# *Full Papers*

## Antileukemic Activity of Genistein, a Major Isoflavone Present in Soy Products

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Soy has been used in traditional medicine for the treatment of various diseases, including cancer. The isoflavones present in soy have been shown in animal models to have cancer-preventing activity. However, the therapeutic effects of isoflavones against cancer are still unclear. We have evaluated the in vitro and in vivo antileukemic activity of genistein (**1**), a major isoflavone present in soy. We observed that it produced a dose- and time-dependent antineoplastic activity against myeloid and lymphoid leukemic cell lines. In addition, genistein treatment of the leukemic cells reactivated tumor suppressor genes that were silenced by aberrant DNA methylation. A genistein-enriched diet produced a moderate, but significant, antileukemic effect in mice. The limited extent of this in vivo response may have been due to the rapid metabolic inactivation of genistein in mice. Due to the longer half-life of genistein in humans, a soy-enriched diet has the potential to produce plasma levels of this isoflavone in the range of the concentrations used in vitro that produced an antileukemic activity.

Soy (*Glycine max* L.; Leguminosae) has been used in traditional medicine for the treatment of various diseases.[1] Previous research indicates that genistein (**1**, Figure 1), a major isoflavone in soy, has some activity for the chemoprevention of cancer.[2] Recent epidemiological studies showed a significant correlation between the plasma level of genistein (**1**) and a reduction of the risk of breast cancers in Asian populations, who consume 20–50 times more soy than Western populations.[2,3] A recent comprehensive study in Japan showed that soy intake significantly reduced the incidence of prostate cancers.[4]

Plasma levels of total genistein (**1**) in high soy consumers are in the micromolar range.[3,5] Genistein in the plasma can reach 4–16 $\mu$M with an elimination half-life between 15 and 22 h.[6] Higher plasma levels of 20–25 $\mu$M can be reached after ingestion of high-dose supplements.[7,8]

Genistein (**1**) produces pleiotropic effects against cancer cells that can influence cell proliferation, cell cycle progression, and apoptosis.[9–11] Its effects on a number of cellular targets have been proposed to be responsible for its anticancer activity.[3] In breast carcinoma cells, its antiproliferative effects were shown to be mediated through the formation of an intracellular metabolite that inhibits cell cycle progression.[12] Normal lymphocytes survived after treatment with concentrations that were cytotoxic to HL-60 leukemic cells, indicating that genistein is nontoxic to normal cells.[13] No significant genotoxicity was observed in tumor patients treated with genistein.[8]

Genistein (**1**) was reported to inhibit cell proliferation and induce differentiation of human leukemic cell lines.[13,14] In these latter studies, the in vitro antileukemic activity of genistein was assessed at very high concentrations, which are not obtainable in humans.[6] To our knowledge the action of genistein on colony formation of the leukemic cell lines has yet to be investigated. This property is important for the complete evaluation of its in vitro antineoplastic activity.[15] In comparison to other in vitro methods, the clonogenic assay is the method of choice to assess antineoplastic activity,[15] since the major objective of chemotherapy is the irreversible eradication of the clonogenic potential of leukemic stem cells.[16,17] Moreover, the antileukemic activity of genistein, including dietary supplementation, has not been tested in animals. This study aimed to assess the effect of genistein on in vitro growth inhibition, loss of clonogenicity, and gene reactivation of both myeloid and lymphoid leukemic cells. The concentrations used in this study were those that are obtainable in plasma of high soy consumers or people treated by genistein supplements. In addition, our second objective was to evaluate in an in vivo model the antineoplastic activity of genistein administrated in the diet of mice with L1210 leukemia. We observed significant antileukemic activity for genistein in both models.

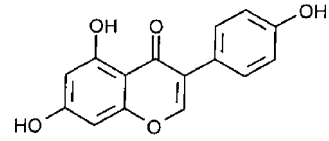


**Figure 1.** Molecular structure of genistein (**1**): 5,7,4′-trihydroxyisoflavone.

### Results and Discussion

The effect of genistein (**1**) on growth inhibition of human HL-60, MOLT-3, Raji, and KG1a leukemic cells is shown in Figure 2. The growth inhibitory activity of this isoflavone was dose- and time-dependent in all four cell lines. MOLT-3 lymphoid cells were the most sensitive cell line to the antiproliferative effect of genistein, as shown by growth inhibition (>10%) produced by 2 $\mu$M after 48 h exposure (Figure 2B). For the other cell lines, the growth inhibitory effect of genistein (>10%) was obtained at concentrations starting at 5 $\mu$M. The concentrations that produced a 50% inhibition of growth ($IG_{50}$) for 48 h exposure were 18.2 $\mu$M for HL-60 (Figure 2A), 12.7 $\mu$M for MOLT-3 (Figure 2B), 18.6 $\mu$M for Raji (Figure 2C), and 23 $\mu$M for KG1a (Figure 2D) leukemic cells.

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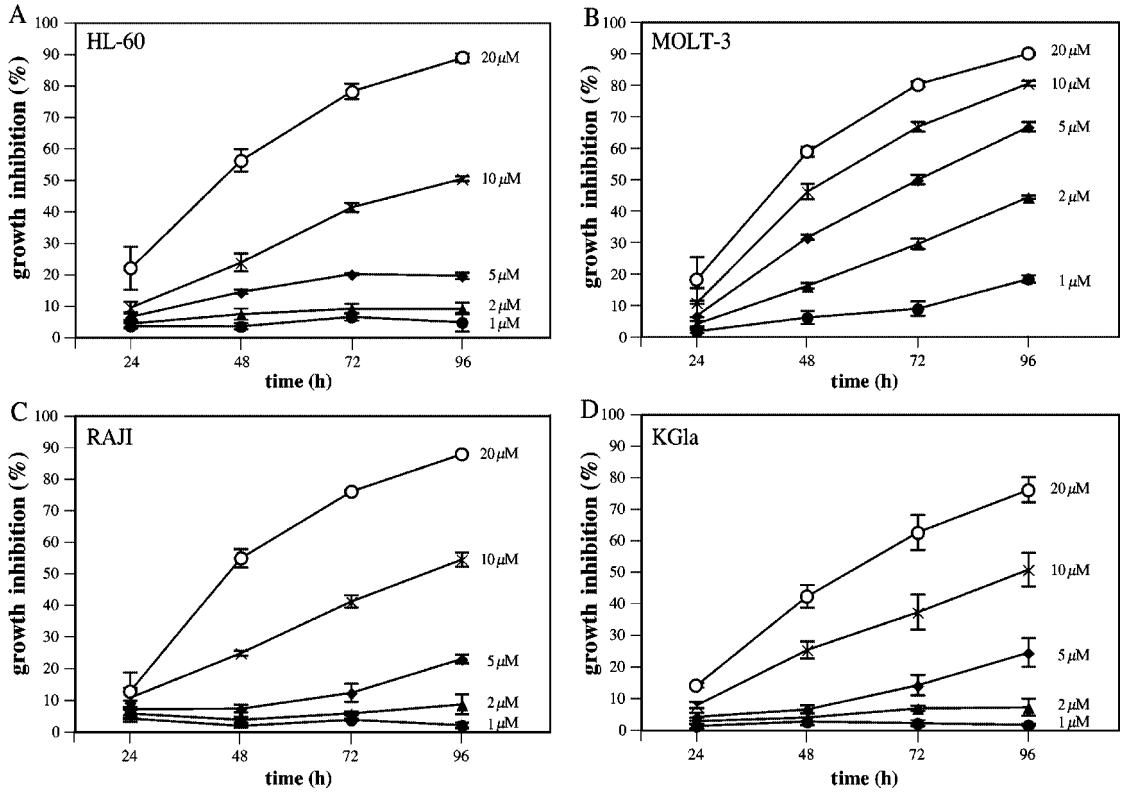


**Figure 2.** Effect of genistein (**1**) on growth inhibition in (A) HL-60, (B) MOLT-3, (C) Raji, and (D) KG1A human leukemic cell lines. Cells were exposed to genistein at indicated concentrations (1–20 $\mu$M), and cell counts were performed at 24, 48, 72, and 96 h after exposure. Graphs represent the percentage of growth inhibition relative to control cells. Data shown are mean values ± SEM, $n = 3$.

The effect of genistein (**1**) on loss of clonogenicity as determined by a colony assay (Figure 3) was investigated for HL-60, MOLT-3, and Raji leukemic cell lines, but not in KG1a cells (due to their inability to grow in soft agar). Both lymphoid and myeloid human leukemic cell lines showed a dose- and time-dependent loss of clonogenicity after genistein exposure. After 24 h exposure, **1** produced a loss of clonogenicity (>10%) starting at 1 $\mu$M for MOLT-3 (Figure 3B). The other cell lines required a concentration >2 $\mu$M to produce a loss of clonogenicity >10%. The concentrations that produced a 50% loss of clonogenicity ($LC_{50}$) for 24 h exposure were 6.3 $\mu$M for HL-60 (Figure 3A), 7.5 $\mu$M for MOLT-3 (Figure 3B), and 13.7 $\mu$M for Raji (Figure 3C) leukemic cells.

For L1210 murine leukemic cells, genistein (**1**) at concentrations of 1 to 20 $\mu$M induced a dose- and time-dependent growth inhibition and a loss of clonogenicity at 12, 24, and 48 h (Table 1). Genistein produced growth inhibition and loss of clonogenicity >10% starting at 2 $\mu$M. The $IG_{50}$ and $LC_{50}$ values of genistein for 24 h exposure were 18.5 and 6.5 $\mu$M, respectively.

The effect of different concentrations (1 to 20 $\mu$M) of genistein (**1**) on gene expression was assessed by RT-PCR in human HL-60 and mouse L1210 leukemic cell lines after 48 and 24 h, respectively. For HL-60 cells, the cDNA products of $p57^{KIP2}$ and 18S rRNA (used as the reference standard) are shown in Figure 4A. For L1210 cells, the cDNA products of $p15^{CDKN2B}$ and 18S rRNA are shown in Figure 4B. Genistein induced variable re-expression of these two silenced tumor suppressor genes at concentrations ranging from 1 to 20 $\mu$M. An analysis of the promoter methylation level of $p57^{KIP2}$ gene in HL-60 was performed by methylation-specific PCR (Figure 4C). Genistein at 10 $\mu$M produced a weak demethylation of the promoter region that was shown by the detection of a weak band amplified by the primers for unmethylated DNA (U) and by a decrease in the intensity of the band amplified by the primers for the methylated DNA (M), compared to nontreated cells.

The activity of genistein (**1**) was tested in a mouse model in order to evaluate its potential as a chemotherapeutic agent against leukemia. CD2F1 male mice were placed on a 0.5% genistein-enriched diet (10-day period). The food intake was similar to the mice fed with a control diet. The mice received an i.v. injection of $10^4$ L1210 leukemic cells. Control mice survived 175 ± 2 h after injection of leukemia (Figure 5). The leukemic mice fed with a 0.5% genistein-enriched diet had a moderate, but significant, increase in life span of 7% and died after 187 ± 2 h ($p < 0.05$). In each experiment, it was observed that all the control mice showed symptoms of advanced leukemic disease before the genistein-treated mice.

The objective of this study was to evaluate the in vitro and in vivo antileukemic activity of genistein (**1**), a major isoflavone present in soy that has been used in traditional medicine.[1] In this study, genistein was shown to inhibit growth of human and murine leukemic cell lines. Our results confirmed the growth inhibitory activity on leukemic cells as reported by other investigators.[13,14] However, these investigators used very high concentrations of genistein, which are not obtainable in humans, as compared to our lower concentrations that are reachable for a person on a high-soy diet. In addition, these reports did not use a clonogenic assay, which is of primary importance for the evaluation of the in vitro activity of antineoplastic agents. This ability in reducing the clonogenicity of leukemic cells by genistein is very important in the evaluation of its antineoplastic activity, since the current focus of therapy is the inactivation of the clonogenic potential of leukemic stem cells.[16,17]

In this report, we show that genistein induced a time- and dose-dependent effect on both myeloid and lymphoid leukemic cell lines. Loss of clonogenicity by the leukemic cells was observed at levels (1–20 $\mu$M) that are in the range of the observed plasma concentration of genistein by a soy-enriched diet.[8] Interestingly, low concentrations (2 $\mu$M) were able to induce loss of clonogenicity >10% after 24 h exposure. It has been reported that genistein is a cytotoxic agent for neoplastic but not for normal cells.[13] In addition, genistein shows no genotoxicity in patients.[8] We also investigated

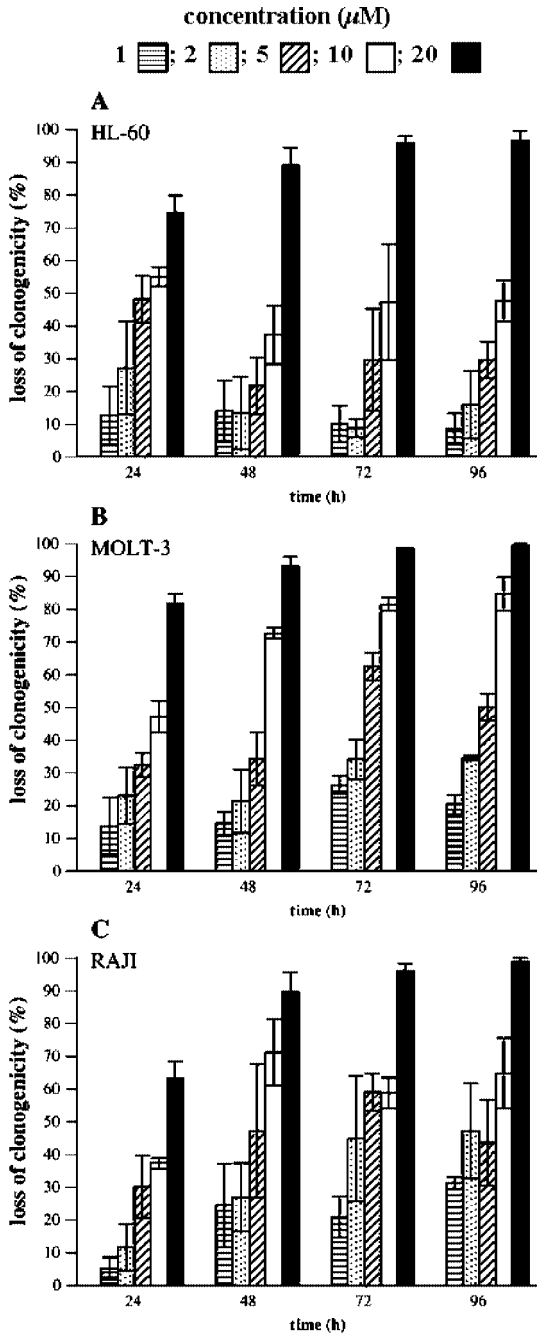


**Figure 3.** Effect of genistein (**1**) on loss of clonogenicity in (A) HL-60, (B) MOLT-3, and (C) Raji human leukemic cell lines. Cells were treated with the indicated concentrations of **1**. At different times (24, 48, 72, and 96 h) genistein was removed and colony formation was determined on day 17–20. Loss of clonogenicity (%) was expressed relative to control cells. Data shown are mean values ± SEM, $n = 3$.

the effects of genistein (**1**) on the reactivation of tumor suppressor genes, which were reported to be silenced by promoter hypermethylation in leukemic cell lines.[18,19] Genistein (1–20 μM) induced a re-expression of two silenced tumor suppressor genes, $p57^{KIP2}$ in a human cell line and $p15^{CDKN2B}$ in a mouse cell line. The variable cDNA amplification of these genes after genistein exposure may have been due to the pleotropic action of this isoflavone on various targets. At the higher concentrations of genistein, the expression appeared to decrease, which was probably due to the perturbation of cellular function. Our data are in accord with the report that genistein on esophageal squamous carcinoma cells reactivated the expression of several tumor suppressor genes, which were silenced

**Table 1.** Effect of Genistein (**1**) on Growth Inhibition and Loss of Clonogenicity in L1210 Murine Leukemic Cell Line[a]

| genistein (μM) | growth inhibition (%) | | | loss of clonogenicity (%) | | |
|---|---|---|---|---|---|---|
| | 12 h | 24 h | 48 h | 12 h | 24 h | 48 h |
| 1 | 3.2 ± 1.7 | 4.1 ± 0.1 | 13.2 ± 0.1 | 11 ± 6.1 | 6 ± 0.5 | 11.9 ± 6.0 |
| 2 | 6.2 ± 3.2 | 11.4 ± 0.1 | 21.9 ± 1.6 | 14 ± 6.9 | 13 ± 4.8 | 8.9 ± 5.7 |
| 5 | 15.1 ± 2.7 | 28.5 ± 1.4 | 55.9 ± 0.6 | 44 ± 4.9 | 16 ± 3.8 | 32.9 ± 7.3 |
| 10 | 23.8 ± 0.2 | 42.8 ± 0.9 | 79.2 ± 1.6 | 94 ± 0.8 | 63 ± 8.1 | 81.1 ± 5.1 |
| 20 | 54.2 ± 2.0 | 66.7 ± 2.2 | 93.1 ± 1.2 | 100 ± 0.0 | 97 ± 1.3 | 99.7 ± 0.3 |

[a] Cells were treated with genistein at indicated concentrations (1–20 μM) for 12, 24, or 48 h. Cell counts and colony formation assay were expressed in percentage relative to control cells. Data shown are mean values ± SEM, $n = 3$.

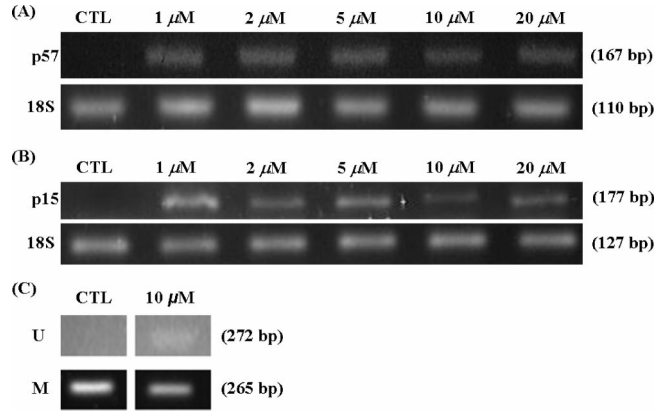


**Figure 4.** Effect of genistein (**1**) on gene expression. The reactivation of tumor suppressor genes (A) $p57^{KIP2}$ in HL-60 and (B) $p15^{CDKN2B}$ in L1210 leukemic cells treated with indicated concentrations of genistein was determined by RT-PCR for 48 and 24 h, respectively. (C) Methylation-specific PCR of $p57^{KIP2}$ promoter was performed in HL-60 cells exposed 48 h to genistein at 10 μM. Unmethylated (U) and methylated (M) primers amplified DNA when the promoter region is demethylated or methylated, respectively. Experiments were repeated two times. CTL, control nontreated cells.

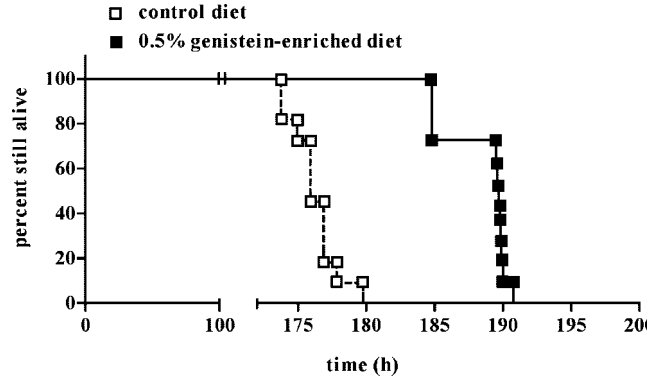


**Figure 5.** Effect of genistein-enriched diet on the survival time of mice with L1210 leukemia. Control mice on normal diet and mice on genistein-enriched diet were injected i.v. with $10^4$ L1210 leukemic cells at 0 h. Leukemic mice ($n = 11$) fed with control diet died after 175 ± 2 h. Leukemic mice ($n = 11$) fed with 0.5% genistein-enriched diet survived 187 ± 2 h ($p < 0.05$). The time of death (h) was plotted on a survival chart.

by aberrant DNA methylation.[20] The reactivation of tumor suppressor genes by genistein and its demethylation effect on one of these genes suggest that part of the antileukemic activity of this agent is related to its epigenetic action. Its actions on other targets, such as an inhibition of tyrosine kinases, may also contribute to its antileukemic effect.[3] For example, inhibitors of tyrosine kinases

have been reported to have antineoplastic activity and are under clinical investigation in patients with cancer.[21]

In our in vivo model, the leukemic mice fed with a 0.5% genistein-enriched diet showed a significant increase in survival time as compared to control mice on a normal diet. This effect was moderate but significant. This mouse model is very sensitive in detecting the antineoplastic activity of experimental agents because of an excellent correlation between the number of surviving leukemic cells and the survival time of the mice.[22] The major cause for this moderate in vivo response was probably that genistein did not exceed 1 $\mu$M in the blood of the mice as reported by others in mice fed with 0.6% soy extract supplement.[23] Indeed, the metabolism of genistein is very rapid in the strain of mice (CD2F1) used in our in vivo leukemic model as shown by its short half-life of less than 15 min.[24] Therefore, we and other investigators have found it very difficult to obtain adequate concentrations of genistein, a lipophilic agent, to produce potent antineoplastic activity in mice.[23] In this regard, it was recently published that genistein (injected i.p.) did not produce a significant increase in survival time ($p = 0.06$) of nude mice bearing subcutaneous HL-60 tumors.[25]

In conclusion, the present data show that genistein (**1**), a key isoflavone present in soy, has promising potential against leukemia. It can produce a significant loss of clonogenicity of both myeloid and lymphoid leukemic cells. Genistein was shown to produce the reactivation of tumor suppressor genes associated with a weak effect on promoter demethylation. In a preclinical model, the antileukemic effects of genistein administrated directly in the diet were evaluated. The moderate in vivo antileukemic effect of genistein is probably due to its very rapid inactivation in mice. The longer half-life of genistein in humans can produce plasma levels of this isoflavone after a soy-enriched diet in the range of the concentrations used in vitro, which produced a potent antileukemic activity. The interesting antileukemic activity of genistein merits further investigation.

## Experimental Section

**Cell Lines and Culture Conditions.** Human myeloid leukemic cells HL-60 and KG1a and lymphoid leukemic cells MOLT-3 were obtained from ATCC (Manassas, VA). The Raji lymphoid leukemic cell line was donated by Dr. J. Menezes (Hôpital Saint-Justine). Human cell lines were cultured in RPMI-1640 medium (Invitrogen, Burlington, Ontario, Canada) supplemented with 10% heat-inactivated fetal bovine serum (Wisent, St-Bruno, Quebec, Canada). The doubling times of HL-60 and Raji cells were 16–18 h, whereas those of MOLT-3 and KG1a leukemic cells were 23–24 and 35–36 h, respectively. The murine lymphoid leukemic cell line L1210 was obtained from Dr. T. Khwaja (University of Southern California, Los Angeles) and cultured in RPMI-1640 with 5% heat-inactivated fetal calf serum and with 6 $\mu$M 2-mercaptoethanol. The doubling time of the L1210 cells was about 10 h. All cell lines were incubated at 37 °C in 5% $CO_2$ atmosphere. Genistein (LC Laboratories, Woburn, MA) was dissolved in DMSO (Sigma, Oakville, Ontario, Canada) to prepare stock solutions of 2 and 20 mM and kept frozen at −20 °C until use.

**Inhibition of Growth and Clonogenic Assays.** Human and murine cell lines in log phase at $2 \times 10^4$ cells/mL were placed in tissue culture flasks. Genistein (**1**) was added at 1, 2, 5, 10, and 20 $\mu$M. For the growth inhibition assay, aliquots of cells were counted at 12, 24, 48, 72, and 96 h using a Beckman Z1 Coulter particle counter (Hialeah, FL). For the clonogenic assay, **1** was removed by centrifugation and cells were suspended in drug-free medium. Then 100 cells were placed in 2 mL of 0.45% soft agar RPMI-1640 medium containing 10% serum for the murine cell line or 20% serum for human cell lines. After 7 days of incubation for the murine cell line or 15 days for human cell lines, the number of colonies (>50 cells) was counted. The cloning efficiency in soft agar without drug treatment was in the range 50–60% for all cell lines. The inhibition of growth and loss of clonogenicity (%) were expressed relative to control cells without treatment. All the experiments were performed in triplicate, and the mean values were divided by the control mean value. The experiments were repeated three times and expressed as mean ± SEM.

**Isolation of RNA and RT-PCR Analysis.** Total RNA from human (HL-60) and mouse (L1210) leukemic cell lines was isolated using RNeasy mini kit (Qiagen, Mississauga, Ontario, Canada) and reversed-transcribed using Omniscript reverse transcriptase kit (Qiagen) at 37 °C for 60 min. DNA amplifications were performed using HotStar Plus Taq polymerase (Qiagen) in a Whatman Biometra T gradient thermocycler (Göttingen, Germany). For each gene, the number of cycles was selected during the exponential phase of DNA amplification. The cDNA products were electrophoresced on 2% agarose gel and detected by ethidium bromide staining. Specific primers spanning different exons and conditions for DNA amplification of human 18S rRNA and $p57^{KIP2}$ and for mouse 18S rRNA and $p15^{CDKN2B}$ were described previously.[26,27]

**Isolation of DNA and Methylation Specific-PCR Analysis.** HL-60 cells were exposed for 48 h to genistein at 10 $\mu$M. After treatment, drug was removed and cells were harvested after an additional 24 h. Genomic DNA was isolated using DNeasy tissue kit (Qiagen), and 2 $\mu$g was treated with sodium bisulfite as per the manufacturer's instructions (EpiTect Bisulfite, Qiagen) for methylation analysis. Using specific primers and PCR conditions, the methylation status of the *p57* promoter region was assessed as previously described.[26] The number of cycles used corresponded to the exponential phase of DNA amplification. The PCR products were electrophoresced on 2% agarose gel and detected by ethidium bromide staining.

**In Vivo Experiments.** Male CD2F1 mice (24–28 g) were purchased from Taconic Biotechnology (Germantown, NY). Mice were acclimatized to housing conditions at least 2 weeks before experiments. They received food and water ad libitum. The institutional animal committee approved the experimental protocol, and animals were handled in accordance with the institutional guidelines. For transplantation of leukemia in mice, i.p. injections of $10^4$ L1210 cells in RPMI-1640 medium were performed weekly into the CD2F1 mice. Seven days later, the ascetic fluid was obtained from the mice and a cell count of the leukemic cells was performed with a hemocytometer prior to subsequent transplantation. Mice were separated in two groups. The control group ($n = 11$) received the 2016 diet (Harlan Teklad, Madison, WI), and treated mice ($n = 11$) received the 2016 diet supplemented with 0.5% genistein (**1**) (Harlan Teklad). Treated mice were acclimated to the genistein-enriched regimen over a 10-day period before injection of leukemic cells. Control and treated mice were injected i.v. with 0.1 mL of L1210 ($10^4$) cells and were kept on either control or 0.5% genistein-supplemented diet. Toxicity was evaluated by body weight loss. The survival times of each group of mice were monitored 2 or 3 times per day, and the increase in life span was calculated.

**Statistical Considerations.** In order to evaluate whether the variations between groups were random, a Student's *t* test was performed. A *p* value of 0.05 was taken for statistical significance.

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## References and Notes

(1) Chen, X.; Zhou, H.; Liu, Y. B.; Wang, J. F.; Li, H.; Ung, C. Y.; Han, L. Y.; Cao, Z. W.; Chen, Y. Z. *Br. J. Pharmacol.* **2006**, *149*, 1092–1103.
(2) Park, O. J.; Surh, Y. J. *Toxicol. Lett.* **2004**, *150*, 43–56.
(3) Ravindranath, M. H.; Muthugounder, S.; Presser, N.; Viswanathan, S. *Adv. Exp. Med. Biol.* **2004**, *546*, 121–165.
(4) Kurahashi, N.; Iwasaki, M.; Sasazuki, S.; Otani, T.; Inoue, M.; Tsugane, S. *Cancer Epidemiol. Biomarkers Prev.* **2007**, *16*, 538–545.
(5) Mathey, J.; Lamothe, V.; Coxam, V.; Potier, M.; Sauvant, P.; Pelissero, C. B. *J. Pharm. Biomed. Anal.* **2006**, *41*, 957–965.
(6) Takimoto, C. H.; Glover, K.; Huang, X.; Hayes, S. A.; Gallot, L.; Quinn, M.; Jovanovic, B. D.; Shapiro, A.; Hernandez, L.; Goetz, A.; Llorens, V.; Lieberman, R.; Crowell, J. A.; Poisson, B. A.; Bergan, R. C. *Cancer Epidemiol. Biomarkers Prev.* **2003**, *12*, 1213–1221.
(7) Izumi, T.; Piskula, M. K.; Osawa, S.; Obata, A.; Tobe, K.; Saito, M.; Kataoka, S.; Kubota, Y.; Kikuchi, M. *J. Nutr.* **2000**, *130*, 1695–1699.
(8) Miltyk, W.; Craciunescu, C. N.; Fischer, L.; Jeffcoat, R. A.; Koch, M. A.; Lopaczynski, W.; Mahoney, C.; Crowell, J.; Paglieri, J.; Zeisel, S. H. *Am. J. Clin. Nutr.* **2003**, *77*, 875–882.
(9) Matsui, J.; Kiyokawa, N.; Takenouchi, H.; Taguchi, T.; Suzuki, K.; Shiozawa, Y.; Saito, M.; Tang, W. R.; Katagiri, Y. U.; Okita, H.; Fujimoto, J. *Leuk. Res.* **2005**, *29*, 573–581.
(10) Park, S. S.; Kim, Y. N.; Jeon, Y. K.; Kim, Y. A.; Kim, J. E.; Kim, H.; Kim, C. W. *Cancer Chemother. Pharmacol.* **2005**, *56*, 271–278.
(11) Sarkar, F. H.; Adsule, S.; Padhye, S.; Kulkarni, S.; Li, Y. *Mini Rev. Med. Chem.* **2006**, *6*, 401–407.

(12) Nguyen, D. T.; Hernandez-Montes, E.; Vauzour, D.; Schonthal, A. H.; Rice-Evans, C.; Cadenas, E.; Spencer, J. P. *Free Radical Biol. Med.* **2006**, *41*, 1225–1239.
(13) Traganos, F.; Ardelt, B.; Halko, N.; Bruno, S.; Darzynkiewicz, Z. *Cancer Res.* **1992**, *52*, 6200–6208.
(14) Constantinou, A.; Kiguchi, K.; Huberman, E. *Cancer Res.* **1990**, *50*, 2618–2624.
(15) Momparler, R. L. *Pharmacol. Ther.* **1980**, *8*, 21–35.
(16) Kvinlaug, B. T.; Huntly, B. J. P. *Expert Opin. Ther. Targets* **2007**, *11*, 915–927.
(17) Ravandi, F.; Estrov, Z. *Clin. Cancer Res.* **2006**, *12*, 340–344.
(18) Shen, L.; Toyota, M.; Kondo, Y.; Obata, T.; Daniel, S.; Pierce, S.; Imai, K.; Kantarjian, H. M.; Issa, J. P.; Garcia-Manero, G. *Blood* **2003**, *101*, 4131–4136.
(19) Toyota, M.; Kopecky, K. J.; Toyota, M. O.; Jair, K. W.; Willman, C. L.; Issa, J. P. *Blood* **2001**, *97*, 2823–2829.
(20) Fang, M. Z.; Chen, D.; Sun, Y.; Jin, Z.; Christman, J. K.; Yang, C. S. *Clin. Cancer Res.* **2005**, *11*, 7033–7041.
(21) Nichols, G. L. *Cancer Investig.* **2003**, *21*, 758–771.
(22) Momparler, R. L.; Gonzales, F. A. *Cancer Res.* **1978**, *38*, 2673–2678.
(23) Hewitt, A. L.; Singletary, K. W. *Cancer Lett.* **2003**, *192*, 133–143.
(24) Supko, J. G.; Phillips, L. R. *J. Chromatogr. B, Biomed. Appl.* **1995**, *666*, 157–167.
(25) Shen, J.; Tai, Y. C.; Zhou, J.; Stephen Wong, C. H.; Cheang, P. T.; Wong, F. W. S.; Xie, Z.; Khan, M.; Han, J. H.; Chen, C. S. *Exp. Hematol.* **2007**, *35*, 75–83.
(26) Lemaire, M.; Momparler, L. F.; Bernstein, M. L.; Marquez, V. E.; Momparler, R. L. *Anticancer Drugs* **2005**, *16*, 301–308.
(27) Lemaire, M.; Momparler, L. F.; Farinha, N. J.; Bernstein, M.; Momparler, R. L. *Leuk. Lymphoma* **2004**, *45*, 147–154.

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