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Presence of Leukemia-maintaining Cells in Differentiation-resistant Fraction of K562 Chronic Myelogenous Leukemia

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The present study investigated whether leukemia-maintaining cells reside in a differentiation-resistant fraction using a megakaryocytic differentiation model of K562 cells. Treatment with phorbol-12-myristate-13-acetate (PMA) significantly inhibited the colony-forming efficiency of the K562 cells. At a PMA concentration of 1 nM or higher, colony was not formed, but approximately 40% of K562 cells still survived in soft agar. Approximately 70% of colony-forming cells that were isolated following the removal of PMA after exposure to the agent were differentiated after treatment with 10 nM PMA for 3 days. The differentiation rate of the colony-forming cells was gradually increased and reached about 90% 6 weeks after colony isolation, which was comparable to the level of a PMA-treated K562 control. Meanwhile, imatinib-resistant variants from the K562 cells, including K562/R1, K562/R2, and K562/R3 cells, did not show any colony-forming activity, and most imatinib-resistant variants were CD44 positive. After 4 months of culture in drug-free medium, the surface level of CD44 was decreased in comparison with primary imatinib-resistant variants, and a few colonies were formed from K562/R3 cells. In these cells, Bcr-Abl, which was lost in the imatinib-resistant variants, was re-expressed, and the original phenotypes of the K562 cells were partially recovered. These results suggest that leukemia-maintaining cells might reside in a differentiation-resistant population. Differentiation therapy to eliminate leukemia-maintaining cells could be a successful treatment for leukemia if the leukemia-maintaining cells were exposed to a differentiation inducer for a long time and at a high dose.

Key words : Chronic myelogenous leukemia, differentiation-resistance, imatinib, leukemia-maintaining cells, phorbol-12-myristate-13-acetate

Introduction

Although modern tumor therapy has achieved considerable progress, cancer is still one of the leading causes of death. Current failure with cancer treatment is not usually due to a lack of primary clinical responses including complete remission, but to recurrence or metastasis after initial therapies. The concept that cancer is driven by cancer-initiating or maintaining cells (popularly known as cancer stem cells, CSC) has recently attracted a great deal of attention [37]. While conventional cancer therapies have targeted well the bulk tumor cells, there is now compelling evidence that cancer-initiating cells may be responsible for recurrence or metastasis of cancers after successful initial induction of remission due to their resistance to traditional cancer treatments such as surgery, radiotherapy and chemotherapy [46]. It has been demonstrated that the cancer stem cells share several important characteristics of normal stem cells, including the capacity for self-renewal, the ability to differentiate, migrate and metastasize, a relative quiescence, activation of telomerase and antiapoptotic pathways, the increased expression of multidrug-resistance proteins, and robust DNA repair activity [25, 44], and consequently show high resistance to anticancer drugs [11] and radiotherapy [13].

Current therapies have been developed largely against the bulk tumor cells, since they have been usually developed by their ability to shrink tumors. Therefore, even therapies that cause complete regression of tumors might spare enough cancer stem cells to allow regrowth of the tumors.

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A number of therapeutic strategies directed at CSCs are being studied experimentally. The approaches include ablation using antitumor agents that target prospective markers of CSCs (e.g., monoclonal antibodies and activated immune cells), reversal of chemo- or radioresistance mechanisms operative in CSC, CSC pathway interference, differentiation therapy, disruption of protumorigenic CSC-microenvironment interactions, antiangiogenic or antivasculogenic therapy, and disruption of immunoevasion pathways [18, 19]. These strategies can be potentially combined with current anticancer treatments to enhance responsiveness and reduce the possibility of recurrence and dissemination.

Among these strategies, differentiation therapy may be attractable at least in leukemia, since acute promyelocytic leukemia (APL) can be successfully treated with *all-trans* retinoic acid (ATRA), a natural derivative of vitamin A, which unlike other chemotherapies, does not directly kill the malignant cells. ATRA induces the terminal differentiation of the leukemic cells, after which these differentiated leukemic cells undergo spontaneous apoptosis [1, 2, 22]. In addition, it has been demonstrated in experimental models that the quiescent CSCs could be differentiated into more mature tumor cells in various solid tumors, such as human glioblastoma by activation of bone morphogenic protein-signaling pathways [35], medulloblastoma by inhibition of Notch pathway [16], breast cancer by expression of the let-7 miRNA [45] and treatment with salinomycin [21], and epigenetic differentiation therapy [29].

However, it has been reported that 2 or 3 years after ATRA plus chemotherapy-based regimens more than 7 % of patients had relapsed [17, 36]. The relapse might be due to the dormant leukemic stem cells, which may be resistant to differentiation induction. Since CSCs have self-renewal activity, they should be resistant to differentiation induction. Or not, CSC population could not be maintained and tumor would be regressed by differentiation therapy. In the present study, it was studied if leukemia-maintaining cells reside in differentiation-resistant fraction, using a megakaryocytic differentiation model of K562 cells, which was characterized as a multipotential leukemia stem cell line [30].

Materials and Methods

Cell and culture

K562 cell line was obtained from the American Type Culture Collection (Manassas, VA, USA). The imatinib-resistant K562 variants, including K562/R1, K562/R2, and K562/R3 cells, were isolated from K562 cells by culturing in the presence of gradually increasing concentrations of imatinib [28]. The cells were grown in suspension in RPMI 1640 medium (GIBCO Invitrogen cell culture, Grand Island, NY, USA) supplemented with 10% (v/v) heat-inactivated FBS (GIBCO Invitrogen cell culture), 100 units/ml penicillin (Sigma-Aldrich Corp. St. Louis, MO, USA) and 100 mg/ml streptomycin (Sigma-Aldrich Corp.). Cells were maintained at 37°C in a humidified atmosphere containing 5% CO_2 in 95% air and fed with fresh medium every 2 or 3 days. Viable cell counts were performed by trypan blue dye exclusion.

Soft agar colony assay

The soft agar colony assay was performed as described below. Each well of a 96-well culture plate was coated with 50 μl bottom agar mixture in RPMI 1640 medium containing 10% FBS, 0.5% agar (Sigma-Aldrich Corp.). The bottom layer was overlaid with 50 μl top agar mixture in RPMI 1640 medium containing 10% FBS, 0.35% agar containing 100 cells for K562 cells or 1×10^4 cells for K562/R3 cells. After incubation at 37°C, 5% CO_2 for 14 days in a humidified atmosphere, colonies larger than 100 μm in diameter were scored by counting under an inverted microscope (Olympus CKX 41, Tokyo, Japan) equipped with a camera (Olympus DP72, Tokyo, Japan) and image analyzer (Olympus DP2-BSW, Tokyo, Japan) to determine colony size. Sometimes, colonies were stained with 1 mg/ml MTT solution (Sigma-Aldrich Corp.).

Cell proliferation assay

Cell proliferation was analyzed with Cell Counting Kit-8 (Sigma-Aldrich Corp.), according to manufacturer's manual. Briefly, cells (2×10^3 cells/200 μl/well) were seeded in a 96-well plate. After incubation, 10 μl of the CCK-8 solution was added to each well of the plate. After incubation for 1 hour in the 37°C incubator, the absorbance was measured at 450 nm using a PowerWave X340 Microplate Reader (Bio-Tek Instruments, Winooski, VT, USA). A calibration curve was prepared using the data obtained from the wells that contain known numbers of viable cells. All experiments were repeated with at least two experiments in triplicate.

Flow cytometric analysis

The flow cytometric analysis was performed on a FACSCanto II Flow Cytometer (BD Biosciences, San Jose,

CA, USA). Cell suspensions were analyzed after staining with mouse anti-human CD44-FITC (BD Biosciences). At least 10,000 events were acquired and analyzed using FACSDiva software (BD Biosciences).

RT-PCR

Total RNA was isolated using an RNeasy Mini Kit (QIAGEN, Valencia, CA, USA) according to the manufacturer's protocol from K562 and its imatinib-resistant variants and one microgram of extracted total RNA was used to synthesize cDNA. RT-PCR was performed with PTC-100 Peltier Thermal Cycler-100 (MJ Research, INC., Waltham, MA, USA) using with the following primers [28]. Bcr-Abl (forward), 5′-GACATGCCATAGGTAGCAATTTCCC-3′, and (reverse), 5′-ACATCACGC CAGTCAACAGTCTGG-3′; Hsp70 (forward), 5′-TCATCTCTGCATGTAGA AACCGGA-3′, and (reverse), 5′-CGAGGCCGACAAGAAG AAGGTG-3′; Bcl-2 (forward), 5′-CCGCTACCGCCGCGACTTC-3′, and (reverse), 5′-AAACAGAGGCCGCATGCTG-3′; ACTB (forward), 5′-TCCATCCTGGCCT CGCTGTC-3′, and (reverse), 5′ -GCATTTGCGGTGGAC GATGG-3′.

Statistical analysis

For comparison of groups, the unpaired Student t-test was performed. A P value below 0.05 was considered statistically significant in all experiments.

Results

Inhibition of soft agar colony forming efficiency of K562 Cells by PMA-induced differentiation

To determine if leukemia-maintaining cells can form colonies in differentiation-inducing condition, K562 cells were treated with phorbol-12-myristate-13-acetate (PMA, Sigma-Aldrich Corp.), which is known to induce megakaryocytic differentiation of K562 cells through activation of the protein kinase C/extracellular signal-regulated kinase/90-kDa ribosomal S6 kinase/nuclear factor-κB pathway [26, 27]. When

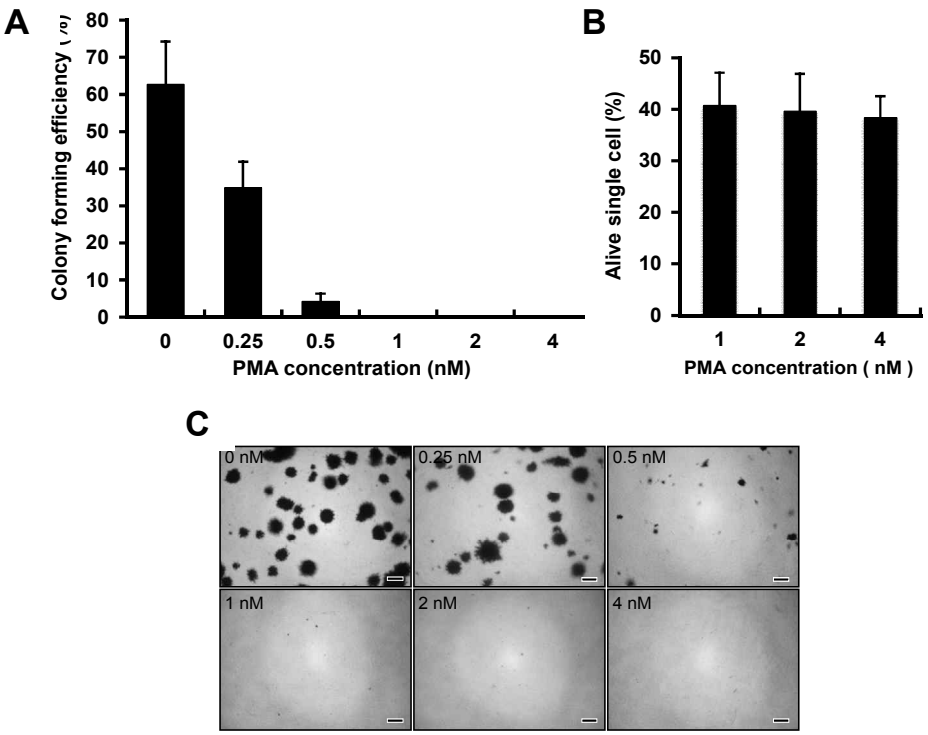

Fig. 1. Inhibition of soft agar colony forming efficiency of K562 cells by treatment with PMA. A) The colony forming efficiency of K562 cells was determined after 2 weeks culture in soft agar in the presence or absence of PMA at various concentrations. The colonies larger than 100 μm in diameter were counted. B) The number of single or double cells, which survived in soft agar in the presence of PMA at high concentrations. The soft agar containing cells was stained with MTT after 2 weeks culture and the stained cells were counted. C) The photographs of MTT-stained colonies in soft agar were taken after 2 weeks culture on inverted microscope at magnification of x40. The length of scale bar is 200 μm. Each bar represents the mean value±SE for 3 independent experiments

K562 cells were treated with PMA at various concentrations for 2 weeks in soft agar, colony forming efficiency and colony size of K562 cells were significantly inhibited and at 1 nM or more concentration of PMA, colony was not formed (Fig. 1 A and C). However, at this high concentration of PMA approximately 40% of K562 cells still survived as single or double cells in soft agar (Fig. 1 B and C). These results demonstrate that induction of differentiation can inhibit colony-forming activity in soft agar, which is highly correlated with tumorigenicity [3, 6].

Recovery of colony forming activity of PMA-treated K562 cells after removal of PMA

Since there were single or double cells surviving in dormant state after exposure to PMA, it was examined if the PMA-treated dormant K562 cells would be able to regrow and form colony after removal of PMA. After exposure of K562 cells to various concentrations of PMA for various peri-

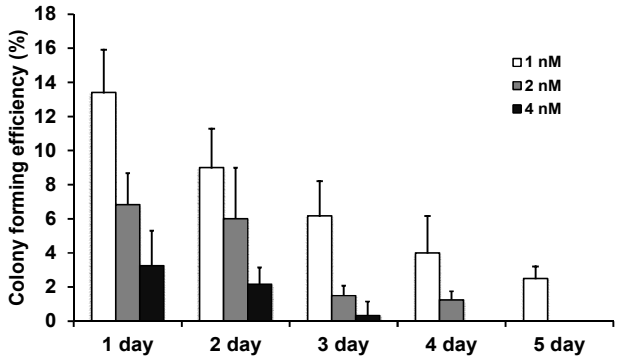

Fig. 2. Recovery of the colony forming activity of PMA-treated K562 cells after removal of PMA. The K562 cells were cultured in the presence of high concentrations of PMA (1 nM, 2 nM, and 4 nM) in soft agar and after the indicated periods PMA was washed out with complete medium 12 times every one hour. After washing out of PMA the cells were cultured further for additional 2 weeks for colony observation. The colonies larger than 100 μm in diameter were counted. Each bar represents the mean value±SE for 3 independent experiments.

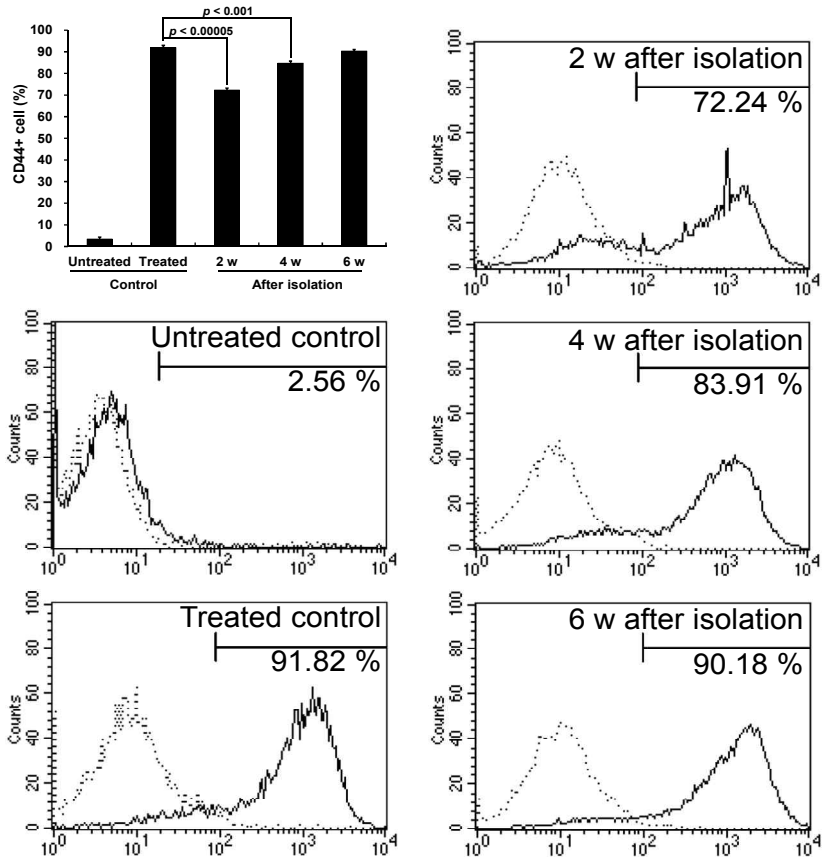

Fig. 3. Resistance of colony forming cells in the presence of PMA to PMA-induced differentiation. The colonies formed by removal of PMA after exposure to 4 nM PMA for 3 days were isolated and expanded for the indicated times. After that, the cells were treated with 10 nM PMA for 3 days and determined with flow cytometer for the expression of CD44 surface marker of PMA-induced differentiation. Each bar represents the mean value±SD for three independent experiments. A representative flow cytometric analysis of three independent experiments was shown. Dot line indicated the isotype control.

ods, PMA was washed-out and colony formation was observed with MTT staining after 2 weeks (Fig. 2). When K562 cells were treated with 1 nM PMA, the colony forming activity could be recovered by removal of PMA after 5 days. At concentrations of 2 and 4 nM PMA, the colony forming activity could not be recovered by removal of PMA after treatment with PMA for 5 and 4 days, respectively. These results suggest that the single or double cells survived after exposure to PMA may be leukemia-maintaining cells, and the colony forming activity of leukemia-maintaining cells could be suppressed by induction of differentiation for long time with high concentration of PMA.

Resistance of colony forming cells in the presence of PMA to PMA-induced differentiation

If the cells, which can form colonies after exposure to PMA, are leukemia-maintaining cells, these cells should maintain the resistance to differentiation induction. Therefore, colonies formed by removal of PMA after exposure to 4 nM PMA for 3 days were isolated and it was determined if the colony forming cells were resistant to PMA-induced differentiation (Fig. 3). When PMA was not treated, the colony forming cells showed an undifferentiated phenotype, as untreated K562 control (data not shown). When K562 cells were treated with 10 nM PMA for 3 days, more than 90% cells were CD44-positive, which is a PMA-induced megakaryocytic differentiation marker [26, 32, 40]. However, approximately 70% of the colony forming cells was positive for CD44 after treatment with 10 nM PMA for 3 days at 2 weeks after colony isolation. This differentiation rate was gradually increased and reached about 90% comparable to the level of the PMA-treated K562 control cells at 6 weeks after colony isolation. This result indicated that K562 cells have leukemia-maintaining cells, which are resistant to PMA-induced differentiation and can recover the original phenotype.

Association of colony forming activity and Bcr-Abl expression in imatinib-resistant K562 variants

Since it was difficult to isolate and expand the leukemia-maintaining cells from K562 cells after treatment with PMA, we tried to identify the leukemia-maintaining cells from the imatinib-resistant K562 variants including K562/R1, K562/R2, and K562/R3 cells, which were isolated from K562 cells in the presence of imatinib [28]. Since it is well known that leukemic stem cells in CML are insensitive

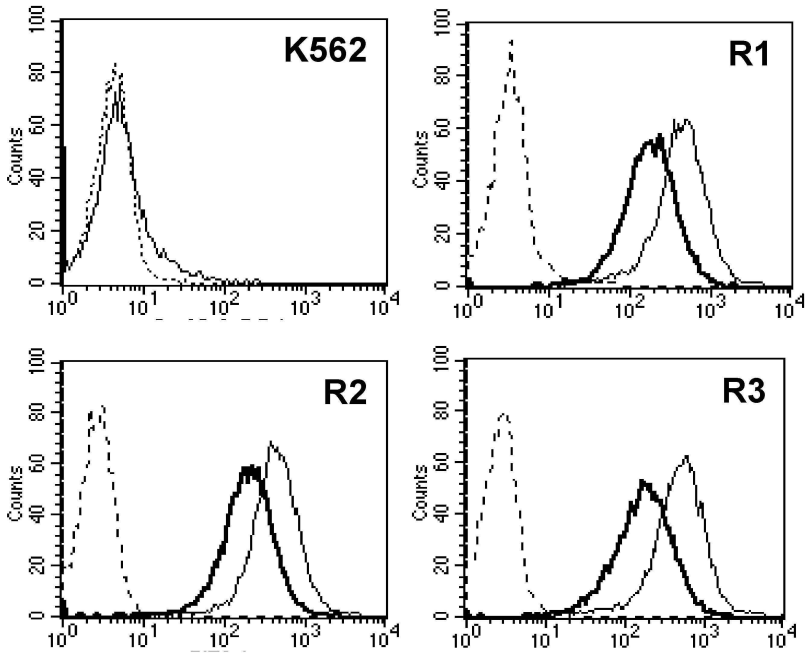

Fig. 4. Change in expression of CD44 surface marker in imatinib-resistant K562 variants during long-term drug free culture. After 4 month culture in drug-free medium, the surface expression level of CD44 was compared between the primary imatinib-resistant variants, including K562/R1, K562/R2, and K562/R3 cells (thin lines) and the secondary imatinib-resistant variants cultured in drug free condition, which were renamed as K562/R1′, K562/R2′, and K562/R3′ cells, respectively (thick lines). Dot line indicated the isotype control.

to imatinib treatment [5, 20], and CML stem cells are quiescent and undifferentiated, resulting in resistance to chemotherapy [33], the imatinib-resistant K562 variants may have more leukemia-maintaining cells and consequently can form more colonies, compared with their parental K562 cells. Unexpectedly, the imatinib-resistant K562 variants did not show any colony forming activity in soft agar (data not shown), and were CD44-positive (Fig. 4. thin line), explaining why the imatinib-resistant variants did not have a colony forming activity. After 4 month-culture in drug-free medium, the surface expression level of CD44 was decreased in comparison with the primary imatinib-resistant variants, including K562/R1, K562/R2, and K562/R3 cells (Fig. 4. thick line), although almost all cells of imatinib-resistant variants remained still as CD44-positive. The secondary imatinib-resistant variants cultured in drug-free condition were renamed as K562/R1′, K562/R2′, and K562/R3′ cells, respectively.

Since the expression level of CD44 was decreased in the secondary imatinib-resistant variants, it was determined if these cells could form colonies in soft agar. A few tiny colonies were observed in K562/R3′ cells (Fig. 5A). Since it has been shown that Bcr-Abl and Hsp70 were decreased and Bcl-2 was increased remarkably in the primary imatinib-resistant variants [28], there was a possibility that recovery of expression of the molecules might be associated with this reversion of clonogenicity, albeit very low. Therefore, the tiny colonies were picked up and cultured in drug-free medium (named as K562/R3″ cells), and RT-PCR was performed to determine the level of Bcr-Abl, Hsp70 and Bcl-2. Bcr-Abl was barely detected in K562/R3 and K562/R3′ cells, but reappeared in K562/R3″ cells (Fig. 5B). In addition, the decreased Hsp70 and the increased Bcl-2 in K562/R3 cells were being increased and decreased in K562/R3″ cells, respectively. These results were followed by an increased clonogenicity of K562/R3″ cells, compared with K562/R3′ and K562/R3 cells (Fig. 5C).

Discussion

Currently cancer research is being focused on the molecular and cellular analysis of the cancer stem cells, and subsequent development of new therapeutic modalities targeting CSCs. It has been demonstrated that several properties of CSCs make them difficult to eradicate, for example, the activity of multiple drug resistance transporters

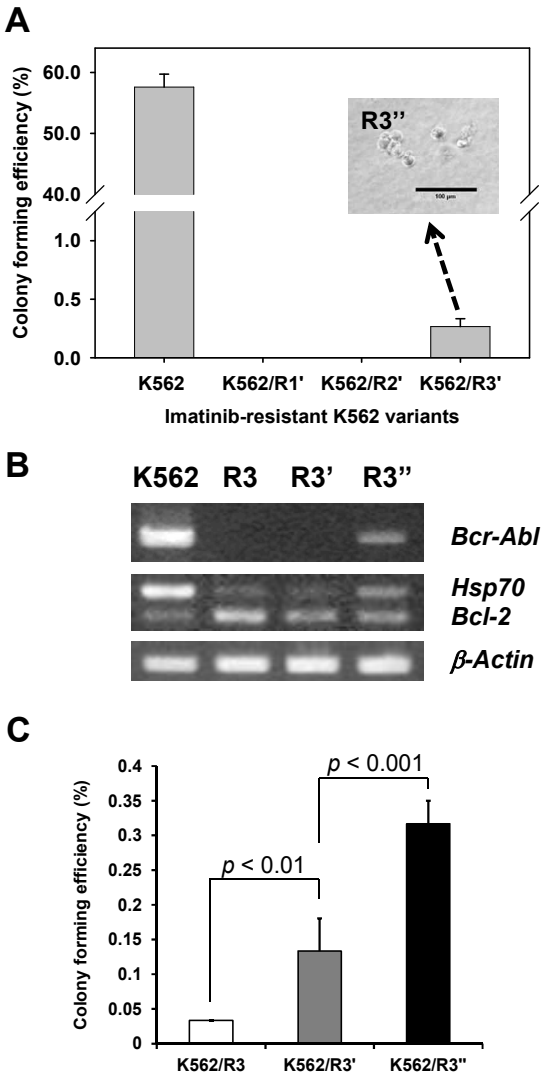

Fig. 5. Slow recovery of colony forming efficiency and molecular changes in the imatinib-resistant K562 variants after long-term drug free culture. A) Clonogenicity of the secondary imatinib-resistant variants cultured in drug-free condition, renamed as K562/R1′, K562/R2′, and K562/R3′ cells, respectively, was determined in soft agar as described in the 'Materials and Methods' section. Each bar represents the means of triplicates±SD. For K562/R1′, K562/R2′, and K562/R3′ cells, colonies larger than 50 μm in diameter were counted. (Inset: a photograph of a small colony formed from K562/R3′ cells, from which K562/R3″ cells were expanded.) B) The mRNA levels of Bcr-Abl, Hsp70 and Bcl-2, which were modulated in the imatinib-resistant K562/R3 variants, were analyzed with RT-PCR in K562/R3′ and K562/R3″ cells. C) Clonogenicity of K562/R3, K562/R3′, and K562/R3″ cells was determined in soft agar as described in the 'Materials and Methods' section. Each bar represents the mean value±SE for 3 independent experiments. The colonies larger than 50 μm in diameter were counted.

[14], overexpression of antiapoptotic proteins [42], great abilities for DNA repair [4, 15, 39], and cell cycle restriction [43]. Since elimination of CSCs is not easy due to these properties, differentiation induction of cancer stem cells will be considered as one way to avoid these CSC problems. In this context, differentiation-inducing drugs such as BMPs (bone morphogenetic proteins), and histone deacetylase inhibitors as well as ATRA, have drawn growing attention [7, 31, 35].

It had been known that K562 cells have the capacity to express characteristics of erythrocytic, monocytic, and megakaryocytic differentiation when exposed to various agents, and can be induced to differentiate into cells with monocytic and/or megakaryoblastic characteristics by PMA [41]. This differentiation process is characterized by changes in cell morphology, adhesiveness, and expression of megakaryocytic markers as well as cell growth arrest [24, 38]. In the present study, when K562 cells were treated with PMA at high concentration for long time, the colony forming activity of K562 cells could be completely suppressed, indicating that the clonogenicity, which is highly correlated with tumorigenicity [3, 6], would be suppressed by inducing differentiation in chronic myelogenous leukemic cells.

It was demonstrated that BMP4 could reduce the tumor-initiating cell pool of glioblastoma multiformes (GBM) by triggering the Smad signalling cascade followed by a reduction in proliferation, and increased expression of markers of neural differentiation, with no effect on cell viability, and the concomitant reduction in clonogenic ability, in the size of the CD133+ population and in the growth kinetics of GBM cells [35]. Although it has been shown that retinoids also induce astrocytic differentiation with down regulation of telomerase activity and enhance sensitivity to taxol for apoptosis in glioblastoma cells [10], the effectiveness of ATRA has been studied in stem-like glioma cells (SLGCs) for the first time, demonstrating that ATRA-based differentiation can target the AC133/CD133-positive SLGCs population, inducing long-term antiangiogenic, antimigratory, antitumorigenic, proapoptotic, and therapy-sensitizing effects [8]. Therefore, these results highlighted the potential of differentiation therapy to target the stem-like cell population in cancers including leukemias as well as glioblastoma.

However, when PMA was removed after inducing megakaryocytic differentiation of K562 cells, some clonogenic cells were reappeared, more easily with lower doses and shorter duration of PMA treatment. ATRA is able to induce complete remission in about 90% of newly diagnosed patients with acute promyelocytic leukemia through *in vivo* differentiation of APL blasts. However, it cannot eliminate completely the leukemic clone and to be more effective must be used in combination with anthracycline-based chemotherapy [12, 34]. The reappearance of clonogenic cells may be probably due to resistance of leukemic stem cells to differentiation. To reduce the incidence of relapse of APL, maintenance treatment with ATRA, and possibly in combination with low-dose chemotherapy is required [12]. Since it was demonstrated in the present study that the colony forming activity of K562 cells could be completely suppressed by treatment for long time with high concentration of PMA, long-term maintenance therapy with high dose of differentiation inducer would be required to prevent recurrence of cancers.

Previously, we reported that three imatinib-resistant K562/R1, R2 and R3 variants showed a gradual loss of Bcr-Abl at mRNA and protein levels, consequently unresponsiveness to imatinib [28]. Since it has been demonstrated that the primitive, quiescent, Philadelphia-positive stem cells from patients with chronic myeloid leukemia are insensitive to STI571 *in vitro* [20] and *in vivo* [5], and that human CML stem cells do not depend on BCR-ABL activity for survival and are thus not eliminated by imatinib therapy [9], it was expected that leukemic stem cells with self-renewal capacity might be enriched in imatinib-resistant K562/R1, R2 and R3 variants and consequently colony forming efficiency would be higher, compared with their parental K562 cells. However, they did not have any colony forming activity in soft agar and were CD44-positive, explaining the absence of colony forming activity in the imatinib-resistant variants. We could isolate some tiny colonies from K562/R3′ cells after culture in drug-free medium for more than 4 months. This result suggests that the imatinib-resistant K562 variants contain the dormant leukemia-maintaining cells, although rare.

In response to therapy, cancer cells may reduce their dependence on a particular hallmark capability, becoming more dependent on another and consequently obtain a quite different form of acquired drug resistance [23]. The imatinib-resistant variants of K562 showed several changes in levels of apoptosis-modulating molecules such as an increase in antiapoptotic Bcl-2 and Ku70 levels, and a decrease in proapoptotic Bax and Hsp70, which might be involved in the compensation for Bcr-Abl loss during the obtaining of

imatinib resistance [28]. After isolation of clonogenic cells from K562/R3 variant, the level of CD44 was being decreased and Bcr-Abl was reappeared in K562/R3″ cells, compared with primary imatinib-resistant variants. In addition, the decreased Hsp70 and the increased Bcl-2 also began to return slowly to the original level of K562 cells. Therefore, these results suggest that megakaryocytic differentiation and loss of Bcr-Abl of K562 cells induced by long-term culture in the presence of imatinib could be responsible for loss of clonogenicity of imatinib-resistant K562 variants, and the reappearance of original phenotypes of K562 cells during culture in imatinib-free medium may confer the clonogenicity to K562/R3″ cells, again.

Whereas a number of differentiating agents have been studied over the past decades, only ATRA is being used to treat APL by causing immature blood cells to differentiate, and the capability of drugs to induce differentiation in CSCs has not yet been seriously considered [37]. From the present study including two models of differentiation of K562 cells, it could be suggested that differentiation therapy may be effective to eliminate cancer maintaining cells, when differentiating agents would be able to be used to get rid of cancer maintaining cells at high dose and for long time without side effects, albeit differentiation-resistant population might be cancer maintaining/initiating/stemcells.

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초록 : 만성 골수성 백혈병 K562세포의 분화 내성 분획에서 백혈병 유지 세포의 동정

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본 연구에서는 K562 만성 골수성 백혈병 세포를 이용하여, 분화 유도에 의해 암 유지/개시 세포의 자기 재생 능력이 소실되는 지를 조사하였다. K562 세포의 집락(colony) 형성 능력은 PMA 처리에 의하여 현저히 억제되었고, 1 nM 이상의 PMA 처리시에는 집락이 형성되지 않았으나, 약 40%의 세포는 여전히 연한천(soft agar)에서 살아 있었다. PMA 4 nM을 3일간 처리하고 제거한 후 분리한 집락 형성 세포에 다시 10 nM PMA를 3일간 처리하였을 때, 약 70% 정도의 세포가 분화되었고, 6주 후에 PMA를 처리하였을 때는 분화율이 약 90%로 K562 모세포에 PMA를 처리한 수준에 도달하였다. 한편, imatinib-내성 K562 변종 세포들은 연한천에서 집락을 형성하지 않았으며, 대부분의 세포가 CD44 양성이었다. Imatinib 무첨가 배지에서 4개월 배양 후, 이 세포들의 표면 CD44 발현량은 감소하였고, K562/R3 imatinib-내성 변종 세포에서는 연한천에서 작은 집락이 형성되었다. 이 세포에서는 imatinib-내성 변종 세포에서 소실되었던 Bcr-Abl이 다시 발현되기 시작하였고, 다른 표현형들도 부분적으로 회복되었다. 이러한 결과는 백혈병 유지 세포가 분화에 내성을 나타내는 세포이며, 분화 유도제를 오랜 기간 동안 고농도로 처리할 수 있다면 백혈병 줄기 세포를 제거하기 위한 분화 요법이 백혈병 치료에 적용될 수 있음을 시사하였다.