Genistein Protects Hematopoietic Stem Cells against G-CSF–Induced DNA Damage

Liliana R. Souza1, Erica Silva1, Elissa Calloway1, Omer Kucuk1, Michael Rossi2, and Morgan L. McLemore1

Abstract

Granulocyte colony-stimulating factor (G-CSF) has been used to treat neutropenia in various clinical settings. Although clearly beneficial, there are concerns that the chronic use of G-CSF in certain conditions increases the risk of myelodysplastic syndrome (MDS) and/or acute myeloid leukemia (AML). The most striking example is in severe congenital neutropenia (SCN). Patients with SCN develop MDS/AML at a high rate that is directly correlated to the cumulative lifetime dosage of G-CSF. Myelodysplastic syndrome and AML that arise in these settings are commonly associated with chromosomal deletions. We have demonstrated in this study that chronic G-CSF treatment in mice results in expansion of the hematopoietic stem cell (HSC) population. In addition, primitive hematopoietic progenitors from G-CSF–treated mice show evidence of DNA damage as demonstrated by an increase in double-strand breaks and recurrent chromosomal deletions. Concurrent treatment with genistein, a natural soy isoflavone, limits DNA damage in this population. The protective effect of genistein seems to be related to its preferential inhibition of G-CSF–induced proliferation of HSCs. Importantly, genistein does not impair G-CSF–induced proliferation of committed hematopoietic progenitors, nor diminishes neutrophil production. The protective effect of genistein was accomplished with plasma levels that are attainable through dietary supplementation. Cancer Prev Res; 7(5); 534–44. ©2014 AACR.

Introduction

Severe congenital neutropenia (SCN) is a rare, heritable disorder characterized by isolated neutropenia from birth (1). Before the clinical use of granulocyte colony-stimulating factor (G-CSF), individuals typically died before the age of 2 from overwhelming infections. With G-CSF treatment, patients with SCN now routinely survive until adolescence or even adulthood. Unfortunately, a substantial number of patients with SCN now develop myelodysplastic syndrome (MDS) and/or acute myeloid leukemia (AML; refs. 1, 2). After 10 years of G-CSF treatment, the rate of MDS/AML in patients with SCN is estimated to be 2% to 3% per year (2). The etiology of AML in SCN is not well defined. The initial hypothesis was that a molecular defect responsible for SCN predisposed individuals to AML. Because accumulating evidence has demonstrated that MDS/AML arises from hematopoietic stem cells (HSC), this hypothesis seems less likely as the most frequently mutated gene in SCN, ELA2, is not expressed in HSCs. Another hypothesis is that chronic G-CSF treatment promotes expansion of a malignant myeloid clone. This remains a concern as the risk of myelodysplastic syndrome and/or AML roughly correlates with lifetime cumulative dosage of G-CSF (1, 3). Other researchers have suggested that G-CSF usage in certain settings may promote leukemic transformation (4–9). These studies are far from conclusive, as others have shown no increase in the risk of leukemic transformation and/or relapse with G-CSF therapy (10). In particular, a prospective study has shown that G-CSF usage did not increase the rate of relapse or decrease complete remission rates in AML (11). However, SCN is unique in its cumulative lifetime dosage of G-CSF. Patients with SCN typically receive G-CSF multiple times per week for life, as opposed to patients undergoing chemotherapy who may receive a short-term treatment.

G-CSF signals through the granulocyte colony-stimulating factor receptor (G-CSFR). The G-CSFR is a non-tyrosine kinase receptor that is present at low levels on HSCs (12). In mice, G-CSF treatment results in an increase in HSCs (13). It is possible that chronic G-CSF treatment results in HSC proliferation and acquisition of mutations. Several lines of evidence lend support to this hypothesis. First, acquisition of hyperproliferative mutations of the G-CSFR increases the risk of leukemic transformation (14). Second, it has been demonstrated that HSCs preferentially use error-prone DNA repair pathways when entering cell cycle, and chromosomal deletions are often seen not only in patients with SCN and AML, but also in patients with aplastic anemia treated with G-CSF (7, 9, 14, 15).
In this article, we provide evidence that prolonged G-CSF treatment results in genomic instability in murine HSCs. In addition, we demonstrate that treatment with the soy isoflavone genistein lessens DNA damage. This effect is achieved by using genistein at a dosage that can be easily attainable by dietary supplementation, suggesting that genistein may be an effective preventive agent for those patients who require prolonged G-CSF treatment.

Materials and Methods

Mouse strain

C57BL/6J mice were obtained from The Jackson Laboratory. All mice were housed in a specific pathogen-free environment. We used 6- to 10-week-old mice in all studies and the experiments were approved by the Emory University and Institutional Animal Care and Use Committee (IACUC protocol number, 2000678).

In vivo G-CSF treatment

C57BL/6J mice were treated subcutaneously 5-times a week with G-CSF (10 μg/kg; Neupogen; Amgen) or diluent alone (control mice) for different amounts of time varying up to 1 year.

Array comparative genomic hybridization analysis

Bone marrow cells were harvested from mice treated with G-CSF or diluent for 4 months. Lin–Sca+ cells were isolated using the instructions provided by the manufacturer (Easy Sep 18756, 19756A; STEMCELL Technologies). Genomic DNA was extracted from Lin–Sca+ cells using the Qiagen DNeasy kit, and quantification and quality assessment were performed with Picogreen, NanoDrop, and a standard agarose gel. DNA was analyzed by array comparative genomic hybridization (aCGH) at the Florida State University NimbleGen Microarray Facility using NimbleGen 3720K mouse whole-genome tiling arrays. Copy-number abnormalities were identified using NimbleScan and BioDiscovery Nexus softwares. Ablerrant segments were queried in the UCSC Genome Browser (GRCm38/mm10) for overlapping BAC alignments to be used for FISH validation of copy-number loss and gain. Data from the aCGH are available on the Gene Expression Omnibus (GEO) repository under accession number GSE54737.

Immunofluorescence microscopy

Bone marrow cells from mice were harvested and LSK cells (lineage-negative, cKit+, Sca+) were sorted on a BD FACSaria (BD Biosciences) and spotted on a slide. The cells were fixed using 10% methanol and 10% formalin in PBS and washed twice with PBS. Cells were then permeabizalized, blocked, and incubated with antibodies overnight at 4°C. The antibodies used individually were: pH2AX (07–164; Millipore), pGSK3 A555 (bs-5367R-A555; Bioss), cyclin D1 A555 (bs-0623R-A555; Bioss), and cyclin D3 A555 (bs-0660R-A555; Bioss). The following day, the sample was washed with PBS, incubated with a fluorescent secondary antibody when necessary (cat#A21429, Invitrogen), and stained with DAPI (4,6-diamidino-2-phenylindole; cat#D3571; Invitrogen). For quantification of immunohistochemistry, images from more than 100 cells were captured using a Carl Zeiss LSM 510 META confocal microscope (Zeiss) with a Plan-Apo 63 × 1.4 oil immersion lens. The maximum intensity per nucleus was determined and background was subtracted using Metamorph software (Molecular Devices).

Reactive oxygen species analyses

LSK cells were sorted, stained with dihydroergotoxine (DHET) (final concentration 50 μg/mL; cat# 7008; Sigma-Aldrich) at 37°C for 20 minutes, and analyzed by FACS-Canto (BD Biosciences).

FISH

Lineage-negative Sca+ (Lin–Sca+) bone marrow cells were isolated, fixed in a 1:3 mixture of methanol:acetic acid, and spotted onto a glass slide. For FISH analysis, the chromosome region-specific BAC clone for Ab1 (RP23-156H9) on mouse chromosome 2, and Tsc2 (RP23-438P15) on mouse chromosome 17, were labeled with 5-ROX dUTP by nick translation (Empire Genomics). The denaturation, hybridization, and signal detection procedures were carried out as described by the Oncology Cytogenetics Facility at Emory University. Hybridization was visualized on a LSM 510 META confocal microscope (Zeiss).

In vivo genistein treatment

C57BL/6J mice were treated subcutaneously 3-times a week for 6 weeks as follows: genistein (10 mg/kg; Cayman Chemical Company; genistein + 25 μL Peg400 + 75 μL 0.1% BSA in PBS), G-CSF (10 μg/kg; Neupogen; Amgen; G-CSF + 25 μL Peg400 + 75 μL 0.1% BSA in PBS), genistein + G-SCF (genistein + 25 μL Peg400 + G-CSF + 75 μL 0.1% BSA in PBS), diluent [DMSO (dimethyl sulfoxide) + 25 μL Peg400 + 75 μL 0.1% BSA in PBS; refs. 16, 17].

Cell sorting and flow cytometric analysis

We used a BD FACSAria and FACSCanto (BD Biosciences) for cell sorting and flow cytometric analysis, respectively, followed by analysis using FlowJo Software (TreeStar Inc). The following antibodies were used for cell sorting and flow cytometric analysis: MAC1 (cat#00112), GR1 (cat#5931), Ter119 (cat#5921), B220 (cat#0452), CD3 (cat#0031), c-Kit (cat#1171), Sca1 (cat#5981), CD48 (cat#0481), and CD150 (cat#1501); all were obtained from eBioscences.

BrdUrd incorporation

To examine 5-bromo-2′-deoxyuridine (BrdUrd) incorporation, we used the protocol previously described (18). Briefly, mice were given three daily intraperitoneal injections of BrdUrd in 0.1% BSA in PBS (Sigma; 3 mg/24 hours) and maintained on 0.2 mg/mL of BrdUrd in the drinking water for 72 hours. Mice were euthanized and bone marrow cells were stained with antibodies against lineage markers, c-Kit, Sca, cd48, and cd150. Cells were fixed, permeabilized, and stained with anti-BrdUrd-PE (all from eBioscences) and analyzed by FACSCanto.
Statistical analysis
Data were analyzed with Microsoft Excel. Paired t tests were used to test for evidence of differences in groups. Values were considered statistically significant at \( P \) less than 0.05.

Results
G-CSF treatment induces DNA damage in hematopoietic progenitors
Mice were treated with G-CSF (10 \( \mu \)g/kg 5 x week) for 4 months and bone marrow cells were analyzed by flow cytometry. The results demonstrated a 3-fold increase in the lineage-negative, Sca-positive, and cKit-positive (LSK) population of G-CSF–treated mice (Fig. 1A). As phosphorylation of histone H2AX (p-H2AX) is an indicator of DNA damage (19), we quantified the amount of nuclear pH2AX in individual LSKs using immunostaining. Our results demonstrate that there is a 2-fold increase in double-strand breaks (DSB) in LSK cells of treated animals in comparison with control animals (Fig. 1B and C). The LSK population was also analyzed for the presence of reactive oxygen species (ROS). LSK cells from G-CSF–treated animals displayed a significant increase in ROS levels (Fig. 1D).

G-CSF induces chromosomal instability
To determine whether the increased proliferation and DNA damage induced by G-CSF treatment lead to chromosomal alterations, we performed aCGH. Bone marrow cells were harvested from 3 mice treated with G-CSF and 3 mice treated with diluent for 4 months. Genomic DNA from Lin−Sca+ cells of G-CSF–treated mice and respective controls were analyzed in triplicate using NimbleGen 3 \( \times \) 720K mouse whole-genome tiling arrays. Copy-number abnormalities were identified using NimbleScan and BioDiscovery Nexus software. Aberrant segments were queried in the UCSC Genome Browser (GRCm38/mm10) for overlapping BAC alignments to be used for FISH. Significant deletions in

![Figure 1. G-CSF induces proliferation, DNA damage, and ROS production in LSK cells. C57BL/6J mice (n = 4 per group) were given 5 doses/week of G-CSF or diluent for 4 months. Bone marrow LSK cells were isolated and analyzed by FACS Aria. A, fold change in bone marrow LSK cells between both groups. Data represent the mean \pm SD of 4 mice per group; \( a, P < 0.05 \). B, fold change in DNA damage in sorted LSK cells quantified by intracellular staining of pH2AX; \( a, P < 0.05 \). For quantification, images from more than 100 cells were captured using a Carl Zeiss LSM 510 META confocal microscope (Zeiss) with a Plan-Apo 63 \( \times \) 1.4 oil immersion lens. The maximum intensity per nucleus was determined and background was subtracted using Metamorph software (Molecular Devices). C, immunofluorescence staining of pH2AX (red) in sorted LSK cells of G-CSF–treated mice. Images were captured using a Carl Zeiss LSM 510 META confocal microscope with a Plan-Apo 63 \( \times \) 1.4 oil immersion lens. D, fold change in ROS levels measured by staining sorted bone marrow LSK cells with DHET; data represent the mean \pm SD of 4 mice per group; \( a, P < 0.05 \).]
chromosome 2 and 17 were present in all G-CSF samples and were chosen for validation (Fig. 2A and B; GEO accession number, GSE54737).

Validation of the copy-number abnormalities detected by CGH were examined using FISH analysis in Lin−/Sca+ bone marrow cells from animals treated with G-CSF for 4 months using BAC clones RP23-156H9 (chromosome 2, Abl1) and RP23-438P15 (chromosome 17, Tsc2) within the regions of interest. Two sets of 100 cells per mouse (n = 44) were counted and the average was calculated.

FISH analysis confirmed alterations in chromosome 2 in 17% of cells, compared with 2% in equivalent control mice (Fig. 2C). Hybridizations also confirmed deletions in chromosome 17 in 31% of cells compared with 3% in equivalent control mice (Fig. 2D). Greater than 90% of abnormal cells showed loss of one or two probe signals. In less than 10% of abnormal cells, a gain of signal was noted.

**Genistein decreases G-CSF–induced DNA damage**

Previous studies have demonstrated that genistein protects hematopoietic progenitor cells from ionizing radiation and cytotoxic chemotherapy; therefore, the following protocol was designed to examine whether genistein could protect HSCs against the deleterious effects of chronic G-CSF treatment.

Mice were concomitantly treated with G-CSF (10 mg/kg 5 × week) and genistein (10 mg/kg) subcutaneously 3 times a week for a total of 6 weeks. At 6 weeks of G-CSF treatment, the maximum expansion of HSCs was observed, with statistically significant evidence of genomic instability.
The mean concentration of genistein in plasma reached 1.70 μmol/mL 24 hours after the last treatment. Mice were euthanized and bone marrow cells were evaluated by flow cytometry. The LSK cell population in the bone marrow increased 3.4-fold in mice treated with G-CSF; however, concurrent treatment with genistein lessened the expansion of LSK cells by 2.3-fold (Fig. 3A and Supplementary Fig. S1). The production of ROS increased after G-CSF treatment (Fig. 1D); however, we found that the levels of intracellular ROS were significantly lower in mice treated simultaneously with genistein and G-CSF (Fig. 3B). These cells were sorted and then analyzed for the amount of DNA DSBs via the presence of nuclear pH2AX. We found that LSKs from mice treated concurrently with genistein and G-CSF exhibited less DNA damage than LSKs from G-CSF–treated mice (Fig. 3C).

Genistein decreases chromosomal instability

Because we determined that LSKs from mice treated concurrently with genistein and G-CSF had less DNA damage, we verified the copy number of aberrant genomic segments.

Lin–Sca+ cells were analyzed by FISH using BAC clones for chromosome 2 and chromosome 17. Two sets of 100 cells per mouse (n = 44) were counted and the average was calculated. Our results demonstrated that after 6 weeks of G-CSF treatment, Lin–Sca+ bone marrow cells have alterations in chromosome 2 (6%) and chromosome 17 (20%), whereas cells from animals treated with genistein combined with G-CSF had fewer chromosomal abnormalities based on alterations in chromosome 2 (2%; Fig. 3D) and chromosome 17 (9%; Fig. 3E).

Bone marrow differentials showed that G-CSF increases the percentage of granulocyte and concomitant treatment with genistein did not interfere with granulocytes expansion (Fig. 4A). Animals treated simultaneously with genistein and G-CSF had a similar level of total neutrophils as the animals treated only with G-CSF (Fig. 4B). Therefore, our data suggest that genistein protects against the deleterious effects of G-CSF–induced excessive HSC proliferation and at the same time permits the desired increase in the neutrophil population.

Genistein limits DNA damage through inhibition of proliferation

To elucidate the mechanism of action of genistein, we compared its effect to that of a known antioxidant, N-acetyl-cysteine (NAC; ref. 20). Animals were treated with G-CSF for
mice (LSK population in mice treated with G-CSF or NAC 5 days of G-CSF treatment (Fig. 5A–C). The expansion in the 5 days and with genistein or NAC (50 mg/kg) on the last 2 days of G-CSF treatment (Fig. 5D). Genistein, however, blocked GSK3 phosphorylation and cyclin D1 and D3 induction in G-CSF–treated mice. To evaluate the degree of G-CSF signal activation in LSK cells, the levels of pGSK3 (Fig. 6E), cyclin D1 (Fig. 6F), and D3 (Fig. 6G) were measured. LSK cells were sorted by flow cytometry directly on glass slides and analyzed by immunofluorescence. G-CSF treatment induces GSK3 phosphorylation and increases cyclin D1 and D3 levels (Fig. 6E–G). Genistein treatment inhibited G-CSF–induced phosphorylation of GSK3 and cyclins D1 and D3 in LSK cells.

Taken together, these results suggest that genistein modulates G-CSF induction of the GSK3-cyclinD1/D3 pathway in LSK and myeloid progenitors; nonetheless, it does not interfere with myeloid proliferation and differentiation of neutrophils (Fig. 4A and B).

Discussion

The effects of prolonged G-CSF treatment on HSCs are not well understood. We hypothesized that excessive HSC proliferation induced by G-CSF could lead to deleterious consequences. Genistein is a soybean-derived isoflavone with antioxidant effects (27). It also has tyrosine kinase inhibitory properties that attenuate proliferation of both normal and cancerous cells (28). On the basis of these properties, we hypothesized that genistein could counteract the deleterious effects of excessive HSC proliferation induced by G-CSF.

G-CSF is widely used in multiple clinical settings to lessen the effects of neutropenia. Although clearly beneficial, there are concerns about the long-term effects of G-CSF. A particular concern is that G-CSF therapy may increase the risk of myelodysplastic syndrome and/or AML. G-CSF utilization in both aplastic anemia and Fanconi’s anemia has been associated with clonal evolution to AML (7, 9). Furthermore, usage of G-CSF has been associated with an increased
risk of developing MDS/AML in women who undergo chemotherapy for breast cancer (29). However, use of G-CSF during treatment of AML in one large prospective study had no impact on complete response rate or relapse rate (11).

The most compelling evidence for the increased risk of MDS/AML through G-CSF therapy comes from SCN. Although G-CSF clearly improves survival, there are several lines of evidence to suggest that G-CSF treatment contributes to the development of leukemia in these patients. First, the risk of leukemia seems to correlate with the cumulative dose of G-CSF (3). Second, of all the congenital marrow failure syndromes predisposed to AML, SCN alone does not seem to be an HSC disorder. Because AML seems to rise from sequential mutations in HSC, this would suggest that therapy, not the intrinsic cell defect, is causal (30). It has been demonstrated that G-CSF does initiate signaling pathways in HSCs (31). In addition, the presence of hyperproliferative truncation mutations and an activating mutation of the G-CSFR have been associated with the development of AML in SCN (1).

This article provides evidence that prolonged G-CSF exposure results in genomic instability in HSCs. After extended treatment with G-CSF, there is a significant increase in DNA damage in LSK cells. HSCs from mice treated \textit{in vivo} with G-CSF displayed consistent loss of regions of chromosome 2 and 17. Interestingly, the chromosome 2 deletions align with ABL1, which has previously been demonstrated to be involved in several rearrangements and chromosome translocations in various types of human leukemia (32). A mouse model of acute promyelocytic leukemia and a radiation-induced model of AML show deletions on chromosome 2 in a region containing the gene \textit{PU.1}, a transcription factor critical for myeloid development (33). Previous reports have implicated heterozygous loss of \textit{PU.1} as contributing to the development of leukemia in the aforementioned settings and in other mouse models (34). Although similar deletions in \textit{PU.1} are rare in human AML, mutations in \textit{RUNX1}, an important regulator of \textit{PU.1} expression, are commonly seen.

Consistent deletions on chromosome 17 that include the tumor suppressor gene \textit{TSC2} were also detected. \textit{TSC2}
is a negative regulator of mTOR and recent studies have demonstrated that the expression of TSC2 is downregulated in patients with acute leukemia (35). The mTOR pathway is frequently activated in blasts from patients with AML (36) and high-risk myelodysplastic syndrome (37). Furthermore, constitutive activation of the AKT/mTOR pathway has been shown to induce acute leukemia in mice (38). Collectively, the results suggest that prolonged G-CSF treatment induces DNA damage in HSCs and genistein acts as a genoprotective agent in this setting.

Despite evidence of genomic instability in HSCs, none of the mice treated with G-CSF developed leukemia. Transgenic mice overexpressing G-CSF also do not develop leukemia (39). This is not unexpected, as patients with SCN only develop AML after years of G-CSF treatment and the prevalence even after 10 years of treatment is less than 50%. As expected, the number of HSCs at risk for developing a leukemogenic mutation is significantly higher in a human than in a mouse.

Although prolonged G-CSF exposure promotes genomic instability in HSCs and is associated with the development of AML, it remains the only effective treatment for SCN, besides a HSC transplant. An ideal treatment would promote late myeloid differentiation without affecting HSCs. An alternative strategy would be to coadminister a complex that selectively blocks the effect of G-CSF on HSCs. On the basis of previous studies, genistein is an attractive...
compound. Genistein is a natural soy isoflavone with excellent bioavailability that has both antioxidant and antiproliferative properties (16, 40, 41). Both oxidative stress and excessive proliferation have been postulated to result in genomic instability in HSCs. In reality, previous studies have demonstrated that genistein protects HSCs from radiation and chemotherapy (17, 42, 43).

G-CSF treatment leads to a modest increase in ROS in LSK cells, which is reduced by genistein (Fig. 3B). Although oxidative stress has been implicated in genomic instability in HSCs, NAC treatment, while reducing ROS, did not reduce DNA damage. In addition, we did not observe increased 8-oxo-guanine levels in LSK cells treated with G-CSF (data not shown). Interestingly, ROS and cell-cycle progression seem to be linked in HSCs and the reduction in ROS may merely reflect decreased proliferation (44).

In the current study, we used a dose of genistein that results in serum levels that can easily be obtained through oral supplementation (16, 45). At this dosage, genistein partially blocked the G-CSF–induced expansion of LSK cells and significantly reduced pH2AX levels in this population. This was also accompanied by a reduction in the level of cells with an abnormal FISH signal. Importantly, genistein did not block the G-CSF–driven expansion of mature neutrophils, as the total number of neutrophils in mice treated with G-CSF and genistein were the same as in mice treated with G-CSF alone (Fig. 4A and B). Genistein seems to inhibit the G-CSF–driven expansion of LSKd48–cd150+ cells (Supplementary Fig. S2). This population, termed LSK-SLAM, is highly enriched for HSCs.

Genistein did not block G-CSF–induced expansion of a population enriched for myeloid progenitors (Lin–Sca–cKit+; Fig. 6B). Collectively, these results would suggest that the effects of genistein are mediated primarily through preferential inhibition of HSC proliferation, while not impairing myeloid progenitor proliferation and differentiation. Genistein inhibits G-CSF–induced GSK3 phosphorylation and cyclin D1 and D3 induction in LSK and myeloid progenitors (Fig. 6D–C). Our results suggest that there is a distinct requirement for GSK3/cyclin D1/D3 in G-CSF-modulated pathways in myeloid progenitors and HSCs.

It has been shown that the absence of GSK3β impairs long-term self-renewal capacity of HSCs, although it is not essential for myeloid development (23). While HSCs from the triple cyclin knockout mouse (cyclins D1, D2, and D3) display delayed cell-cycle entry and multilineage hematopoietic failure (46, 47), single knockouts for D1 or D2 display normal granulocyte counts. Mice lacking cyclin D3 show impaired neutrophil development, however, all D1−/−, D2−/−, D3−/− single knockout mice present normal myelopoiesis (48).

The data imply that genistein treatment prevents G-CSF–induced GSK3 phosphorylation, thus activating GSK3. GSK3 has been shown to regulate cyclin D1 and D3 through various mechanisms including mRNA transcription, protein localization, and ubiquitin-dependent proteolysis (49); however, the mechanism involved in genistein modulation of GSK3/cyclin D1/D3 in G-CSF–treated mice requires further elucidation.

Collectively, our results indicate that prolonged G-CSF treatment induces DNA damage in HSCs by initiating cell-cycle progression. HSCs are long-lived, quiescent cells that preferentially use nonhomologous end joining (NHEJ) for DNA repair when progressing from G0 to G1 (19, 50). NHEJ is a relatively error-prone DNA repair mechanism, and its preferential use by HSCs has been postulated as a reason that chromosomal deletions and translocations are often seen and are frequently causal in the development of acute leukemia (19). Further evidence is provided by recent whole genome wide array sequencing that has shown that HSCs accumulate mutations over time. Importantly, we demonstrate that genistein, at levels obtainable through dietary supplementation, is able to reduce DNA damage by attenuating G-CSF–induced HSC proliferation without compromising the ability of G-CSF to accelerate terminal neutrophilic differentiation. These results suggest that genistein may be an effective preventive agent in patients with SCN who require prolonged G-CSF support.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors’ Contributions

Conception and design: L.R. Souza, O. Kucuk, M.L. McLemore
Development of methodology: L.R. Souza, O. Kucuk, M. Rossi, M.L. McLemore
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): L.R. Souza, E. Silva, E. Calloway
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): L.R. Souza, E. Silva, M. Rossi
Writing, review, and/or revision of the manuscript: L.R. Souza, E. Silva, O. Kucuk, M. Rossi, M.L. McLemore
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): L.R. Souza, M. Rossi, M.L. McLemore
Study supervision: L.R. Souza, M.L. McLemore

Acknowledgments

Omer Kucuk is a Georgia Cancer Coalition Distinguished Scholar. The authors thank Dr. Debra F. Saxe and Faith Sheff (Emory University) for their assistance with the FISH assays. Dr. Daniel R. Doerge (U.S. Food and Drug Administration, Jefferson, AR) for analyzing the total isoflavone levels on mice serum; Dr. Hanna J. Khoury and Leon Bernal-Matizachi (Emory University) for their critical reading of this article; Anthea Hammond (Emory University) for the proofing and editing of this article; Dr. Adam Marcus and Deborah Eltzroth Martinson at the Winship Cell Imaging Core, for their expertise on imaging acquisition; and Aaron Rae at the Flow Cytometry Core at Emory Children’s Pediatric Research Center, for his assistance in cell sorting and analysis. The authors also thank Vinicius Mieses de Andrade Carvalho, PhD student at UNICAMP-Brazil, for his assistance on some of the assays using G-CSF–treated and untreated mice during the summer of 2011.

Grant Support

This work was supported by the ‘Charles Harris Leukemia’ & the ‘Kelly Wilhite Aplastic Anemia’ Research Fund. No governmental grants funded this research.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received August 15, 2013; revised February 17, 2014; accepted February 18, 2014; published OnlineFirst March 10, 2014.

Published Online First: March 10, 2014; DOI: 10.1158/1940-6207.CAPR-13-0295
References


Genistein Protects Hematopoietic Stem Cells against G-CSF–Induced DNA Damage

Liliana R. Souza, Erica Silva, Elissa Calloway, et al.


Updated version
Access the most recent version of this article at:
doi:10.1158/1940-6207.CAPR-13-0295

Supplementary Material
Access the most recent supplemental material at:
http://cancerpreventionresearch.aacrjournals.org/content/suppl/2014/03/12/1940-6207.CAPR-13-0295.DC1

Cited articles
This article cites 50 articles, 16 of which you can access for free at:
http://cancerpreventionresearch.aacrjournals.org/content/7/5/534.full.html#ref-list-1

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.