

## The modulation of radiation-induced cell death by genistein in K562 cells: Activation of thymidine kinase 1

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### ABSTRACT

Ionizing radiation is one of the most effective tools in cancer therapy. In a previous study, we reported that protein tyrosine kinase (PTK) inhibitors modulate the radiation responses in the human chronic myelogenous leukemia (CML) cell line K562. The receptor tyrosine kinase inhibitor, genistein, delayed radiation-induced cell death, while non-receptor tyrosine kinase inhibitor, herbimycin A (HMA) enhances radiation-induced apoptosis. In this study, we focused on the modulation of radiation-induced cell death by genistein and performed PCR-select suppression subtractive hybridization (SSH) to understand its molecular mechanism. We identified human thymidine kinase 1 (TK1), which is cell cycle regulatory gene and confirmed expression of TK1 mRNA by Northern blot analysis. Expression of TK1 mRNA and TK 1 enzymatic activity were parallel in their increase and decrease. TK1 is involved in G1-S phase transition of cell cycle progression. In cell cycle analysis, we showed that radiation induced G2 arrest in K562 cells but it was not able to sustain. However, the addition of genistein to irradiated cells sustained a prolonged G2 arrest up to 120 h. In addition, the expression of cell cycle-related proteins, cyclin A and cyclin B1, provided the evidences of G1/S progression and G2-arrest, and their relationship with TK1 in cells treated with radiation and genistein. These results suggest that the activation of TK1 may be critical to modulate the radiation-induced cell death and cell cycle progression in irradiated K562 cells.

**Keywords:** K562, radiation, cell death, genistein, thymidine kinase 1, cell cycle.

### INTRODUCTION

The signaling pathway, which is triggered by cancer therapy, includes both apoptotic and survival pathways and the efficacy of cancer therapy results from the balance between both pathways [1]. In radiotherapy of cancer treatment, especially, it sometimes causes a problem because of the property of resistance to radiation in several types of cancer. To overcome this limitation, two main strategies have been employed. One is to minimize radiation-induced damage of normal tissue to radiation in physical studies and the other is to analyze the cancer-related genes using molecular biological techniques and then to apply it to actual radiotherapy.

Chronic myelogenous leukemia (CML) is a myelopro-

liferative disorder resulting from the clonal expansion of transformed hematopoietic stem cells. CML is caused by the translocation of chromosomes (9:22), resulting constitutively activated BCR-ABL tyrosine kinase, which may cause resistance to radiotherapy as well as chemotherapy [2-3]. Although several groups reported that several drugs, such as pentoxifyllin or caffeine, can regulate the CML response to radiation [4-5], the mechanism of radioresistance of CML is not clearly understood.

In a previous study, we demonstrated the modulation of radiation-induced cell death with PTK inhibitors, herbimycin A (HMA) or genistein [6]. Cell cycle analysis showed that K562 cells lack a G1 phase checkpoint after X-irradiation, and the vast majority of cells appear to arrest at G2/M phase. However, p53-deficient K562 cell line is unable to sustain cell cycle arrest after irradiation. Interestingly, the addition of genistein to irradiated cells maintains a prolonged G2 arrest, which correlates with normal radiation-induced cell death of K562 cells.

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**Tab. 1** Nucleotide sequences used for this study

Name	Nucleotide sequences
For suppression subtractive hybridization	
cDNA synthesis primer	5'-TTTTGTACAAGCTT <sub>30</sub> N <sub>1</sub> N-3'
Adaptor 1	5'-AATACGACTCACTATAGGGCTCGAGCGGCCGCCGGGCAGGT-3' 3'-GGCCCGTCCA-5'
Adaptor 2R	5'-CTAATACGACTCACTATAGGGCCAGCGTGGTCGCGGCCGAGGT-3' 3'-GCCGGTCCA-5'
PCR primer 1	5'-CTAATACGACTCCACTATAGGGC-3'
Nested PCR primer 1	5'-TCGAGCGGCCGCCGGGCAGGT-3'
Nested PCR primer 2R	5'-AGCGTGGTCGCGGCCGAGGT-3'

Thymidine kinase 1 (TK 1) is an important enzyme that controls cell cycle progression and is highly expressed in leukemia cell lines. The amount of cytosolic TK1 increased significantly in cells during transition from G1 to S phase [7]. In tumor cells, the expression of TK1 is associated with cell growth control and TK1 get to be phosphorylated when cells are in mitosis state [8-11]. Interestingly, TK1 expression is related to regulation of radiation response in the acute lymphatic leukemia (ALL) HL60 cell line [12]. Recent papers reported the differences in the changes of TK1 and 2 phosphorylation after irradiation among spleen, thymus and ascites tumors [13-14]. Although TK1 is regulated at many levels, it is still unclear how TK1 regulates cell responses to radiation in radioresistant CML cells.

In this study, we performed PCR-select suppression subtractive hybridization (SSH) to find differentially expressed genes involved in the modulation of radiation-induced cell death in K562 cell line. The result showed that human TK1 is highly expressed when cells are treated with radiation and genistein compared to irradiated alone or treated with radiation and HMA. This study suggests that TK 1 may play an important role to modulate radiation-induced cell death and cell cycle progression in K562 cells.

## MATERIALS AND METHODS

### Cell culture and drug treatments

K562 (ATCC CCL 243) was obtained from the American Type Culture Collection and cultured in RPMI 1640 (Gibco BRL, Rockville, MD) supplemented with 10% fetal bovine serum (FBS) (Hyclone, Logan, UT) and penicillin (100 units/ml)/streptomycin (100 µg/ml) (Gibco BRL, Rockville, MD). Cell cultures were kept in a humidified incubator with 5% CO<sub>2</sub> at 37°C. For X-ray irradiation and drug treatments, cells were prepared at approximately 3.5×10<sup>5</sup> cells/ml. Cells were irradiated by using a 6-MV X-ray machine (Clinac 1800C, Varian). Genistein or herbimycin A (HMA) (Calbiochem, San Diego, CA) prepared in dimethyl sulfoxide (DMSO) (Sigma, St. Louis, MO)

as 10 mM or 1 mM of stock solution, respectively. Cells were treated with genistein or HMA at 25 µM or 250 nM of final concentration (IC<sub>50</sub>), respectively.

### Total RNA and mRNA isolation

Cells were harvested and washed with phosphate buffered saline (PBS). Total RNA was isolated using UltraspecII RNA isolation system (Biotech Laboratories, Houston, TX) according to the manufacturer's instructions. mRNA was isolated from total RNA using PolyTract mRNA isolation system (Promega, Madison, WI). Briefly, 500 µg of total RNA and biotin-labelled oligo(dT)<sub>20</sub> probe were hybridized, mixed with streptavidin magnetic particles and incubated at 37°C for 30 min. Then magnetic particles were separated from the fluid and washed three times with washing buffer (10 mM Tris-Cl, 0.2 M LiCl, 1 mM EDTA, pH 7.5). mRNA was eluted by resuspending the magnetic particles in water, followed by incubation at 65°C for 2 min. The supernatant containing the mRNA was transferred to fresh tube.

### Polymerase chain reaction (PCR)-select suppression subtractive hybridization (SSH)

Suppression subtractive hybridization was performed using the PCR-select cDNA Subtraction Kit (Clontech Laboratories, Palo Alto, CA). cDNA from cells treated with radiation alone was used as driver and cDNA from cells treated with radiation and genistein was used as tester. Briefly, driver and tester cDNAs were synthesized from 2 µg of mRNA, purified by phenol/chloroform extraction, and digested with *Rsa*I at 37°C for 1.5 h. Tester cDNA was ligated with adaptor1 and adaptor2R using T4 DNA ligase and incubated at 16°C overnight. Then samples were heated at 70°C for 5 min to inactivate the ligase. The adaptor1-ligated tester cDNA and adaptor2R-ligated tester cDNA were separately hybridized with excessive driver cDNA at 68°C for 8 h. Then, 1.5 µg of fresh denatured driver cDNA in hybridization buffer was added in both samples at 68°C overnight. The reaction mixture was prepared for PCR and consisted of 2.5 units *Taq* DNA polymerase (Promega, Madison, WI), 10 mM dNTP, 10 µM PCR primer, 4 mM MgCl<sub>2</sub> in a final volume 25 µl. The first round of PCR was based on suppression PCR to amplify only cDNA with different adaptors at both ends, which were further enriched by

the second round of PCR amplification with nested PCR primers. Both PCRs were carried out in a Perkin-Elmer 2400 PCR machine and the products were analyzed by 2% agarose gel electrophoresis. Secondary PCR products were transformed using pGEM-T easy vector system (Promega, Madison, WI). Tab. 1 displays the nucleotide sequences used for this study.

### DNA isolation and DNA sequencing

We used the PCR-select Differential Screening Kit (Clontech laboratories, Palo Alto, CA) to compare differentially expressed clone in driver and tester cDNA. Isolation and purification of plasmid DNAs were performed using Wizard Plus SV Minipreps DNA purification system (Promega, Madison, WI) and purified by using the Qiaex II Gel Extraction Kit (Qiagen, Valencia, CA). DNAs were sequenced with the ALPexpress Auto Cycle Sequencing kit (Amersham Pharmacia Biosciences, Piscataway, NJ). Homologous sequences were compared in the EMBL and GenBank databases. The above three procedures were performed according to the manufacturer's instructions.

### Northern hybridization and RT-PCR

Northern hybridization was performed with [ $\alpha$ - $^{32}$ P] ATP-labelled cDNA probe. 30  $\mu$ g of total RNA was fractionated on a 1.5% formaldehyde-agarose gel and blotted in 10 $\times$ SSC overnight onto Hybond N-plus membrane (Amersham Pharmacia Biosciences, Piscataway, NJ). The filter was cross-linked using the Spectrolinker XL-1000 UV cross-linker (Spectronics corp., Westbury, NY) and was hybridized in hybridization solution containing 50% deionized formamide, 125 mM sodium phosphate, pH 7.2, 7% SDS, and 250 mM NaCl, at 42°C overnight followed by two washes (10 min each) at 42°C in 2 $\times$ SSC/0.1% SDS. The probe was labeled with Rediprime Random Primer Labeling Kit (Amersham Pharmacia Biosciences, Piscataway, NJ) according to the manufacturer's instructions. RT-PCR was performed using the AccuPower RT-PCR Premix (Bioneer, Daejeon, Korea) as described by the manufacturer. Primer sequences are as follows: GAPDH forward 5'-CCTCCCGCTTCGCTCTCTGC; reverse 5'-GGGTGGCAGTGATGGCATGG; TK1 forward 5'-AGACACTCGCTACAGCAGCA; reverse 5'-AATGGCTTCCTCTGG-AAGGT.

### In vitro thymidine kinase assay

Cells were lysed by sonication with ice-cold lysis buffer consisting of 50 mM Tris-HCl, pH 7.9, 50 mM NaF, and 1 mM  $\beta$ -mercaptoethanol. The cytoplasmic extracts were obtained by centrifugation at 10,000 g for 10 min. 30 mg of cytoplasmic extract was incubated for 30 min at 37°C in a final volume of 100  $\mu$ l in a reaction mixture containing 50 mM Tris-HCl, pH 7.9, 3 mM  $\beta$ -mercaptoethanol, 2.5 mM MgCl<sub>2</sub>, 5 mM ATP, 0.1% bovine serum albumin, 90  $\mu$ M thymidine, 5 mM NaF, and 2  $\mu$ Ci [ $^3$ H]-thymidine (25 Ci/mmol, Amersham Pharmacia Biosciences, Piscataway, NJ). Each sample was spotted onto p81 phosphocellulose disc filters (Gibco BRL, Rockville, MD), washed extensively with 4 mM ammonium formate, and incorporated [ $^3$ H] thymidine was determined by Beckman LS 5801 Liquid Scintillation System.

### Trypan blue dye exclusion assay

Cells were stained with a 0.4% trypan blue (Sigma, St. Louis, MO) solution for 5 min. The number of cells that took up trypan

blue was expressed as a percentage of the total cell number.

### Hoechst 33258 staining

Cytospin preparations of cells were stained with a 4  $\mu$ g/ml Hoechst 33258 (2'-[4-ethoxyphenyl]-5-[4-methyl-1-piperazinyl]-2,5'-bi-1H-benzimidazole) (Sigma, St. Louis, MO) solution at 37°C for 30 min. At least 300 cells were counted under differential interference contrast (DIC) optics, and the cells with nuclear fragmentation were counted under an epifluorescence microscope.

### Cell cycle analysis

Flow cytometric analysis was performed to analyze cell cycle progression. At each time, cells were harvested, and ice-cold 95% ethanol with 0.5% Tween 20 was added to the cell suspensions to a final concentration of 70% ethanol. Fixed cells were pelleted, washed with 1% BSA-PBS, resuspended in 1 ml PBS containing 11 Kunitz U/ml RNase, incubated at 4°C for 30 min, and sequentially resuspended in 50  $\mu$ g/ml of propidium iodide (Sigma, St. Louis, MO) solution. The content of DNA per cell was estimated by an Epics XL flow cytometry system (Becton Dickinson, Fullerton, CA) and analyzed by Modifit software.

### Western blot analysis

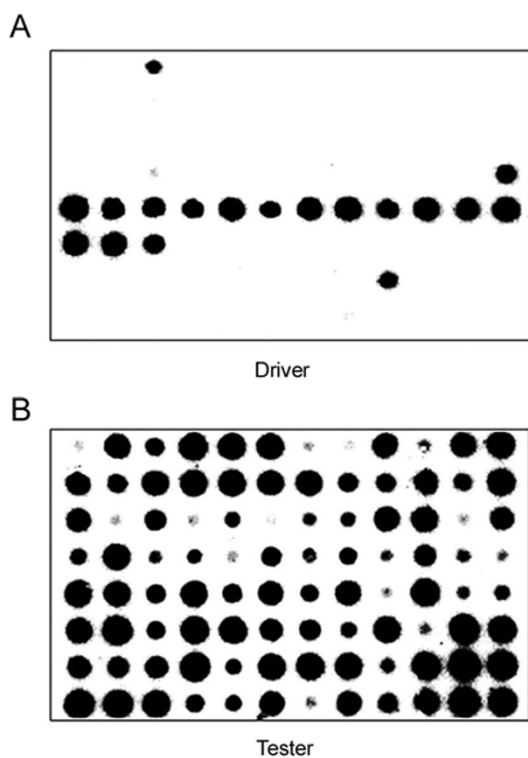
Cells were lysed by EBC buffer (120 mM NaCl, 40 mM Tris, pH 8.0, and 0.5% Nonidet P-40) on ice and the cell lysates were clarified by centrifugation. Cell lysates of each sample were subjected on 10% tris-glycine gel, transferred onto PVDF membrane and immunoblotted with appropriated antibodies; anti-CDK2 and Cdc2 (Santa Cruz Biotechnologies, Santa Cruz, CA). The blots were developed using Enhanced Chemiluminescent (ECL) system (Amersham Pharmacia Biosciences, Piscataway, NJ) and detected by LAS-1000 PLUS (Fujifilm, Japan).

## RESULTS

### PCR-select subtractive hybridization (SSH) and mRNA expression of selected clone RG4-37

In a previous study, we have shown that PTK inhibitors, genistein and HMA, modulates radiation-induced cell death in K562 cells. The receptor tyrosine kinase inhibitor, genistein, delayed radiation-induced cell death, while non-receptor tyrosine kinase inhibitor, HMA, shortened the induction time of apoptosis [6]. Thus, to identify differentially expressed genes that modulate radiation-induced cell death in CML cell line K562, we performed SSH in the cells treated with radiation alone and the cells treated with radiation and genistein.

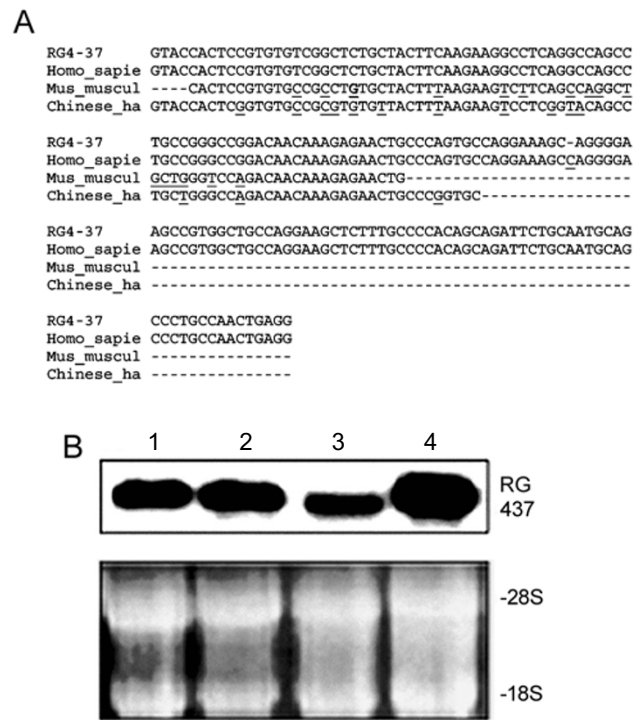
Two steps of hybridization and PCR were carried out to compare driver, which is the reference cDNA, and tester, cDNA in which is specifically transcribed. We set the cells treated with radiation alone as driver and the cells treated with radiation and genistein as tester. Final PCR products were subcloned into TA-vector and all displayed clones were applied to dot hybridization using tester cDNA as probe. The 124 dots showed the differential expression



**Fig. 1** A representative blot showing differential display by dot blot analysis of subtractive cDNA clones. Two identified membranes were hybridized with unsubtracted driver cDNA probes (irradiated cells) (A) and subtracted tester cDNA probes (cells exposed to combined treatment of radiation and genistein) (B).

between driver and tester and a representative result of dot hybridization is shown in Fig. 1. DNAs of these clones were sequenced with either ARFred M13-40 primer or ALFred MB reverse primer. A computer search against GenBank and EMBL DNA databases revealed that clone RG4-37 was identical to human TK1 (Fig. 2A). The clone RG4-37 was also highly homologous with mouse and Chinese hamster TK. The percentages of sequence homology are 99% with human TK1, 85% with mouse TK, and 83% with Chinese hamster TK.

To confirm the differential expression of the identified clone RG4-37, we analyzed TK1 mRNA expression by Northern hybridization (Fig. 2B). We compared the expression between driver and tester and used cells treated with radiation and HMA as a control. Cells treated with radiation and genistein (tester) displayed a significant increase of mRNA expression (lane 4). However, irradiation alone (driver) had little effect on TK1 mRNA expression and the treatment with radiation and HMA (control) caused

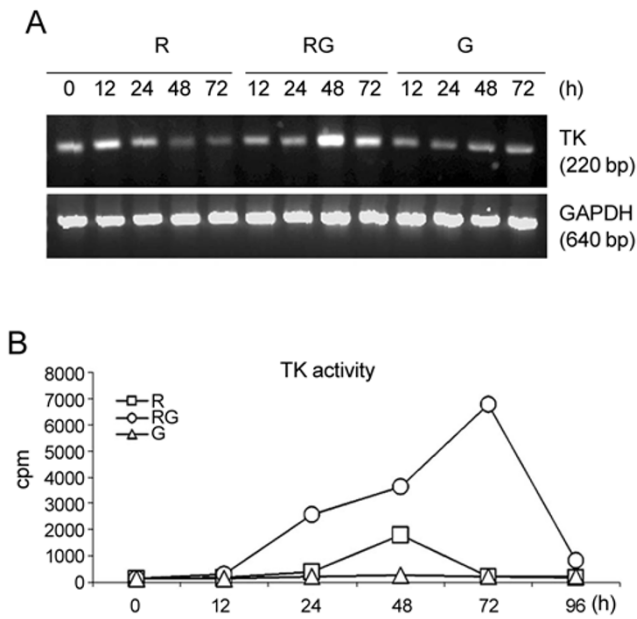


**Fig. 2** Identification of human thymidine kinase (TK) 1 in K562 cells treated with radiation and genistein. (A) Partial sequence alignment of clone RG4-37. Nucleotide sequences of clone RG4-37 are compared with the TK1 of human (NM\_003258), Chinese hamster (L00369 M 13622), and mouse (XM\_125105). Unmatched bases with clone RG4-37 were underlined. (B) mRNA expression of clone RG4-37 by Northern hybridization. Cells were untreated (lane 1), irradiated alone (lane 2), treated with radiation and 250 nM HMA (lane 3), or with radiation and 25 μM genistein (lane 4) for 24 h.

a decreased expression of TK1 mRNA (lane 2 and 3). From these results, it was predicted that genistein efficiently induced TK1 expression in irradiated K562 cells.

### TK1 activity is regulated at the transcriptional level in K562 cells treated with radiation and genistein

To insure that the increasing mRNA expression of TK1 in cells treated with radiation and genistein was not due to genistein itself, RT-PCR was performed. Cells were incubated from 0 to 72 h with stimulation and their RNAs were isolated. In cells treated with radiation and genistein, TK1 mRNA expression revealed a time-dependent increase which peaked at 48 h. In contrast, cells treated with radiation alone were induced a time-dependent decrease and genistein-treated cells had no significant change. The expression of house keeping gene, GAPDH, was constant (Fig. 3A). This result provides another evidence that, in K562 cells, the combined treatment with radiation and genistein efficiently induced the expression of TK1 but

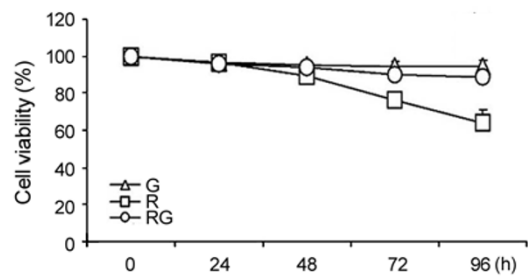


**Fig. 3** The expression of mRNA and enzymatic activity of TK1 in K562 cells. (A) RT-PCR was performed with cells treated with irradiation alone (R), genistein alone (G), or both radiation and genistein (RG) during indicated time courses. (B) The kinase activity was determined by [<sup>3</sup>H]-thymidine nucleotide formation by *in vitro* kinase assay. Cells were irradiated alone (□), treated with genistein alone (△), or treated with radiation and genistein (○). Three independent assays were performed and representative data are shown.

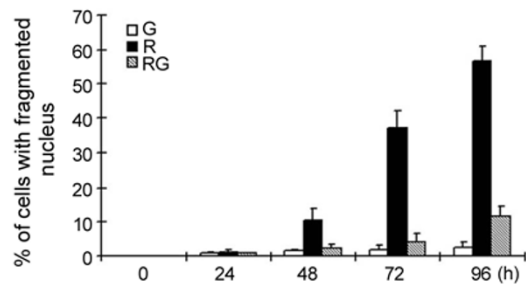
genistein alone was not sufficient. It has been demonstrated that the TK1 enzymatic activity was mainly controlled at the transcription level [15-16]. To test whether the expression of TK1 mRNA correlates with TK1 activity, we performed an *in vitro* kinase assay. The kinase activity was determined by [<sup>3</sup>H]-thymidine nucleotide formation. As expected, cells treated with radiation and genistein significantly increased TK1 activity in a time-dependent manner up to 72 h and then decreased after 96 h (Fig. 3B). However, cell treated with radiation showed a small and transient increase of TK1 activity at 48 h and cells treated with genistein alone were unaffected. Of interest, the increase and decrease of TK1 enzymatic activity was almost paralleled with that of TK1 mRNA expression in cells treated with radiation and genistein, suggesting that TK1 activity was controlled at the transcription level.

**The role of TK1 in radiation-induced cell death and cell cycle regulation**

We have previously reported the inhibitory effect of irradiation (2 to 10 Gy) and genistein (5 to 100 μM) on the *in vitro* growth of K562 cells [6]. Cell growth was delayed

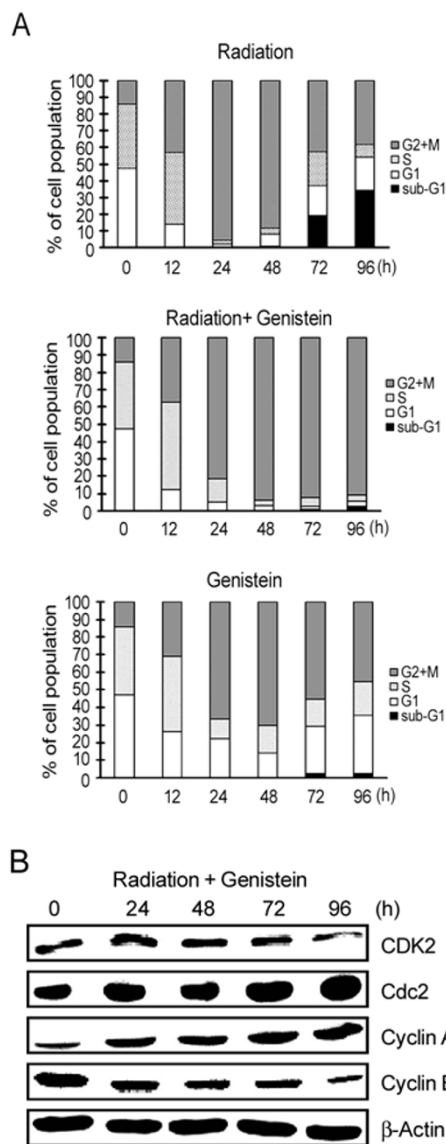


**Fig. 4** Viability of K562 cells as determined by trypan blue dye exclusion. Cells treated with radiation alone (□), genistein alone (△), or radiation and genistein (○) and then incubated for the indicated time points. Cells were stained with trypan blue and then counted using a hemocytometer. Three independent assays were performed and data shown are the means ± SEM obtained from the triplicates experiment.



**Fig. 5** Quantitation of K562 cells with nuclear fragmentation as determined by Hoechst 33258 staining. Cells treated with radiation alone (R), genistein alone (G), or radiation and genistein (RG) and then incubated for the indicated time points. Cells with atypical fragmented nuclei were counted continuously over the course of the experiment by Hoechst 33258 staining. Three independent assays were performed and data was represented as the means ± SEM obtained from the triplicates experiment.

by irradiation in a dose-dependent manner, and was completely inhibited at 10 Gy of irradiation. Genistein inhibited cell growth by approximately 50% but did not induce a significant cell death until 120 h at 25 μM. To further evaluate the effect of genistein on radiation-induced cell death, we used 10 Gy of irradiation with or without 25 μM of genistein. Irradiation alone decreased the viability of K562 cells in a time-dependent manner as measured by the trypan blue dye exclusion assay. Genistein had no significant effect on the viability of irradiated K562 cells (Fig. 4). Dead or dying cells was determined by nuclear morphology using Hoechst 33258 staining. As expected, the nuclear fragmentation of irradiated K562 cells was increased in a time-dependent manner and that of genistein-treated cells was



**Fig. 6** Cell cycle analysis and the expression of cyclins in K562 cells. Cells were treated with genistein immediately after irradiation and then incubated for the indicated time points. **(A)** Flow cytometric analysis was performed to analyze cell cycle progression. Cells were stained with propidium iodide and then DNA contents were measured on Epics XL flow cytometry system. The results presented are representatives of three independent experiments. **(B)** Western blotting analysis was performed to analyze protein expression. Cells were treated with radiation and genistein for various time points. Proteins of 20 µg of were separated on SDS-PAGE and probed with indicated antibodies. The data shown here is a representative from three independent experiments.

negligible. Of interest, genistein dramatically decreased nuclear fragmentation of irradiated K562 cells (Fig. 5). These results suggested that genistein prevented radiation-induced cell death.

TK1 is one of important factors for cell cycle regulation. In order to investigate cell cycle progression, DNA content of cell nuclei was measured by flow cytometric analysis (Fig. 6A). When the cells were irradiated, they showed a typical cell cycle arrest at G2 phase. The percentage of cells in G1 and S phases gradually decreased and the vast majority of cells appeared to arrest at G2/M phase at 24 h. However, G2 phase arrest was not sustained after 48 h. The p53 mutation-burdened K562 cells exhibited a significant G2 checkpoint deficit when exposed to radiation; a fraction of cells underwent G2 checkpoint abrogation in the presence of DNA damage and subsequently had a DNA content of less than 2n. At 96 h, sub-G1 phase cells were approximately 35%, indicating that they are undergoing nuclear fragmentation. Genistein-treated cells showed only slight G2 phase arrest. However, K562 cells treated with radiation and genistein were not recovered from G2 phase arrest, suggesting that the synergistic effect of radiation and genistein on G2/M block.

Since cell cycle progression is specifically regulated by a series of cyclin-dependent kinases (CDKs) and cyclins at different phase, we measured the expression of these cell cycle regulatory proteins in K562 cells treated with radiation and genistein. Cyclin A is produced in late G1 and accumulates during S and G2 phase. It is associated with and primarily activates CDK2. Expression of B-type cyclins is typically maximal during the G2 to M phase transition and controls passage through the M phase. B-type cyclins are the major activators of Cdc2. As shown in Fig. 6B, the expression of cyclin A was time-dependently increased but the expression of cyclin B1 was decreased. As expected, no significant changes were observed in the expression of CDK2 and Cdc2. The expression of β-actin as a control was constant.

## DISCUSSION

It is important to analyze and understand known or unknown genes to apply as therapeutic tools for cancer treatment. As one approach, PCR-select SSH is a useful method to selectively detect differentially expressed genes in two different types of tissues or cells. It is also effectively used for cloning uniquely expressed genes which play an important role in cell differentiation or tumorigenesis.

We have previously demonstrated that PTK inhibitors, genistein and HMA, modulate radiation-induced cell death in CML K562 cells which are strikingly resistant to apoptosis induced by radiation [6]. In this study, we showed that irradiation led to decreased viability and increased nuclear fragmentation in K562 cells. In cell cycle analysis, irradiated cells were arrested at G2 checkpoint and sub-G1 phase appeared after 72 h, indicating that these cells may undergo nuclear fragmentation. However, cell death

and cell cycle progression induced by radiation alone was modified by the combined treatment with radiation and genistein. As shown in Fig. 4, 5 and 6A, the viability of the cells were sustained and fragmented nucleus began to appear with maintenance of G2 phase arrest. The treatment with genistein alone showed somewhat different effect. The treatment of genistein alone had little effect on nuclear fragmentation and G2 phase arrest was slightly affected in K562 cells.

In order to clarify the different phenomena induced by the irradiation alone and by the combined treatment of radiation and genistein, we performed PCR-select SSH and their gene expressions were compared. We identified that human TK1 gene was significantly induced in K562 cells treated with radiation and genistein (Fig. 2A). TK is a crucial enzyme in the salvage pathway of thymidine triphosphate formation and is indirectly involved in DNA replication. In eukaryotic cells, there are two TK isozymes: TK1 and TK2, also called cytosolic and mitochondrial TK, respectively. TK1 is involved in the transformation of cancer cells caused by abnormal control of S phase [15]. The expression of TK1 increases at G1/S phase and reaches the maximum during M phase.

Although many reports have described the relationship between TK1 and radiosusceptibility, the mechanism still remains unclear and is cell-type dependent. In this study, Northern hybridization and RT-PCR showed that K562 cells treated with radiation and genistein induced a significant increase of TK1 mRNA (Fig. 2B and 3A). The treatment with genistein alone did not facilitate to induce TK1 mRNA expression. *In vitro* TK1 kinase assay showed that cells treated with radiation and genistein significantly increased TK1 enzymatic activity in a time-dependent manner with the peaking at 72 h (Fig. 3B). Importantly, the change of TK1 enzymatic activation was parallel with that of TK1 mRNA expression in cells treated with radiation and genistein (Fig. 3A and B). These results suggest that the induction of TK1 activity is regulated at its transcription level. A similar report was proposed by Stuart *et al* [16].

As demonstrated above, cell cycle arrest of K562 cells might be critical to modulate radiation-induced cell death by genistein. Therefore, we predicted that TK1 might play an important role in the mechanisms of radiation-induced cell cycle progression and subsequent cell death. It has been reported that the activity of TK1 is associated with cell cycle control [17]. When cells were M phase-arrested by the treatment of nocodazole, a microtubule-depolymerizing drug, TK became hyperphosphorylated in HL60, K562, and HeLa cells [18]. At this phase, TK activity is no longer required, since DNA replication is completed. Hyperphosphorylated TK in mitotically blocked cells exists as the less active form and is accompanied by a decrease in its affinity for its substrate thymidine, which is con-

sistent with the loss of TK1 activity at 96 h in our data (Fig. 3B). In general, cells do not allow progression into the next phase of the cell cycle before completing all events associated with the previous phase. We have shown that the expression of cyclin B1 was time-dependently decreased following genistein treatment after irradiation, probably due to sustained arrest of cell cycle progression in G2/M phase (Fig. 6A and B). In addition, the expression of cyclin A was also increased in K562 cells treated with radiation and genistein, the time frame of which was in consistent with that of the TK1 (Fig. 3B and 6B).

The role of TK1 in the modulation of radiation-induced cell cycle progression and cell death by genistein may correlates with other changes of cell. Recent papers demonstrated that apoptosis is insufficient to explain cancer cell death [19-20]. Cell death through mitotic catastrophe and terminal growth arrest through senescence or differentiation have been introduced in chemotherapy and radiotherapy of tumor cells. Mitotic catastrophe is the product of premature mitosis, which is an apoptosis-like process that begins in prophase after dissolution of the nuclear membrane [21]. By contrast, the hallmark of senescence is the failure of initiation of DNA synthesis during the progression of cell cycle. Senescence induces an undefined cell cycle arrest and cells which undergo senescence eventually die [22-24]. The process of senescence is accompanied by the down-regulation of the G1/S-regulatory genes, TK1 and dihydrofolate reductase (DHFR) [25-27] and some of the transcription factors, E2F and nuclear factor (NF)-Y [24, 25]. Cell differentiation is also associated with the withdrawal of cells from cell cycle to the G0/G1 stage and CDK inhibitors (CDKIs), such as p16<sup>INK4A</sup> or p21<sup>CIP1</sup>, is considered to serve as the initiator of differentiation [28, 29]. The repression of the transcription of TK1, S-phase specific DNA polymerase, and dihydrofolate reductase play a initial and crucial role in the hormone-induced differentiation of embryonic carcinoma cells and oligodendrocyte precursor cells [30]. Chemically induced monocytic/macrophagic and granulocytic differentiation of HL-60 leukemia and U-937 cells showed a declined activity of TK1 [31, 32]. In K562 cells, Filanovskaia *et al* reported that TK1 activity was decreased during differentiation in the myeloid cells but it was much higher in lymphoid cells [33].

This study demonstrates that the alteration of cell cycle by controlling the genes responsible for the modulation of radiation-induced cell death. We offers an effective way to improve and enhance the effects of radiotherapy in CML.

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