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Retrovirus-mediated conditional immortalization and analysis of established cell lines of osteoclast precursor cells ☆

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Abstract

Osteoclast precursor cells (OPCs) have previously been established from bone marrow cells of SV40 temperature-sensitive T antigen-expressing transgenic mice. Here, we use retrovirus-mediated gene transfer to conditionally immortalize OPCs by expressing temperature-sensitive large T antigen (tsLT) from wild type bone marrow cells. The immortalized OPCs proliferated at the permissive temperature of 33.5 °C, but stopped growing at the non-permissive temperature of 39 °C. In the presence of receptor activator of NFκB ligand (RANKL), the OPCs differentiated into tartrate-resistant acid phosphatase (TRAP)-positive cells and formed multinucleate osteoclasts at 33.5 °C. From these OPCs, we cloned two types of cell lines. Both differentiated into TRAP-positive cells, but one formed multinucleate osteoclasts while the other remained unfused in the presence of RANKL. These results indicate that the established cell lines are useful for analyzing mechanisms of differentiation, particularly multinucleate osteoclast formation. Retrovirus-mediated conditional immortalization should be a useful method to immortalize OPCs from primary bone marrow cells.

Keywords: SV40 T antigen; Retrovirus; Conditional immortalization; Osteoclasts

SV40 T antigen (T) has been widely adopted to establish cell lines because its expression immortalizes rodent [1,2] and human primary cells when combined with hTERT (human telomerase reverse transcriptase) [3]. T comprises large T antigen (LT) and small T antigen (ST) [4]. LT binds to and inactivates p53 [5] and RB [6], thus contributing to immortalization of primary cells. ST also promotes the transformation of human cells in conjunction with LT [3].

The forced expression of a temperature-sensitive mutant of T (tsT) has been shown to conditionally immortalize primary fibroblasts [7]. At the permissive temperature of 33.5 °C, the cells continue to proliferate, but at the non-permissive temperature of 39 °C they stop growing. Transgenic mice expressing tsT have been used to generate various cell lines including osteoclast precursor cells (OPCs) [8–10].

Osteoclasts are multinucleate giant cells which have differentiated from monocyte/macrophage precursor cells [11]. For the differentiation of osteoclasts, both receptor activator of NFκB ligand (RANKL) and macrophage colony-stimulating factor (M-CSF) are necessary and sufficient for OPCs to form mature osteoclasts [12–14]. RAW264 cells [13,15] or bone marrow cells prepared from tsT-transgenic mice [8,9] have been used to analyze osteoclast differentiation. As an alternative to primary bone

☆ *Abbreviations:* RANKL, receptor activator of NFκB ligand; TRAP, tartrate-resistant acid phosphatase; M-CSF, macrophage colony-stimulating factor; FACS, fluorescent activated cell sorter; GST, glutathione *S*-transferase; FITC, fluorescein isothiocyanate; PE, phycoerythrin; BrdU, bromo deoxyuridine; PI, propidium iodide; OPCs, osteoclast precursor cells.

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marrow cells, which cannot be maintained in long-term, a method to control the growth and differentiation of primary bone marrow cells is desirable. Therefore, because of future applications of human OPCs, we conditionally immortalized OPCs using retrovirus-mediated gene transfer of tsLT, a derivative of tsT which expresses LT but not ST. Furthermore, we cloned two types of cell lines having different cell fusion capabilities. These cell lines will be useful in analyzing the mechanism of differentiation into multinucleated osteoclasts.

Materials and methods

Plasmid construction. tsLT was constructed using tsT as a template and primers described for the construction of Tns [16]. Full-length tsLT was cloned into pCRBlunt (Invitrogen) and sequenced. The *Bam*HI fragment of tsLT was subcloned into the *Bam*HI site of the pCX4 puro vector [17].

Preparation of M-CSF conditioned medium. The M-CSF expression vector pCAhMCSF (RDB: 1524, RIKEN DNA Bank) [18] was transfected into NIH 3T3 cells using Fugene 6 transfection reagent (Roche). Transfected cells were selected using 1 mg/ml G418 and cloned. After cloning, the culture supernatant of the cells was used as M-CSF conditioned medium (M-CSF-CM).

Establishment of OPCs by retroviral infection. Platinum-E cells (a gift from Toshio Kitamura) [19] were used to produce retrovirus encoding tsLT, essentially as described [16]. White blood cells were separated from murine bone marrow by Ficoll–Paque Plus (Amersham) density separation [20]. The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) and 2% M-CSF-CM. The following day, the cells were infected with retrovirus encoding tsLT. Infected cells were selected using 2 μg/ml puromycin at 33.5 °C. The surviving population was expanded in DMEM supplemented with 10% FBS and 2% M-CSF-CM at 33.5 °C, and designated MDBMT (M-CSF-dependent bone marrow cells immortalized by tsLT). MDBMTs were cultured in DMEM supplemented with 10% FBS and 2% M-CSF-CM at 33.5 °C, unless otherwise indicated.

Cloning of MDBMTs. Semi-confluent MDBMTs in 100-mm dishes were collected, and limiting dilution was performed in 96-well plates to obtain single colonies. Seven different cell clones were obtained. Among these cell lines, two types of cells (MBC-1 and MBC-2) were further analyzed. MBC-1 and MBC-2 were expanded in DMEM supplemented with 10% FBS and 2% M-CSF-CM at 33.5 °C.

Growth curves. Semi-confluent MDBMT, MBC-1, and MBC-2 cells in 100-mm dishes were collected, seeded at 1×10^5 cells per 35-mm plate, and cultured at 33.5 °C. The culture temperature was shifted to 39 °C for 24 h after plating. Cell number in each plate was counted at 24, 48, 72, and 96 h after seeding. Cells were also cultured in the presence or absence of M-CSF-CM at 33.5 °C, and counted at 24 and 96 h after seeding. The growth assay was carried out in triplicate for each sample and averaged.

Cell cycle analysis. Cells growing at 33.5 or 39 °C were treated with 2 μg/ml bromo-deoxy-uridine (BrdU) for 1 h and then harvested. The cells were fixed in ice-cold 70% ethanol overnight and then stained with FITC-conjugated anti-BrdU antibody (Chemicon) and 5 μg/ml propidium iodide (PI) (Sigma). After staining, the cell cycle was analyzed using a flow cytometer (FACScan, Becton–Dickinson).

Detection of senescence. Cell cultures at 39 °C were fixed and stained for senescence-associated β-galactosidase (SA-β-gal) activity using a senescence detection kit (BioVision Inc.). The percentage of SA-β-gal-positive cells was determined by counting the number of blue cells per 100 cells. The assay was carried out in triplicate for each sample and averaged.

Immunoblotting. Immunoblotting (IB) was carried out as previously described [16]. Briefly, total cells were lysed in lysis buffer (1% Triton X-100, 10 mM Tris–HCl, pH 7.6, 50 mM NaCl, 30 mM sodium pyrophosphate, 50 mM sodium fluoride, 20 mM β-glycerophosphate, 1 mM EDTA, 1 mM EGTA, 1 mM sodium vanadate, 20 μg/ml aprotinin, and 1 mM phenylmethylsulfonyl fluoride). Aliquots of lysate were suspended in SDS–PAGE sample buffer at concentrations of 5 μg/μl (for LT), 10 μg/μl (for NFAT2), or 1 μg/μl (for actin). Samples containing 20 μl lysate were separated by 10% SDS–PAGE, followed by IB with the appropriate antibody: anti-LT antibody (pAB101; Pharmingen), anti-NFAT2 antibody (7A6; Santa Cruz Biotechnology), or anti-β-actin antibody (Ac-74; Sigma). After incubation with a secondary antibody, the proteins were visualized with ECL Plus enhanced chemiluminescence solutions (Amersham).

Cell surface marker analysis. FITC-conjugated anti-F4/80 (BMA), PE-conjugated anti-Mac-1 (PharMingen), and PE-Cy5-conjugated anti-CD25 antibodies (eBioscience) were used to stain RAW264, MDBMT, MBC-1, MBC-2, and bone marrow cells. Cell surface marker expression was monitored by FACScan (Becton–Dickinson).

In vitro differentiation of MDBMT, MBC-1, and MBC-2 cells into osteoclasts. Soluble recombinant GST-RANKL was prepared as described [15]. For the differentiation of MDBMTs into osteoclasts, the cells were plated at 1×10^5 (for MDBMTs) or 2.5×10^4 (for MBC-1 and MBC-2) per 35-mm plate, incubated for 24 h, and then GST-RANKL was added to a final concentration of 250 ng/ml. The medium was changed 96 h (for MDBMTs) or 72 and 144 h (for MBC-1 and MBC-2) after addition of

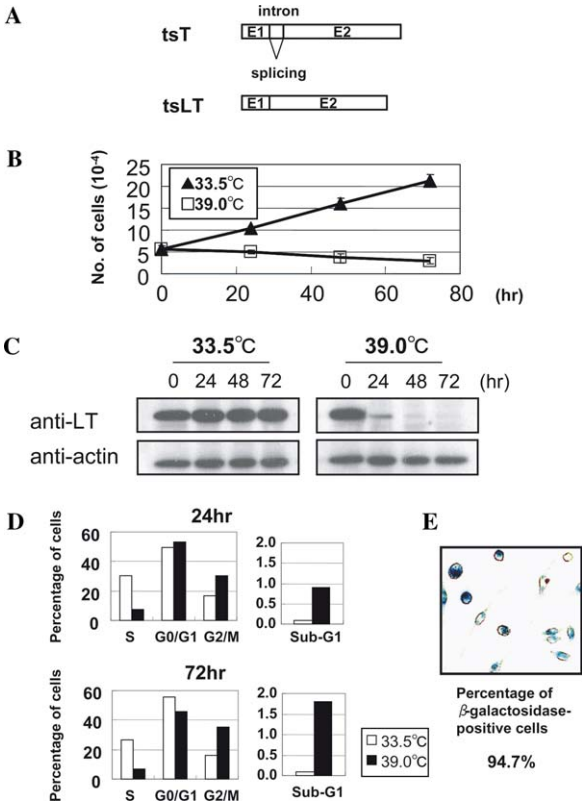

Fig. 1. Temperature-dependent immortalization of primary bone marrow cells. (A) Schematic representation of tsT and tsLT. The intron region of tsT was deleted in tsLT. E1 and E2 represent exon1 and exon2, respectively. (B) Growth curves of MDBMTs. MDBMTs were cultured at 33.5 °C (▲) and at 39 °C (□), and counted at the indicated time of incubation. Three independent experiments were averaged. (C) Western blot analysis of tsLT following temperature shift. Lysates were collected at the indicated time of incubation, separated by 10% SDS–PAGE, and immunoblotted with anti-LT or anti-actin antibodies. (D) Cell cycle analysis of MDBMTs. MDBMTs were grown in culture medium containing BrdU, harvested, and stained with an FITC-labeled anti-BrdU antibody and propidium iodide (PI). Cells grown at 33.5 °C (▲) or 39 °C (■) for 24 h (upper panels) and 72 h (lower panels) were analyzed by a flow cytometer. (E) Senescence-associated β-galactosidase (SA-β-gal) activity of MDBMTs. Cells were cultured at 39 °C for 72 h. MDBMTs express SA-β-gal enzyme with high frequency.

GST-RNAKL and re-stimulated by adding GST-RANKL to a final concentration of 250 ng/ml. At 120 h (for MDBMTs) or 192 h (for MBC-1 and MBC-2) after the initial stimulation by GST-RANKL, the cells were fixed in 100% methanol and subjected to a TRAP staining assay as described [15].

Results and discussion

Conditional immortalization of bone marrow cells by tsLT-expressing retrovirus

Previous studies used bone marrow cells of tsT-transgenic mice to amplify OPCs [8,9]. Because retrovirus-mediated gene transfer of tsT has the advantage of being able to amplify primary bone marrow cells, including those of human, we adopted this method and further developed a mutant T antigen, tsLT (Fig. 1A). Because tsLT lacks the splicing region for ST, and therefore only expresses LT (Fig. 1A), unpredictable effects of ST can be avoided. The LT itself remains the same. In addition, if tsLT acted in a temperature-dependent manner, we would be able to monitor whether OPCs were immortalized by large T antigen.

White blood cells from murine bone marrow were prepared by Ficoll–Paque density separation [20]. The cells were cultured in DMEM supplemented with 10% FBS and 2% M-CSF-CM. Adherent cells were cultured overnight and infected with the retrovirus expressing tsLT. After drug selection of transduced bone marrow cells, we were able to propagate the culture for at least one year in the presence of M-CSF. The immortalized bone marrow cells were designated MDBMT (M-CSF-dependent bone marrow cells immortalized by tsLT).

To confirm that tsLT could act in a temperature-dependent manner, MDBMTs were cultured at both the permissive temperature of 33.5 °C (Fig. 1B, triangles) and the non-permissive temperature of 39 °C (Fig. 1B, squares). The cells continued to grow at 33.5 °C, but stopped growing and even decreased in number at 39 °C. The levels of expression of LT were determined by Western blotting (Fig. 1C, upper panels), using anti-actin antibody (Fig. 1C, lower panels) to confirm comparable loading for each lane. Whereas, constant expression of LT was observed for MDBMTs at 33.5 °C, a rapid decrease in the level of expression of LT was observed 24 h after the temperature shift to 39 °C, as published [7]. This result indicated that there was a correlation between the suppression of MDBMT growth and the rapid disappearance of LT.

We examined the cell cycle of MDBMTs at both permissive and non-permissive temperatures by flow cytometry

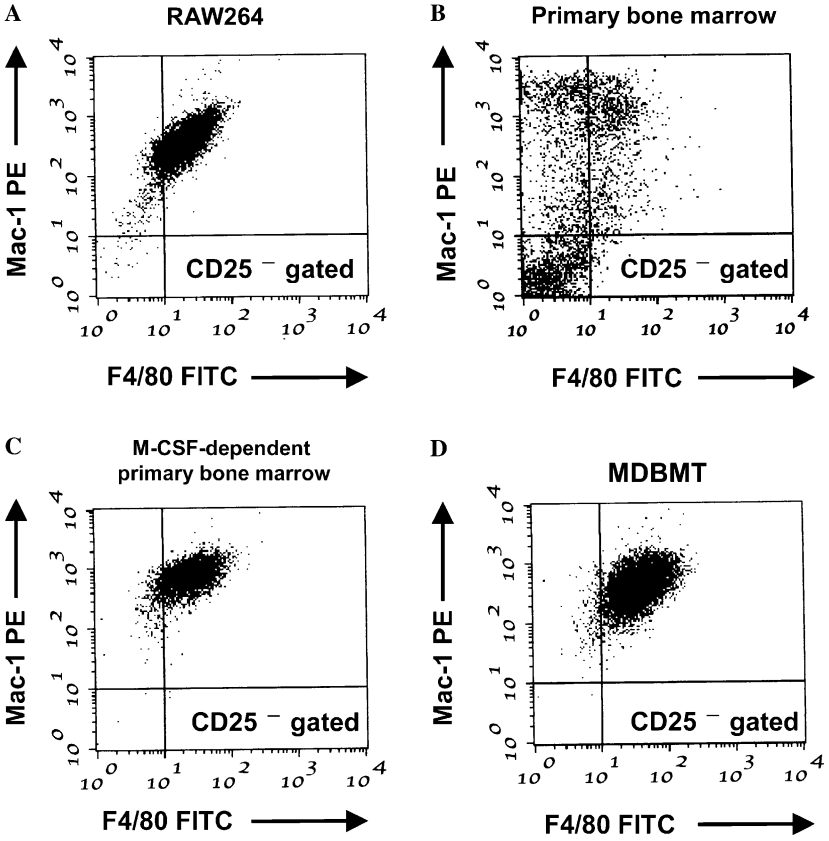

Fig. 2. Cell-surface marker analysis of MDBMTs. Three-color FACS analysis of MDBMTs. The cells were stained with FITC-conjugated anti-F4/80, PE-conjugated anti-Mac-1, and PE-Cy5-conjugated anti-CD25 antibodies, followed by analysis using a flow cytometer. (A) RAW264, (B) primary bone marrow cells, (C) bone marrow cells cultured with M-CSF, and (D) MDBMTs were subjected to FACS analysis. CD25-negative cells were collected and analyzed for Mac-1 and F4/80 expression.

after staining with propidium iodide (PI) and incubating with anti-BrdU antibody. After 24 h (Fig. 1D, upper panels) or 72 h (Fig. 1D, lower panels) of incubation at the non-permissive temperature, the proportion of cells in S phase clearly decreased, suggesting that entry into S phase was blocked (Fig. 1D, left panels). Conversely, the proportion of cells in the G2/M phases increased, indicating that the cell cycle was arrested in G2/M (Fig. 1D, left panels) as previously observed for other types of cells [7,21]. Cells in the G0/G1 phases increased slightly (Fig. 1D, left panels). The Sub-G1 population (Fig. 1D, right panels), composed of cells undergoing apoptosis, also increased slightly, consistent with the reduction of cell numbers in the growth curve for cells cultured at the non-permissive temperature (Fig. 1B). These observations suggest that MDBMTs stopped entering S phase and mainly arrested in the G2/M phases at the non-permissive temperature of 39 °C. We further examined whether MDBMTs had undergone

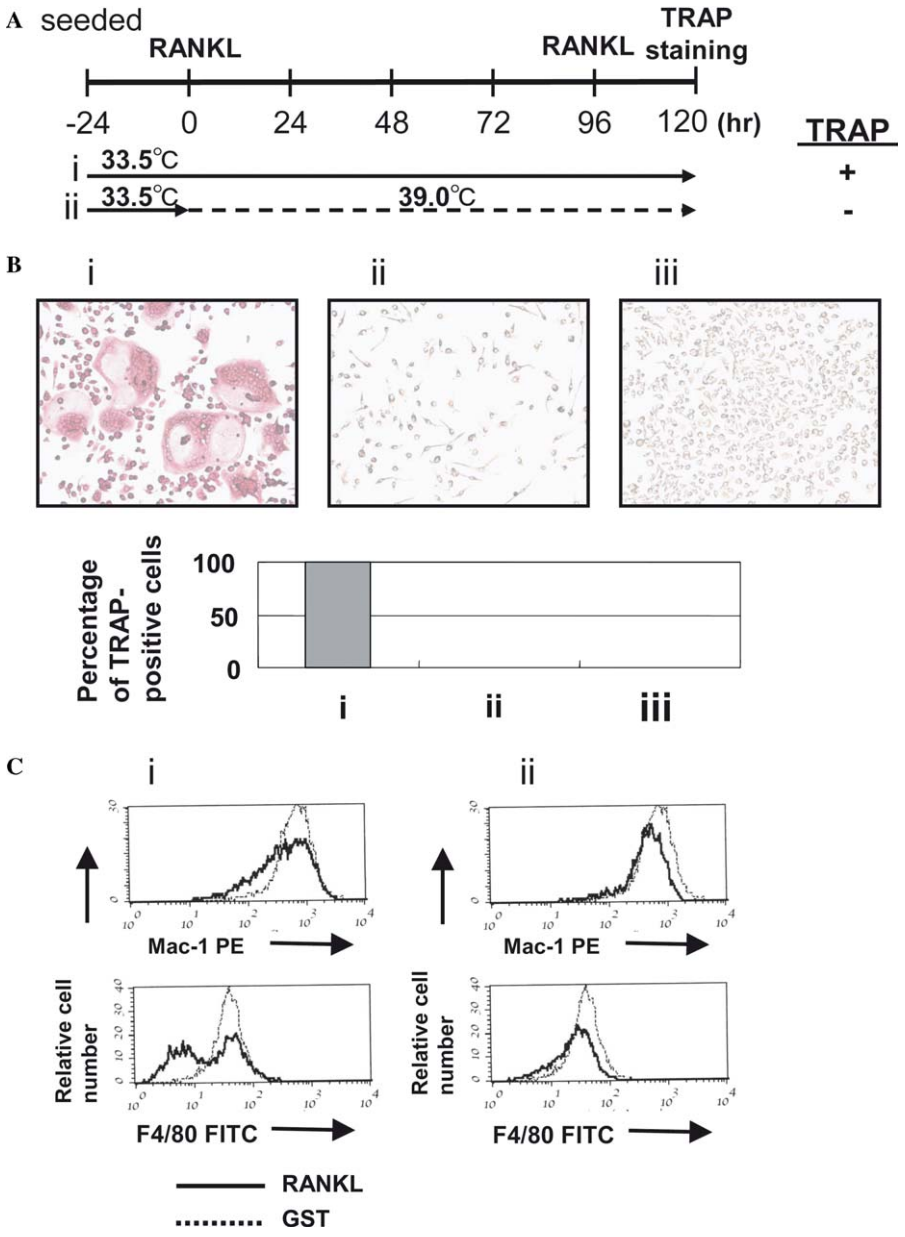

Fig. 3. Analysis of OPC differentiation by TRAP assay and flow cytometry. (A) Schematic representation of the experimental procedure. Cells were seeded 24 h before the initial RANKL stimulation at 0 h. The second stimulation with RANKL was performed 96 h after the first. At 120 h after the initial stimulation, cells were subjected to TRAP staining assay. The results of the TRAP staining assay are summarized at the far right. (B) Photographs of the TRAP staining of MDBMTs for each temperature regime (i,ii) shown in (A). (iii) Photograph of MDBMTs treated with GST instead of RANKL. The results of the TRAP assay are summarized in the lower panel. (C) Cytometry analysis of MDBMTs during differentiation. MDBMTs were cultured at either 33.5 °C (left panels) or 39 °C (right panels) for 72 h after RANKL stimulation (solid lines) or GST stimulation (broken lines). The cells were stained with FITC-conjugated anti-F4/80, PE-conjugated anti-Mac-1, and PE-Cy5-conjugated anti-CD25 antibodies. CD25-negative cells were collected and further analyzed for Mac-1 and F4/80 expression.

premature cellular senescence, by staining for expression of senescence-associated β-galactosidase. After 72 h of incubation at the non-permissive temperature, almost all cells exhibited positive staining for SA-β-gal enzyme. These results suggest that the surviving population of MDBMTs stopped passage through the cell cycle and underwent premature cellular senescence at 39 °C.

Cell surface analysis of MDBMTs

We examined MDBMT surface markers by flow cytometry, using three monoclonal antibodies. Mac-1, encoding the integrin α_M chain, and F4/80, an approximately 125-kDa transmembrane protein, are known to be expressed in macrophages [9,11], while CD25, encoding the IL-2 receptor α chain, is expressed in activated macrophages and activated lymphoid cells [22]. FITC-conjugated anti-F4/80, PE-conjugated anti-Mac-1, and PE-Cy7-conjugated anti-CD25 antibodies were mixed and used to stain the OPC line RAW264 [13,15], primary bone marrow cells, and MDBMTs. In Fig. 2, CD25-negative cells were collected and further analyzed for Mac-1 and F4/80 expression. RAW264 cells (Fig. 2A) and MDBMTs (Fig. 2D) were negative for CD25 but positive for Mac-1 and F4/80. Analysis of primary bone marrow cells (Fig. 2B) showed that 29% of the cells were positive for both Mac-1 and F4/80; 31% were positive for Mac-1 and negative for F4/80; and 39% were negative for both Mac-1 and F4/80. When we cultured the primary bone marrow cells with M-CSF, they proliferated and became positive for both Mac-1 and F4/80 expression (Fig. 2C). The level of Mac-1 expression was slightly higher in these cells (Fig. 2C) than in RAW264 cells and MDBMTs (Fig. 2A and D). These results suggest that MDBMTs were selectively immortalized as a cell population which could proliferate in the presence of M-CSF. In support of this, MDBMTs no longer sustained growth when we cultured them in the absence of M-CSF (data not shown).

Differentiation of MDBMTs into osteoclasts

In previous studies, stimulation of OPCs by RANKL and M-CSF was reported to promote differentiation of the OPCs into osteoclast-like cells [12–14]. To determine whether MDBMTs can differentiate into osteoclasts, they were stimulated by RANKL in culture medium containing M-CSF. Differentiation into osteoclasts was monitored by TRAP activity as previously described [23]. MDBMTs were able to differentiate into TRAP-positive multinucleate osteoclasts when cultured at 33.5 °C (Fig. 3B-i). No TRAP-negative cells were observed in culture. When we cultured MDBMTs at 39 °C, they did not stain for TRAP and failed to form multinucleate cells (Fig. 3B-ii), suggesting that MDBMTs do not differentiate even with RANKL stimulation at 39 °C. The results of TRAP activity assays are summarized in Fig. 3B, and suggest that expression of tsLT during RANKL stimulation is required for MDBMTs to differentiate into TRAP-positive, multinucleate cells. One explanation of these observations is that the cells remain proliferative because of tsLT expression. A satisfactory explanation of the results at the non-permissive temperature during RANKL stimulation is difficult to provide, because the cell number was decreasing due to cell death. However, surviving cells at the non-permissive temperature did not undergo differentiation into TRAP-positive cells because they underwent premature senescence (Fig. 1E).

Changes in MDBMT surface marker expression during RANKL stimulation

To further examine the differentiation of MDBMTs, MDBMTs were stimulated with RANKL (Fig. 3C, solid lines) or GST (broken lines) for 72 h, either at 33.5 °C (Fig. 3C, left panels) or at 39 °C (right panels), and subject-

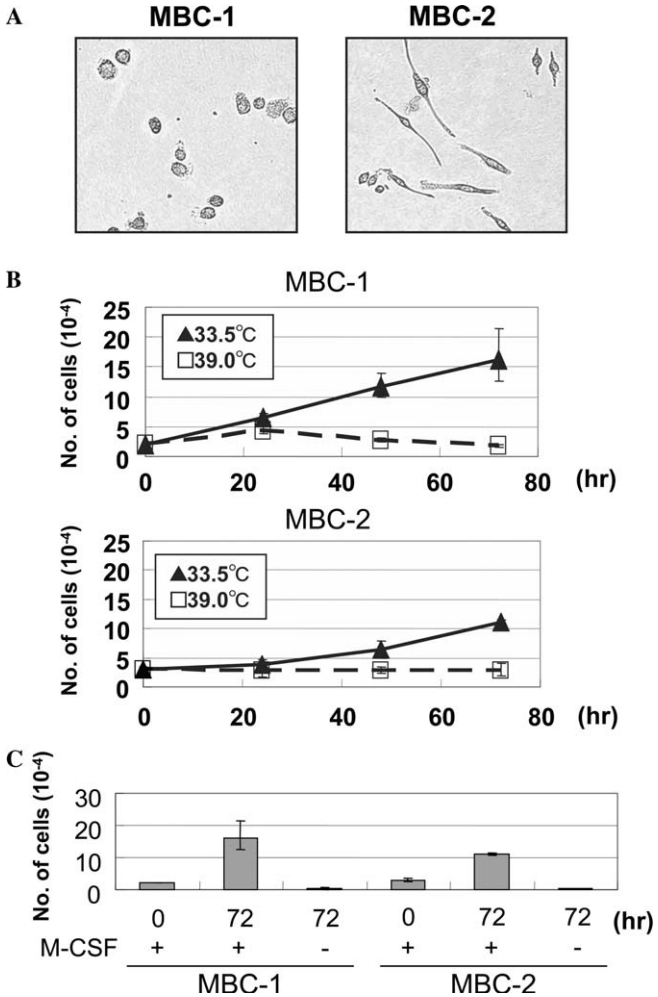

Fig. 4. Characterization of cloned cells. (A) Photographs of MBC-1 (left panel) and MBC-2 (right panel). (B) Growth curves of MBC-1 (upper panel) and MBC-2 (lower panel). MBC-1 and MBC-2 were cultured at 33.5 °C (▲) and at 39 °C (□), and counted at the indicated time of incubation. Three independent experiments were averaged. (C) Growth of MBC-1 and MBC-2 in the presence or absence of M-CSF. MBC-1 and MBC-2 were cultured in the presence or absence of M-CSF-CM at 33.5 °C and counted at the indicated time of incubation. Three independent experiments were averaged.

ed to flow cytometry analysis by staining with the same cell surface markers described in Fig. 2. CD25-positive cells were not observed. CD25-negative cells were collected and further analyzed for Mac-1 and F4/80 expression (Fig. 3C).

This analysis showed decreasing expression of Mac-1 in MDBMTs during RANKL stimulation at 33.5 °C (Fig. 3C, upper left panel). For F4/80 expression, MDBMTs contained two peaks (Fig. 3C, lower left panel). Because these cells became TRAP-positive, the different peaks of F4/80 expression may either reflect differing degrees of osteoclast differentiation among the TRAP-positive OPCs or suggest that these cells consisted of two different cell populations which differ in their ability to differentiate into mature osteoclasts. When MDBMTs were cultured at 39 °C, the levels of Mac-1 and F4/80 expression of the surviving population did not change significantly, even during RANKL stimulation (Fig. 3C, right panels), confirming that the surviving MDBMTs did not undergo differentiation at 39 °C. A previous report showed that cell-cycle progression from G1 to S phase was required after RANKL stimulation for RAW264 cells to differentiate into osteoclasts [24]. Because LT inactivates both p53 and RB, and promotes cell-cycle progression, these observations together suggest that culturing MDBMTs at 39 °C resulted

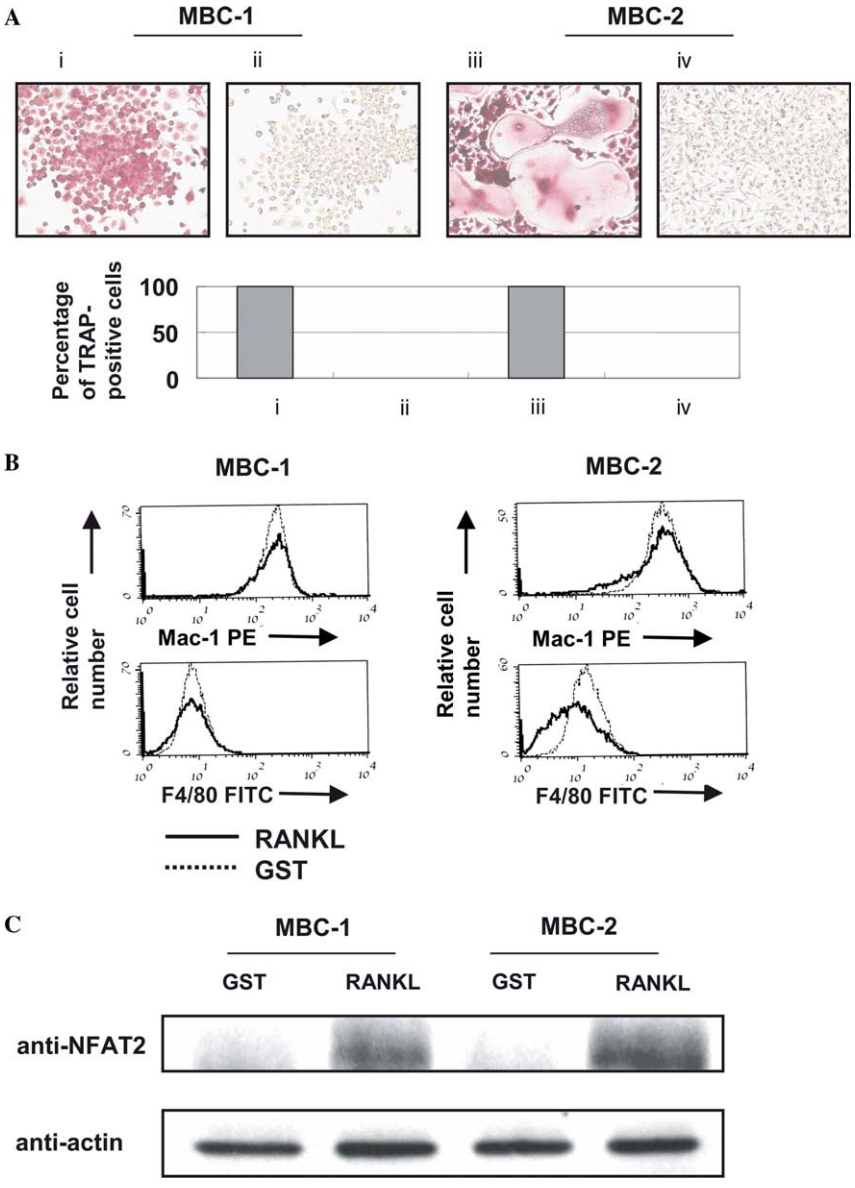

Fig. 5. Analysis of cloned cells by TRAP assay, flow cytometry, and expression of NFAT2. (A) Images of the TRAP staining of MBC-1 (i,ii) and MBC-2 (iii,iv) at 33.5 °C. Images of MBC-1 (ii) and MBC-2 (iv) treated with GST instead of RANKL. TRAP activity results are summarized in the lower panel. (B) Cytometry analysis of MBC-1 and MBC-2 during differentiation. MBC-1 and MBC-2 were cultured at 33.5 °C for 168 h after RANKL stimulation (solid lines) or GST stimulation (broken lines). The cells were stained with FITC-conjugated anti-F4/80, PE-conjugated anti-Mac-1, and PE-Cy5-conjugated anti-CD25 antibodies. CD25-negative cells were collected and further analyzed for Mac-1 and F4/80 expression. (C) Western blot analysis of NFAT2 after RANKL or GST stimulation.

in a decrease in tsLT expression, thereby blocking the cell cycle, and failure to initiate the differentiation program of osteoclastogenesis. Our observation further indicated that cell-cycle progression is required for the differentiation of MDBMTs into osteoclasts. Alternatively, premature senescence induced by loss of LT simply stopped the differentiation stimulated by RANKL.

Establishment and analysis of OPC cell lines from MDBMTs

To further analyze MDBMTs, we cloned seven cell lines from MDBMTs and were able to propagate the seven cultures for at least eight months in the presence of M-CSF. When we observed the cloned MDBMT cells with a microscope, two phenotypes were evident (Fig. 4A). One type grew as a round cell (three clones), while the other adopted an elongated form (four clones). We selected one of each type of cell line from the seven cultures and further analyzed their phenotypes. These cloned MDBMT cells were designated MBC-1 (Fig. 4A, left panel) and MBC-2 (Fig. 4A, right panel). To confirm that tsLT could act on these cells in a temperature-dependent manner, MBC-1 and MBC-2 were cultured at both the permissive temperature of 33.5 °C (Fig. 4B, upper and lower panels, triangles) and the non-permissive temperature of 39 °C (Fig. 4B, upper and lower panels, squares). The cells continued to grow at 33.5 °C, but stopped growing at 39 °C. In MBC-1, there was a decrease in cell number at 39 °C, as we observed for MDBMTs, while in MBC-2 the cell number stayed almost the same. The reason for this difference is unclear. Growth of both MBC-1 and MBC-2 required M-CSF (Fig. 4C).

To determine whether MBC-1 and MBC-2 can differentiate into osteoclasts, the cells were stimulated by RANKL in culture medium containing M-CSF. We compared the cells at 192 h for MBCs after the initial RANKL stimulation, instead of at 120 h for MDBMTs, because the cell lines exhibited significant differences in the timing of multinucleate cell formation. The results of TRAP activity assays are summarized in Fig. 5A. In the presence of RANKL, MBC-1 and MBC-2 were able to differentiate into TRAP-positive osteoclasts at 33.5 °C (Fig. 5A-i, iii); no TRAP-negative cells were observed. MBC-2 was able to differentiate into TRAP-positive multinucleate osteoclasts, and while MBC-1 cells also became TRAP-positive (Fig. 5A-iii), they nonetheless remained as single cells (Fig. 5A-i). To analyze cell surface marker expression during RANKL stimulation, MBC-1 and MBC-2 were stimulated with RANKL (Fig. 5B, solid lines) or GST (broken lines) for 168 h at 33.5 °C, and analyzed by flow cytometry as described in Fig. 2. CD25-positive cells were not observed. CD25-negative cells were collected and further analyzed for Mac-1 and F4/80 expression (Fig. 5B). In MBC-1, there was a slight decrease in the levels of expression of Mac-1 and F4/80 during RANKL stimulation at 33.5 °C. In MBC-2, the level of Mac-1 expression decreased slightly and a relatively greater reduction in the level of F4/80 expression was observed (Fig. 5B), suggesting that MBC-2 has a greater ability to differentiate into mature osteoclasts. Because longer incubation with RANKL did not result in MBC-1 differentiation into multinucleate osteoclasts (data not shown), the reason for the difference between MBC-1 and MBC-2 was probably that MDBMT consists of at least two types of cell lines with varied ability to differentiate into mature osteoclasts.

The transcription factor NFAT2 (also called as NFATc1) is a key regulator of osteoclast differentiation. [16,25]. Therefore, we sought to detect any difference in induction of NFAT2 between MBC-1 and MBC-2. Cell lysates of MBC-1 and MBC-2 in the presence and absence of RANKL stimulation were prepared after 168 h, because beyond this time point multinucleate cell formation was observed for MBC-2, and expression of NFAT2 was determined by Western blotting (Fig. 5C, upper panel). We used anti-actin antibody (Fig. 5C, lower panel) to confirm comparable loading in each lane. The results showed that both lines expressed NFAT2 at a comparable level (Fig. 5C), indicating that variable levels of NFAT2 expression did not explain the differences between MBC-1 and MBC-2.

In conclusion, MDBMTs consist of at least two different types of cells. The cell lines MBC-1 and MBC-2 should be useful in analyzing mechanisms of differentiation, particularly multinucleate osteoclast formation. For example, to detect differences in gene expression between the two cell lines, genes involved in the cell fusion process, including a cell-fusion receptor, may be identifiable.

In this study, we conditionally immortalized OPCs from murine bone marrow cells using retrovirus-mediated gene transfer of tsLT. These results provide essential information for the future use of conditionally immortalized OPCs from primary bone marrow cells, including human cells.

Acknowledgments

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