cell types.

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