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In vitro Proliferation of Mononucleated Suspension and Adherent Cells from Mouse and Human Peripheral Blood System

(Proliferasi *In vitro* Sel Ampaian dan Melekat Mononukleus daripada Sistem Darah Periferi Mencit dan Manusia)

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ABSTRACT

Primary cells have a limited proliferative capacity with a finite number of times as compared with cell line which can grow indefinitely. Therefore, this study was carried out to identify the proliferative capacity of primary mononucleated cells from mouse and human. The mononucleated cells were isolated from mouse and human peripheral blood by density gradient centrifugation using Ficoll-Paque™ Plus. The two types of cells i.e. suspension and adherent forms were obtained after culturing the isolated mononucleated cells for 4 days in the complete medium consists of Alpha Minimal Essential Medium, 10% newborn calf serum and 2% penicillin/streptomycin. The cells were then cultured for another 10 days to observe cell viability using trypan blue exclusion assay (suspension form) and MTT assay (adherent form). NSO and MC3T3-E1 cell lines were selected as control cell for suspension and adherent cells, respectively. Our results showed that the proliferation rate of mouse suspension mononucleated cells increased from 1.31 ± 0.24 cells/day (day 5) to 2.69 ± 0.42 cells/day (day 10) whilst, for human suspension cells, the proliferation rate slightly increased from 0.56 ± 0.20 cells/day (day 5) to 0.76 ± 0.29 cells/day (day 10). However, the proliferation rate of mouse adherent mononucleated cells decreased from 0.23 ± 0.02 cells/day (day 5) to 0.17 ± 0.01 cells/day (day 10). Meanwhile, human adherent cells maintained proliferation rate at approximately 0.67 ± 0.18 cells/day. In conclusion, adherent primary mononucleated cells from both mouse and human have limited capacity to generate more cells in vitro *as compared with suspension mononucleated cells.*

Keywords: Adherent cells; peripheral blood; proliferation; suspension cells

ABSTRAK

Sel primer mempunyai keupayaan yang terhad untuk berproliferasi berbanding titisan sel yang berupaya untuk berproliferasi tanpa had. Oleh itu, kajian ini dilakukan untuk menentukan keupayaan proliferasi sel primer mononukleus daripada mencit dan manusia. Sel mononukleus diasingkan daripada darah periferi mencit dan manusia dengan menggunakan larutan Ficoll-Paque™ Plus melalui kaedah pengemparan kecerunan ketumpatan. Terdapat dua jenis sel iaitu ampaian dan melekat yang diperoleh selepas pengkulturan sel mononukleus diasingkan selama 4 hari di dalam medium lengkap yang mengandungi 'Alpha Minimal Essential Medium', 10% serum anak lembu dan 2% penisilin-streptomisin. Sel ini kemudiannya dikultur selama 10 hari untuk analisis viabiliti menggunakan pengasaian tripan biru (sel ampaian) dan MTT (sel melekat). Sel titisan NSO and MC3T3-E1 masing-masing dipilih sebagai sel kawalan untuk sel ampaian dan melekat. Hasil kami menunjukkan bahawa kadar proliferasi sel ampaian mononukleus mencit meningkat daripada 1.31 ± 0.24 sel/hari (hari ke-5) kepada 2.69 ± 0.42 sel/hari (hari ke-10) manakala, kadar proliferasi sel ampaian manusia meningkat sedikit daripada 0.56 ± 0.20 sel/hari (hari ke-5) kepada 0.76 ± 0.29 sel/hari (hari 10). Walau bagaimanapun, kadar proliferasi sel melekat mononukleus mencit menurun daripada 0.23 ± 0.02 sel/hari (hari ke-5) kepada 0.17 ± 0.01 sel/hari (hari ke-10). Manakala, kadar proliferasi sel melekat mononukleus manusia kekal pada 0.67 ± 0.18 sel/hari. Kesimpulannya, sel primer melekat mononukleus daripada mencit dan manusia mempunyai keupayaan terhad untuk menghasilkan sel secara in vitro *berbanding sel ampaian mononukleus.*

Kata kunci: Darah periferi; proliferasi; sel ampaian; sel melekat

INTRODUCTION

Understanding the basis of cell culture most probably begins with the observation of its morphology and proliferation capacity through *in vitro* study. The modification and development of *in vitro* cell culture techniques are designed to mimic specific *in vivo* micro-environments as close as possible in order to produce reliable results. The capacity of fresh isolated primary mononucleated cells to survive and proliferate enables the cells to be used in further cellular research such as

cells differentiation and development. Any study involved newly potential *in vitro* cells such as cellular cytotoxicity study should begin by obtaining an appropriate *in vitro* cell survival capabilities followed by the rate of cell growth.

Cell proliferation is controlled by growth factors such as epidermal growth factor (EGF) and transforming growth factor-alpha (TGF-α) (Iamaroon et al. 1996) that bind to cell surface receptors which connect to signalling molecules. These molecules activate transcription factors which bind DNA to modulate the production of proteins, resulting in cell division through several pathways, such as Mitogen-Activated Protein Kinase (MAPK) signal pathway. The MAPK pathway includes a series of protein kinase cascades that play a critical role in regulation of cell proliferation (Zhang & Liu 2002). Dysfunction of any step in this regulatory cascade causes abnormal cell proliferation which is the underlying cause of many pathological conditions, including cancer and aging (Sulic et al. 2005).

Primary cell culture is obtained directly from an organism, i.e., tissues or cells. Primary cell culture is generally more difficult to survive than culture of established cell lines. Lennon et al. (2001) have reported that primary cells are far more sensitive than permanent cell lines. It depends on the quality of the culture medium and constituents used including serum quality and concentration (Lennon et al. 2001; van Zglinicki et al. 1995;). *In vitro* primary cell culture has a limited life span with different limitations, depending on the cell type studied, i.e. endothelial and epithelial primary cells. These cultures are capable only in a limited cell passages and they will enter a non-proliferative state called senescence and eventually die (Lennon et al. 2001).

Most of the isolated primary cells contained two types of cells, i.e. adherent and suspension (non-adherent) cells. Adherent cell form is widely used in many research areas especially in therapeutics studies. Nevertheless, Zhang et al. (2008) demonstrate that non-adherent cells derived from bone marrow mesenchymal stem cells can be expanded in suspension and give rise to multiple mesenchymal phenotypes including fibroblastic, osteoblastic and chondrocytic as well as other adherent cells.

Biopsy specimens or samples from surgical cases are another source of primary cells. Nevertheless, their uses are limited in terms of availability, small volume, and heterogeneity due to variations in sample origin. Primary cells are considered by many researchers to be more physiologically similar to *in vivo* cells. They are thought to represent the best experimental models for *in vivo* studies. Therefore, the purpose of the present study was to identify the capacity of *in vitro* expansion for both types of primary mononucleated cells, i.e. adherent and suspension, derived from mouse and human compared to cell line as control.

MATERIALS AND METHODS

ISOLATION OF MOUSE AND HUMAN MONONUCLEATED CELLS FROM PERIPHERAL BLOOD SYSTEM

Aseptic tissue culture technique has been a practice to culture fresh isolated primary mononucleated cells. Primary mononucleated cells obtained from the whole peripheral blood of healthy 4-6 weeks old mice ICR strain and 18-25 year old healthy donors were isolated by density centrifugation with Ficoll Paque™ Plus (G.E Healthcare, UK) according to the manufacturer's standard protocol. Written consent were obtained from the donors before the blood was taken. Briefly, the blood was diluted in ratio of 1:3 with Hanks' Balanced Salt Solution (HBSS) (Sigma, USA). Then, it was laid onto 1.5 mL of Ficoll Paque™ Plus and centrifuged at 400 × g for 20-30 min at room temperature. The diluted blood was separated into four layers according to cells densities, consisting of granulocytes and erythrocytes at the bottom, the polymorphonuclear cells in the Ficoll Paque layer followed by mononuclear cells layer and plasma layer at the top. The peripheral blood mononucleated cells were recovered from its layer and then washed twice with phosphate-buffered saline (PBS) (Sigma, USA). According to the manufacturer's procedure, isolation of mononucleated cells by density gradient centrifugation contains 3% granulocytes, 2% erythrocytes and less than 1% of platelets. Thus, a few washing steps using PBS and Hanks balanced salt solution have to be done to make sure the population are homogenous. Hank's balanced salt solution was used to remove residual platelets whilst, granulocytes and erythrocytes were removed using PBS. After being washed for a few times, the cells were cultured in complete medium for 10 days.

The isolated cells were cultured in selective proliferation media (complete medium) consisted of α-Minimal Essential Medium (α-MEM) (Invitrogen, USA) supplemented with 10% (v/v) newborn calf serum (NBCS) (Invitrogen, USA) and penicillin-streptomycin solution (with final concentration of 500 unit/mL penicillin G sodium and 500 μg/mL streptomycin sulfate) (Invitrogen, USA) in 6 well-plates. After 4 days, the cells were separated into two different forms of cells i.e. adherent and suspension. Both cells were then cultured for another 10 days for proliferation analysis.

MC3T3-E1 AND NSO CELL LINE CULTURE

Both cell lines, MC3T3-E1 subclone C14 (MC3T3-E1/C14) (ATCC No: CRL-2596™) and NSO cell line (ATCC No: HB-276™) were used in this study. MC3T3-E1 cell line was maintained in α-MEM supplemented with 1 mM sodium pyruvate (Sigma, USA) plus 10% (v/v) Fetal Bovine Serum (FBS) (Invitrogen, USA). During MC3T3-E1 cell line subculture, previous complete medium was discarded. The layered cells was washed with 1× PBS and Trypsin/

EDTA was added until cells detach from the T-flask. An equal volume of complete medium was added to stop trypsinization. The cells were sedimented by centrifugation at 97 g for 5 min. The supernatant was removed and the pellet was resuspended in 1 mL of complete medium. Approximately, 1 mL of suspended MC3T3-E1 cell line was cultured in 9 mL complete medium and incubated at 37°C in 5% CO_2 until 70-80% confluent. The medium was changed every 3 to 4 days.

As in suspension form cells, 1×10^5 cell/mL of NSO cell line was cultured in DMEM supplemented with 10% (v/v) FBS and 1% (v/v) penicillin/streptomycin. The cell line was incubated at 37°C until 70%-80% confluent before subculturing.

CELL SEEDING

All types of suspension cell proliferation analysis began with 1×10^5 cell/mL using 24 well-plates. Cell viability was determined daily for 10 days using trypan blue exclusion assay. Meanwhile, both mouse and human primary adherent cells were seeded at 5×10^3 cell/mL in 96 well-plates. Since the 96 well-plates surface were limited and the growth of MC3T3-E1 cell line was rapid among other cells, hence the cells were seeded in a small amount of about 1×10^3 cell/mL to make sure that the cells grow nicely and did not reach a lag phase of cell division for 10 days period.

TRYPAN BLUE EXCLUSION ASSAY

Viability of each suspension mononucleated primary cells and NSO cell lines were counted using haemocytometer (Hirschmann, Germany). The counting chamber is a device used to determine the number of viable cells per unit volume. About 10 µL of complete medium containing a number of viable suspension cells were taken for trypan blue 0.4% (v/v) staining with a dilution ratio of 1:1 (Freshney 2011). Both suspension mononucleated primary cells and NSO cell lines were counted daily for 10 days.

MTT (3-(4,5-DIMETHYLTHIAZOL-2-YL)-2,5-DIPHENYLTETRAZOLIUM BROMIDE) ASSAY

MTT assay was conducted for human and mouse adherent mononucleated cells including MC3T3-E1 cell line. Briefly, about 5×10^3 of primary cells were seeded into 96-well plates and cultured for 10 days while for MC3T3-E1 were seeded at 1×10^3 cells/mL. At the end of the culture, about 20 µL of MTT (2 mg/mL in PBS) was added to each well and incubated for another 4 h at 37°C in dark condition. Then, all the solution consists of complete medium and MTT was discarded. The formazan crystals were dissolved in 100 µL DMSO (v/v) and the absorbance was determined at 540 nm using a multi-plate reader (BIO-RAD model 680). The absorbance value that was determined for cell culture viability in complete medium was based on OD reading in each well (Ariffin et al. 2009). Each OD reading in every well represent total of cells viable and it was assayed in triplicate in three independent experiments. The number of viable MC3T3-E1 cell line throughout 10 days can be determined by extrapolation of MC3T3-E1 cell line standard curve. The standard curve has been constructed by dilution of cell number into 96 well-plates at 1×10^2, 2×10^2, 1×10^3, 2×10^3, 1×10^4, 2×10^4 and 1×10^5 cell/mL. After overnight incubation at 37°C, the cells were subjected to the MTT assay. The data of OD reading were plotted against number of cell.

STATISTICAL ANALYSIS

Data analysis was carried out using paired t-test from Microsoft Office Excel. This programme applies a pairwise comparison to assess any significant difference between mean of the data population. The value of $p<0.05$ indicates significant difference compared with control.

RESULTS AND DISCUSSION

SELECTION BY MORPHOLOGICAL HOMOGENEITY OF FRESHLY ISOLATED PRIMARY MONONUCLEATED CELLS

Freshly isolated primary mononucleated cells obtained from whole blood using Ficoll Paque™ Plus (G.E Healthcare, UK) give rise to a mixed population of cells after short centrifugation at room temperature. All freshly isolated primary cultured cell populations were morphologically similar with one round nucleus (Figure 1). As early as day 1 culture, the population of cells can already be differentiated morphologically i.e., suspension and adherent. Suspension cells produced spherical shape cells (Figure 1(a) and 1(c) whereas adherent cells had long and thin body similar to fibroblast cells (Figure 1(b) and 1(d). Both cells morphologically separated throughout 4 days culture in complete medium continuously before subjected to proliferation studies.

PROLIFERATION OF SUSPENSION MONONUCLEATED CELLS

Cells were observed daily to ensure that they were healthy and grew as expected. Thus, trypan blue exclusion assay was used to determine the viability of cells in suspension forms. Healthy suspension cells appeared as well-rounded and refracting light around their membrane, while dead cells appeared in blue colour as the membrane was lysed and permitted the dye to diffuse into the cells. In this study, those criteria were used as the standard referring viable cells during cell counts. The data were plotted into graph as shown in Figures 2 and 3.

Figures 2 and 3 show the proliferation graphs of mouse and human suspension mononucleated cells for 10 days of culture, respectively. The cells were cultured in complete medium and counted daily to determine the increment of viable cells number. Overall, the number of viable cells was increased upon culturing days. Statistical analysis using paired t-test showed that both mouse and human suspension mononucleated cells were significantly ($p<0.05$) increased

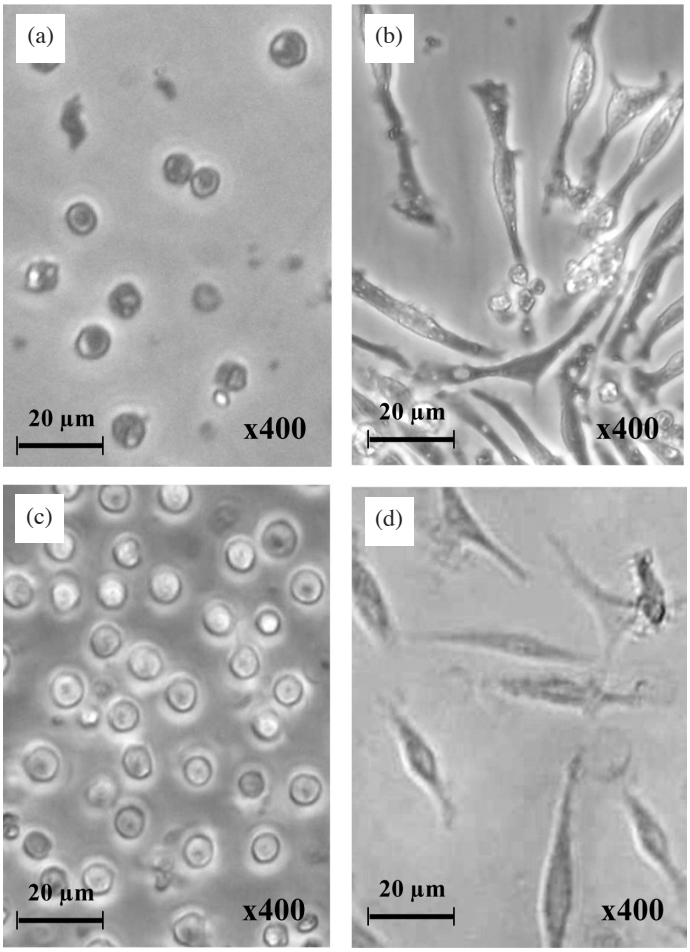

FIGURE 1. Morphological examination of isolated primary mononucleated cells from peripheral blood after 10 days of cultures. (a) Mouse suspension and (b) adherent mononucleated cells. (c) Human suspension and (d) adherent mononucleated cells. Suspension cells were rounded cells with one nucleus whereas adherent cells had long and thin body shape. The morphology of cells was captured using Olympus inverted microscope model IX51

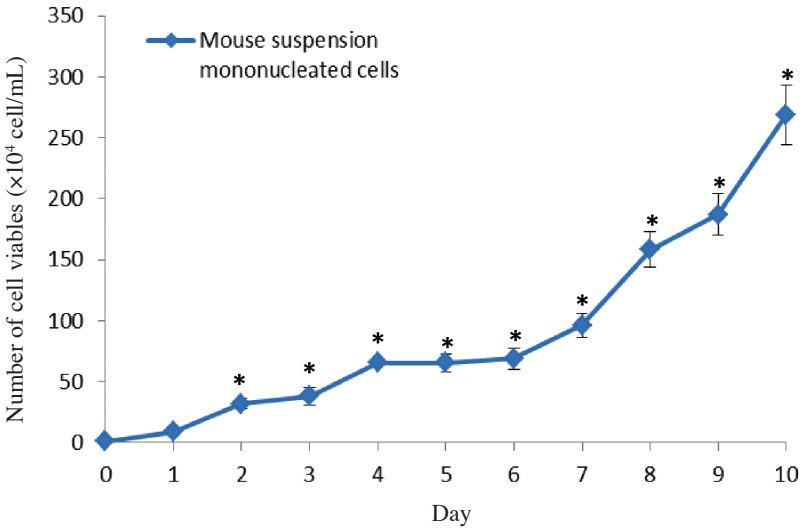

FIGURE 2. Proliferation of mouse suspension mononucleated cells in complete medium for 10 days. About 1×10^5 cells were seeded initially and the cells were daily counted using trypan blue exclusion assay. Results are presented as mean ± SE (n=3). The "*" indicates significant difference (p<0.05) of mononucleated cells as compared with day 0

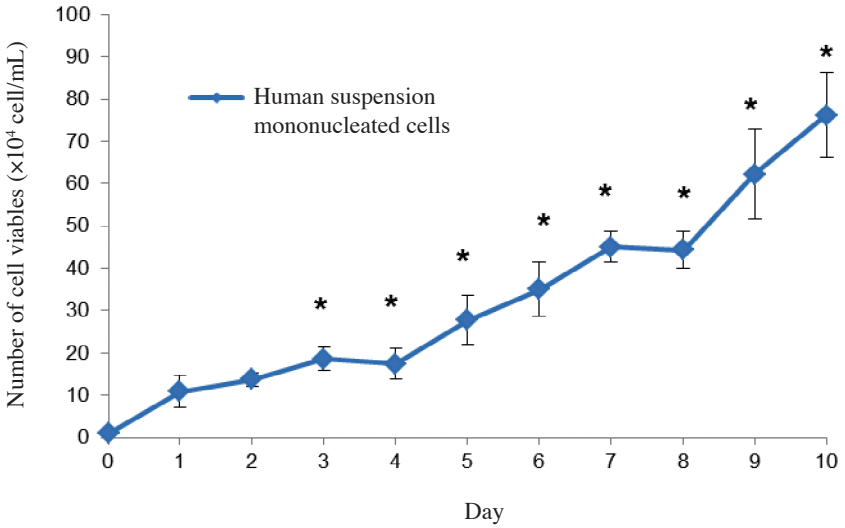

FIGURE 3. Proliferation of human primary suspension cells in complete medium for 10 days. About 1 × 10^5 cells were seeded initially and the cells were daily counted using trypan blue exclusion assay. Results are presented as mean ± SE (n=3). The "*" indicated significant difference (p<0.05) of mononucleated cells as compared with day 0

starting from day 2 to day 10 cultured in complete medium as compared with day 0. Day 0 is the initial culture when specific amount of suspension cells were seeded into plates. The proliferation capacity (proliferation rate) for both cells was observed at day 5 and day 10 of culturing according to the increment of cells number as compared with day 0. The proliferation rate was assessed by dividing the increment of viable cells number to the day of cultures.

Table 1 shows the proliferation analysis data for mouse and human suspension mononucleated cells and also NSO cell line as control cell. The NSO cell line was chosen as control cell due to their non-adherent behaviour similar to both mouse and human primary suspension mononucleated cells. There was an increase in the number of primary suspension cells from 6.5 ± 1.2 (day 5) to 26.9 ± 4.1 (day 10) and 2.8 ± 1.0 (day 5) to 7.6 ± 2.9 (day 10) as compared with day 0 from mouse and human suspension mononucleated cells respectively. Our results also demonstrated that the proliferation rate of mouse suspension increased from 1.31 ± 0.24 cells/day (day 5) to 2.69 ± 0.42 cells/day (day 10) whilst, for human, the proliferation rate increased slightly from 0.56 ± 0.20 cells/day (day 5) to 0.76 ± 0.29 cells/day (day 10). The NSO cell line that act as control for suspension cells exhibited the highest proliferation rate than both mouse and human primary suspension cells which was from 1.70 ± 0.13 cells/day (day 5) to 9.26 ± 3.23 cells/day at 10 days of culture (Table 1).

Based on increment of cell number and proliferation rate on day 5 (Table 1), mouse suspension mononucleated cells gave higher number of viable cell increment and proliferation rate as compared with human suspension mononucleated cells. These results demonstrated that mouse suspension mononucleated cells have higher proliferative capacity as compared with human suspension mononucleated cells. Nevertheless, NSO cell line that act as positive control showed the highest number of viable cell increment and proliferation rate throughout 10 days of culture. These results also showed that even though all the three cells were in suspension forms, they still gave different proliferative capacity on the same culturing day. Different proliferative capacity might be due to different organisms that have a different life span thus, owned a limited number of viable cells divisions. For example, mouse fibroblast cells have higher proliferative capacity compared with human fibroblast cells (Kennedy et al. 2010).

PROLIFERATION OF ADHERENT MONONUCLEATED CELLS

MTT assay is a colorimetric assay that measures the reduction of yellow MTT by mitochondrial succinate dehydrogenase. MTT enters the living cells and passesed into the mitochondria where it is reduced to an insoluble purple formazan product which gave the cells dark purple in colour. The insoluble purple formazan is then solubilised with an organic solvent, i.e. DMSO and the solubilised formazan reagent is measured by spectrophotometer at 540 nm wavelength. Since reduction of MTT could only occur in metabolically active cells, this assay is used to determine viable cell number (Berridge et al. 2005). This method has been used with the assumption that the adherent mononucleated cells did not divide during adherent process early in the culture. In this study, the OD readings represented the viability of the adherent mononucleated cells on different day of proliferation based on the standard curve of viable adherent cells. Final data were in number of cells' viable which varied at different day. Figures 4 and 5 show the proliferation profiles of mouse and human adherent mononucleated cells cultured for 10 days, respectively. Overall, the number of viable cells increased. Statistical analysis using paired t-test showed that both mouse and human adherent

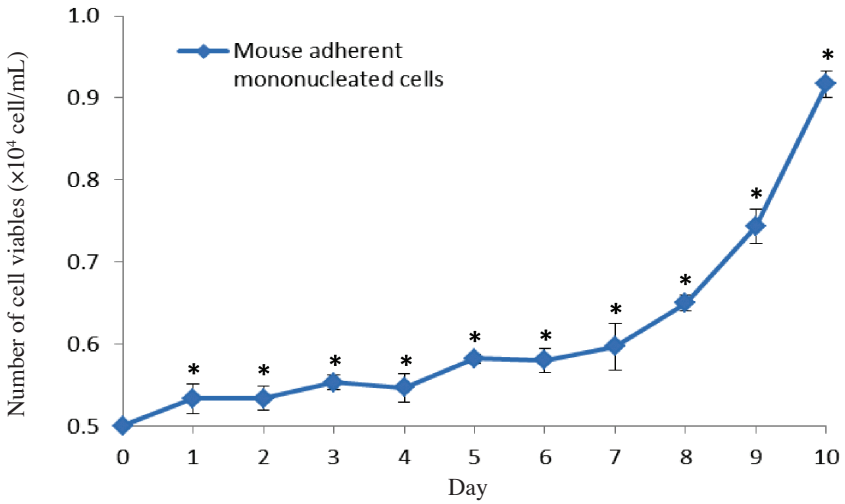

FIGURE 4. Proliferation of mouse adherent mononucleated cells in complete medium for 10 days. Approximately 5×10^3 cells were seeded before subjected for MTT assay. Results are presented as mean ± SE (n=3). The "*" indicated significant difference (p<0.05) of mononucleated cells as compared with day 0

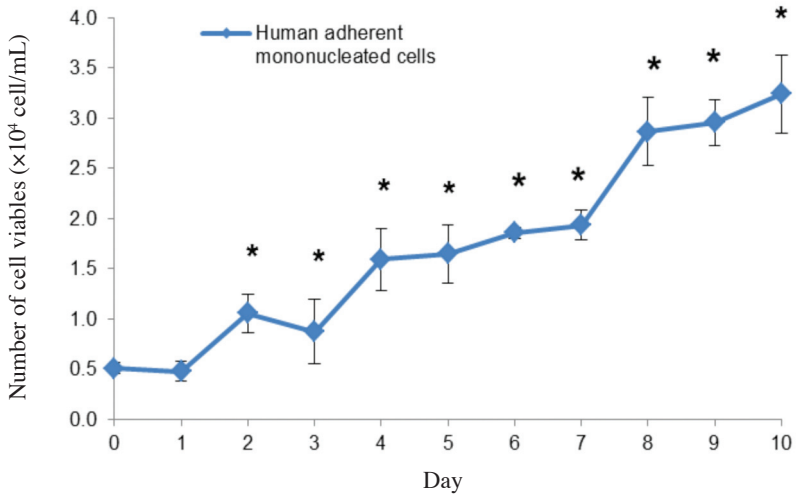

FIGURE 5. Proliferation of human adherent mononucleated cells in complete medium for 10 days. About 5×10^3 cells were seeded before subjected for MTT assay. Results are presented as mean ± SE (n=3). The "*" indicated significant difference (p<0.05) of mononucleated cells as compared with day 0

mononucleated cells were significantly increased (p<0.05) starting from day 1 (mouse) or day 2 (human) until day 10 of culture as compared with day 0. Day 0 is the initial culture when specific amount of adherent cells were seeded into plates.

Table 1 shows the number of viable cell and proliferation rate for mouse and human adherent mononucleated cells including MC3T3-E1 adherent cell line, that act as a control cell. The MC3T3-E1 cell line was chosen as control cell due to their adherent behaviour and attached on well-plate similar to both mouse and human adherent primary mononucleated cells. Our results showed that there was an increase in the number of viable adherent cells from 1.2 ± 0.0 (day 5) to 1.8 ± 0.1 (day 10) and 3.3 ± 0.8 (day 5) to 6.6 ± 2.0 (day 10) as compared with day 0 for mouse and human adherent mononucleated cells respectively. However, the proliferation rate of mouse adherent mononucleated cells was decreased from 0.23 ± 0.02 cells/day (day 5) to 0.17 ± 0.01 cells/day (day 10). Meanwhile, human adherent cells maintained proliferation rate at approximately 0.68 ± 0.17 cells/day. For control cells, the number of viable MC3T3-E1 cell line was dramatically increased from 6.4 ± 1.5 (day 5) to 55.2 ± 8.6 (day 10) as compared with day 0 culture with the increased in proliferation rate from 1.29 ± 0.37 cells/day (day 5) to 5.52 ± 0.86 (day 10).

Our results demonstrated that adherent primary mononucleated cells from both mouse and human have limited capacity to generate more cells *in vitro*. This might be due to their Hayflick limit regarding to the primary behaviour of the fresh isolated cells. The Hayflick limit is the limited number of times for cells' division (Hayflick

& Moorhead 1961) that has been linked to the shortening of telomeres, a region of DNA at the end of chromosomes (Golubev et al. 2003). As control cell, MC3T3-E1 cell line gave the highest number of viable cells increment and proliferation rate than primary cells attributed to the behaviour of established cell line which is immortal i.e, no Hayflick limit.

We also observed that the reduction proliferation rate of mouse adherent mononucleated cells was three times lower than the reduction of human adherent mononucleated cells. Theoretically, Morgan (2007) indicated that human primary adherent cells can be divided approximately 20 to 25 times while mouse primary adherent cells have lower proliferation capacity which was in line with our study due to the cellular replicative senescence. A number of reports about the cause of this phenomenon have been published such as studies on impaired of the signal transduction and mitogenesis (Tresini et al. 2001), cell stress (Soti et al. 2003), apoptosis (Campisi 2003) and telomere changes (de Magalhães & Toussaint 2004). This replicative senescence has also been modelled in hematopoietic development by Marciniak-Czochra et al. (2009).

COMPARISON BETWEEN PROLIFERATION RATE OF SUSPENSION AND ADHERENT MONONUCLEATED CELLS

The proliferation rate of both mouse and human primary suspension cells after 10 days of culture was higher as compared with proliferation of primary adherent cells. The proliferation rate of mouse and human primary suspension cells after 10 days of culture was 2.69 ± 0.42 cells/day and 0.76 ± 0.29 cells/day, respectively, whilst the proliferation

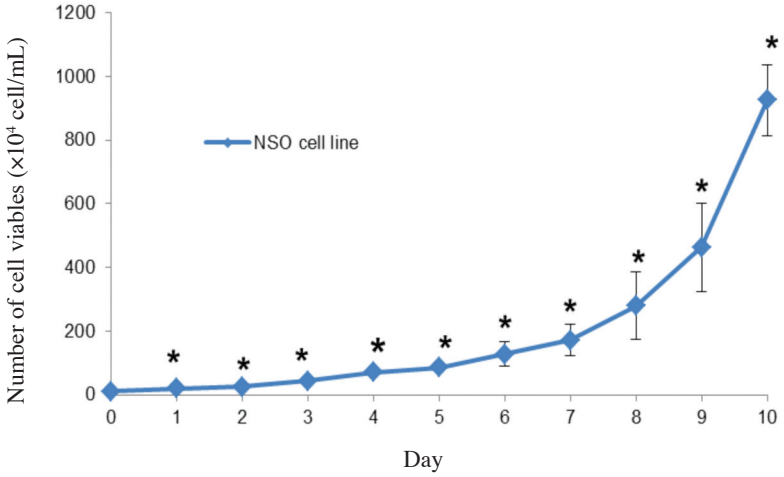

FIGURE 6. Proliferation of control suspension NSO cell line in complete medium for 10 days. Approximately 1 × 10^5 cells were seeded on the first day of culture and the cells were daily counted using trypan blue staining. Results are presented as mean ± SE (n=3). The "*" indicated significant difference (p<0.05) of mononucleated cells as compared with day 0

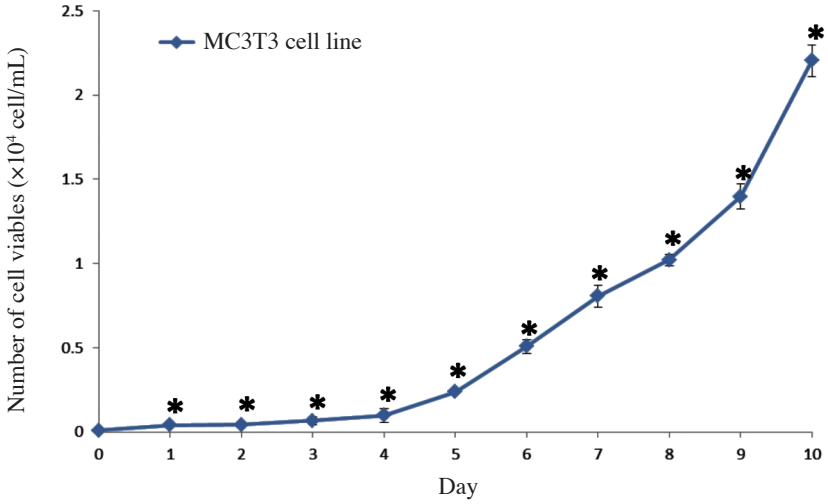

FIGURE 7. Proliferation of control adherent MC3T3-E1 cell line in complete medium for 10 days. Approximately 1 × 10^3 cells were seeded daily for 10 days before subjected for MTT assay. Results are presented as mean ± SE (n=3). The "*" indicated significant difference (p<0.05) of mononucleated cells as compared with day 0

TABLE 1. Proliferation analyses of suspension and adherent mononucleated cells

	Type of cells	Day	The number of viable cells increased compare to initial culture	Proliferation rate (The number of viable cells increased/day)
Suspension form	Mouse	5	6.5 ± 1.2	1.31 ± 0.24
	(*n*=3)	10	26.9 ± 4.1	2.69 ± 0.42
	Human	5	2.8 ± 1.0	0.56 ± 0.20
	(*n*=3)	10	7.6 ± 2.9	0.76 ± 0.29
	NSO cell line	5	8.5 ± 0.6	1.70 ± 0.13
	(*n*=3)	10	92.6 ± 32.9	9.26 ± 3.23
Adherent form	Mouse	5	1.2 ±0.0	0.23 ±0.02
	(*n*=3)	10	1.8 ±0.1	0.17 ±0.01
	Human	5	3.3 ± 0.8	0.68 ± 0.17
	(*n*=3)	10	6.6 ± 2.0	0.66 ± 0.20
	MC3T3-E1 cell line	5	6.4 ±1.5	1.29 ± 0.37
	(*n*=3)	10	55.2 ±8.6	5.52 ± 0.86

rate of mouse and human primary adherent cells after 10 days culture was 0.17 ± 0.01 cells/day and 0.66 ± 0.20 cells/day, respectively. In contrast with proliferation rate of primary suspension cells, the primary adherent cells either gave the reduction (mouse) or maintained with no increase (human) in proliferation rate. These results demonstrate that primary cells in suspension gave higher proliferation capacity as compared with adherent cells. Demsey and Grimley (1976) stated that murine erythroleukimia cells (FLC745) adherent cultures grew slightly slower than the parent FLC745 suspensions which were in line with this research. The result was obtained probably due to the bigger cell surface area during culturing of suspension as compared with adherent cells. This will affect the proliferation capacity of adherent cells due to limited surface area (Ryu et al. 2003).

CONCLUSION

Both mouse and human suspension mononucleated cells have higher *in vitro* expansion capacities than adherent mononucleated cells. In addition, mouse suspension mononucleated cells proliferation capacity was higher than human suspension mononucleated cells. However, suspension and adherent control cells (NSO and MC3T3-E1 cell line) gave the highest proliferation capacity as compared with mouse and human primary cells. Furthermore, characterization and classification of both cells according to morphological differences, specific surface properties and specific cytosolic transcript can be done for future research.

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