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Genistein induces topoisomerase II β - and proteasome-mediated DNA sequence rearrangements: Implications in infant leukemia

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ABSTRACT

Genistein is a bioflavonoid enriched in soy products. However, high levels of maternal soy consumption have been linked to the development of infant leukemia ALL and AML. The majority of infant leukemia is linked to mixed lineage leukemia gene (MLL) translocations. Previous studies have implicated topoisomerase II (Top2) in genistein-induced infant leukemia. In order to understand the roles of the two Top2 isozymes in and the molecular mechanism for genistein-induced infant leukemia, we carried out studies *in vitro* using purified recombinant human Top2 isozymes, as well as studies in cultured mouse myeloid progenitor cells (32Dc13) and Top2 β knockout mouse embryonic fibroblasts (MEFs). First, we showed that genistein efficiently induced both Top2 α and Top2 β cleavage complexes in the purified system as well as in cultured mouse cells. Second, genistein induced proteasomal degradation of Top2 β in 32Dc13 cells. Third, the genistein-induced DNA double-strand break (DSB) signal, γ -H2AX, was dependent on the Top2 β isozyme and proteasome activity. Fourth, the requirement for Top2 β and proteasome activity was mirrored in genistein-induced DNA sequence rearrangements, as monitored by a DNA integration assay. Together, our results suggest a model in which genistein-induced Top2 β cleavage complexes are processed by proteasome, leading to the exposure of otherwise Top2 β -concealed DSBs and subsequent chromosome rearrangements, and implicate a major role of Top2 β and proteasome in genistein-induced infant leukemia.

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1. Introduction

Genistein is a natural bioflavonoid (isoflavone) mostly found in soy-based foods. Genistein exhibits cancer-chemopreventive and antitumor activities, as well as anti-oxidant, anti-inflammatory and anti-*in vitro* angiogenesis effects [1–3]. However, clinical studies have suggested a strong link between a prior exposure to dietary bioflavonoids including genistein and infant leukemia. It was demonstrated that maternal consumption of bioflavonoid-rich foods led to an approximately 10-fold higher risk of infant acute myelogenous leukemia (AML) [4,5].

Infant leukemia is frequently associated with chromosome translocations involving the mixed lineage leukemia 1 (MLL) gene [6]. It has been estimated that up to 80% of infant acute lymphoblastic leukemia (ALL) and 65% of infant AML are linked to MLL translocations [7]. These translocations can take place *in utero* and are associated with poor prognosis, especially in infant ALL [5]. Interestingly, MLL translocations are also hallmarks of more than 70% of t-AML (therapy-related acute myelogenous leukemia) associated with topoisomerase II (Top2)-based chemotherapy in cancer patients [8]. Mapping of chromosomal breakpoints of MLL

translocations has revealed the clustering of breakpoints within an 8.3 kb region of the human MLL gene, known as the breakpoint cluster region (BCR) [8]. The genomic breakpoints in infant leukemia and t-AML tend to co-localize with Top2 cleavage sites, suggesting a possible link between infant leukemia and Top2 [8,9].

Genistein is known to induce Top2-linked DNA breaks (Top2 cleavage complexes) [3]. There are two human Top2 isozymes, Top2 α and Top2 β , that share 70% sequence identity [10]. Top2 α has been suggested to function in cell cycle events such as DNA replication and chromosome condensation/segregation [11], whereas Top2 β has been shown to be involved in transcription [12–15]. Recent studies have shown that cancer chemotherapeutic drugs that target Top2 poison both Top2 α and Top2 β [10]. It has been suggested that Top2 α targeting (poisoning) is primarily responsible for the antitumor activity of these drugs while Top2 β targeting could lead to secondary malignancies such as (Top2 drug) therapy-related acute myelogenous leukemia (t-AML) [16]. Since genistein is a Top2-targeting compound, we envision that genistein may induce infant leukemia through poisoning of the Top2 β isozyme.

Previous studies have demonstrated that the induction of DNA double-strand breaks (DSBs) by Top2-targeting drugs characteristically requires the proteasome activity [17]. It has been shown that Top2-targeting drugs induce preferential degradation of the Top2 β isozyme (Top2 β down-regulation) in various cells, leading to the exposure of the otherwise Top2-concealed DSBs [16,17]. It has

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been proposed that Top2 β down-regulation is the underlying mechanism for Top2 drug-induced DNA sequence rearrangements and carcinogenesis [16].

In the present study, we have tested the role of Top2 β and proteasome in genistein-induced DSBs and chromosome rearrangements. Our results suggest that proteasomal processing of genistein-induced Top2 β cleavage complexes results in DSB formation and DNA sequence rearrangements, thus implicating an important role of Top2 β and proteasome in genistein-induced MLL translocations and infant leukemia.

2. Materials and methods

2.1. shRNA-mediated knockdown of Top2 β in 32Dc13 mouse myeloid progenitor cells

The Control (Ctrl) or Top2 β shRNA sequences were selected using the Whitehead Institute siRNA selection program (<http://jur-a.wi.mit.edu/bioc/siRNAext/>) and the corresponding oligo duplex DNAs were cloned into a LentiLox 3.7 vector with an inserted neomycin-resistant gene. Standard procedures were then followed to generate stable Top2 β - or control-knockdown 32Dc13 cells lines.

2.2. Top2-mediated DNA cleavage assay

The Top2 cleavage assay was performed as described [18].

2.3. Measurement of plasmid integration frequency

For measuring the plasmid integration frequency in MEFs, procedures were followed as previously described [19]. For measuring the plasmid integration frequency in 32Dc13 cells, 2×10^6 cells were seeded 24 h prior to the experiment. Treatment and transfection were performed as described [19]. Six hours post-transfection, cells were washed three times, replenished with fresh medium and incubated for additional 24 h to allow the expression of the blasticidin resistance gene. Cells were then trypsinized and cultured in methylcellulose (Methocult[®] 3134 medium) in RPMI/IL3 medium supplemented with blasticidin (3 μ g/ml). A small aliquot of cells were also cultured in the absence of blasticidin for survival determination. After 10 days, blasticidin-resistant colonies were counted by the IPI MiniCount colony counter. Plasmid integration frequency was determined as the ratio of the number of blasticidin-resistant colonies and the number of surviving cells (number of colonies without blasticidin selection times the dilution factor). The average and standard error of mean were then calculated and plotted.

2.4. Top2 β down-regulation

To determine genistein- or VP-16-induced Top2 β down-regulation, procedures were followed as described previously [17,20].

2.5. Band depletion assay

To determine the level of drug-induced intracellular Top2 cleavage complexes, the band depletion assay was performed as previously described [16].

3. Results

3.1. Genistein induces Top2 cleavage complexes and proteasomal degradation of Top2 β (Top2 β down-regulation)

As shown in Fig. 1A, genistein, like VP-16 (a prototypic Top2 poison), induced both Top2 α and Top2 β -mediated DNA cleavage

in a concentration-dependent manner, most certainly reflecting the formation of cleavage complexes with the two Top2 isozymes (Fig. 1A, note the disappearance of the full length DNA (labeled **) and the appearance of the cleaved DNA fragments (labeled *)). Relative to VP-16, genistein appeared to be slightly more effective in inducing Top2 β - than Top2 α -mediated DNA cleavages. While VP-16 induced comparable levels of Top2 α - and Top2 β -mediated DNA cleavages (lanes 3–8 and lanes 17–22), genistein could induce more extensive Top2 β -mediated DNA cleavages as compared to Top2 α -mediated DNA cleavages at all concentrations of genistein being tested (lanes 9–14 and lanes 23–28). We also tested whether genistein could induce Top2 cleavage complexes in cells. As shown in Fig. 1B, genistein, like VP-16, caused depletion of both Top2 α and Top2 β immunobands within 15 min of treatment as evidenced by a band depletion assay [16], suggestive of the formation of Top2 cleavage complexes (too large to enter the gel due to the large size of the Top2-DNA covalent complexes). As expected for reversible Top2 cleavage complexes [21], genistein- (as well as VP-16-) induced depletion of both Top2 α and Top2 β immunobands was largely abolished following replenishing the cells with drug-free medium and further incubation for 30 min (Fig. 1B, labeled Rev + genistein and Rev + VP-16), demonstrating the reversibility of genistein-induced Top2 cleavage complexes.

The formation of Top2 cleavage complexes (e.g. induced by VP-16) is known to trigger degradation of Top2 β through a proteasome-dependent pathway (Top2 β down-regulation), resulting in the exposure of the otherwise Top2 β -concealed DSBs [17]. To test whether genistein could also induce Top2 β down-regulation, the Top2 β protein levels were monitored in genistein-treated 32Dc13 hematopoietic progenitor cells. As shown in Fig. 1C, genistein (500 μ M) treatment reduced the Top2 β level by about 50% in 2 h. By contrast, the level of Top2 α did not change significantly upon genistein treatment. In addition, genistein-induced Top2 β degradation was prevented by co-treatment with the proteasome inhibitor MG132 (Fig. 1C). These results suggest that similar to VP-16, genistein could induce Top2 β down-regulation through a proteasome-dependent pathway.

3.2. Genistein-induced DNA double-strand breaks (DSBs) require Top2 β and proteasome

Previous studies have linked VP-16-induced Top2 β down-regulation to the formation of DSBs [16,17]. Since our studies have demonstrated that genistein induces Top2 β down-regulation, we tested the possibility that genistein could induce DSBs in a proteasome-dependent manner. As shown in Fig. 1C, genistein, like VP-16, induced γ -H2AX (phosphorylation of histone H2AX at Ser-139, a prototypic DSB marker) in 32Dc13 cells, suggesting that genistein could induce DSBs. Interestingly, genistein-induced DSBs, like VP-16-induced DSBs, were significantly reduced in the presence of the proteasome inhibitor MG132, suggesting that genistein-induced DSBs requires proteasome activity. We also tested the possibility that genistein-induced DSBs require Top2 β . As shown in Fig. 2A, genistein induced γ -H2AX in Top2 β ^{+/+}, but not in Top2 β ^{-/-} MEFs. As a positive control, we showed that VP-16-induced γ -H2AX also required Top2 β (Fig. 2A). By contrast, camptothecin (CPT), a Top1 (topoisomerase I)-targeting drug, induced γ -H2AX independent of Top2 β (Fig. 2A). Together, these results suggest that genistein-induced DSBs, like VP-16-induced DSBs, require both proteasome and Top2 β .

VP-16-induced Top2 β cleavage complexes have been suggested to arrest transcription, triggering proteasomal degradation of Top2 β and concomitant exposure of the Top2 β -concealed DSBs [17]. To determine whether genistein-induced DSBs also specifically involves active transcription, we tested the effect of the transcription inhibitor DRB and the protein synthesis inhibitor

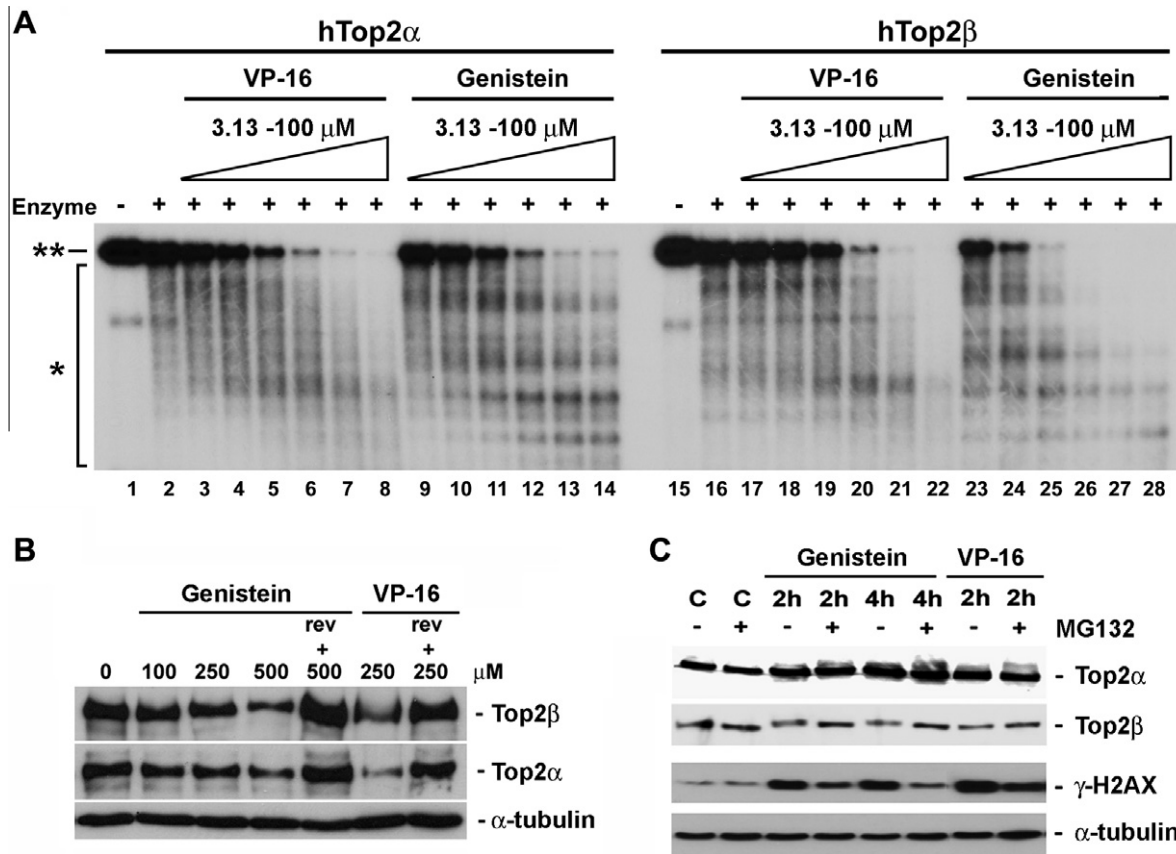


Fig. 1. Induction of Top2 cleavage complexes and Top2 β down-regulation by genistein. (A) Genistein induces Top2 α - and Top2 β -mediated DSBs *in vitro*. VP-16 (0 μ M (lanes 2 & 16), 3.13 μ M (lanes 3 & 17), 6.25 μ M (lanes 4 & 18), 12.5 μ M (lanes 5 & 19), 25.0 μ M (lanes 6 & 20), 50.0 μ M (lanes 7 & 21), 100 μ M (lanes 8 & 22)) and genistein (0 μ M (lanes 2 & 16), 3.13 μ M (lanes 9 & 23), 6.25 μ M (lanes 10 & 24), 12.5 μ M (lanes 11 & 25), 25.0 μ M (lanes 12 & 26), 50.0 μ M (lanes 13 & 27), 100 μ M (lanes 14 & 28)) were incubated with 32 P-labeled linearized plasmid DNA in the presence of purified recombinant hTop2 α (lanes 1–14) or hTop2 β (lanes 15–28) as described in Section 2 (DNA cleavage assay). **Full length 32 P-labeled linearized plasmid DNA; *Cleaved DNA fragments. DNA cleavage products were then analyzed by agarose gel electrophoresis, followed by autoradiography. (B) Genistein traps both Top2 α and Top2 β cleavage complexes in mouse 32Dc13 progenitor cells. The amounts of genistein-induced Top2 α and the Top2 β cleavage complexes were measured by the band depletion assay. 32Dc13 cells, expressing both Top2 isozymes were treated with genistein (100, 250 or 500 μ M) or VP-16 (250 μ M) for 15 min, followed by lysis in 6X sample buffer. Cell lysate were then analyzed by SDS–PAGE and immunoblotted with isozyme-specific antibodies. This assay measures the levels of free Top2 isozymes in the lysate as trapping on DNA leads to the decreased free Top2 levels. To demonstrate the reversibility of genistein- and VP-16-induced Top2 cleavage complexes, drug-treated cells were further incubated in drug-free medium for an additional 30 min, followed by immunoblotting analysis. (C) Mouse 32Dc13 progenitor cells, expressing both Top2 isozymes, were treated with genistein (500 μ M) or VP-16 (100 μ M) for 0 and 2 h in the absence or presence of the proteasome inhibitor MG132 (10 μ M). Cells were then lysed in alkaline lysis buffer followed by neutralization and S7 nuclease digestion. After SDS–PAGE, the amount of the Top2 isozymes was measured by immunoblotting.

cycloheximide (CHX) on the induction of genistein-induced DSB signal, γ -H2AX. As shown in Fig. 2B, genistein-induced γ -H2AX in Top2 $\beta^{+/+}$ MEFs was also largely prevented by the transcription inhibitor DRB (Fig. 2B), but not by the protein synthesis inhibitor cycloheximide (Fig. 2C). Additionally, we showed that the requirement for active transcription and proteasome in DSB induction by genistein and VP-16 was specific since the induction of γ -H2AX by hydrogen peroxide (H_2O_2) was not affected by neither the proteasome inhibitor MG132 nor the transcription inhibitor DRB (Fig. 2B). It indicated that in contrast to Top2-mediated DNA damage, reactive oxygen species (ROS)-induced DNA damage did not involve transcription or proteasome-mediated processing. Furthermore, we have showed that while *N*-acetyl cysteine (NAC, 1 mM) could almost completely block the H_2O_2 -induced γ -H2AX signal, it had minimal effect on the genistein- or VP-16-induced γ -H2AX signal (Fig. 2D). These results further suggested that genistein could induce DNA damage through a mechanism that did not involve ROS.

3.3. Genistein-induced DNA sequence rearrangements are Top2 β - and proteasome-dependent

Genistein is known to induce chromosome translocations involving the MLL locus in CD34⁺ hematopoietic progenitors [22].

However, the molecular basis for genistein-induced rearrangement, other than the involvement of DSBs, is not clear. Since genistein induces Top2 β - and proteasome-dependent DSBs, we hypothesize that genistein-induced chromosome translocations could result from DSBs generated from the proteasomal processing of Top2 β cleavage complexes. To test the possible involvement of Top2 β and proteasome in genistein-induced chromosome translocations, we employed the plasmid integration assay that measures the integration frequency of a plasmid-born resistance gene into chromosomal DNAs in order to score DNA sequence rearrangement events induced by genistein. To test the involvement of Top2 β , we have generated stable Top2 β knockdown and control-knockdown 32Dc13 lines expressing Top2 β shRNA (shTop2 β) and control shRNA (shCtrl), respectively. Immunoblotting analysis showed that 32Dc13-shTop2 β cells expressed much reduced level of Top2 β as compared to 32Dc13-shCtrl cells (Fig. 3A, inset). As shown in Fig. 3A, genistein (100 μ M) stimulated plasmid integration at a much higher frequency in 32Dc13-shCtrl cells than in 32Dc13-shTop2 β cells. A similar trend of decrease in plasmid integration frequency was also observed for VP-16 (0.5 μ M). We have also employed plasmid integration assay using SV-40-transformed Top2 $\beta^{+/-}$ and Top2 $\beta^{-/-}$ MEFs. As shown in Fig. 3B, genistein-stimulated plasmid integration was much less frequent in Top2 $\beta^{-/-}$ cells as

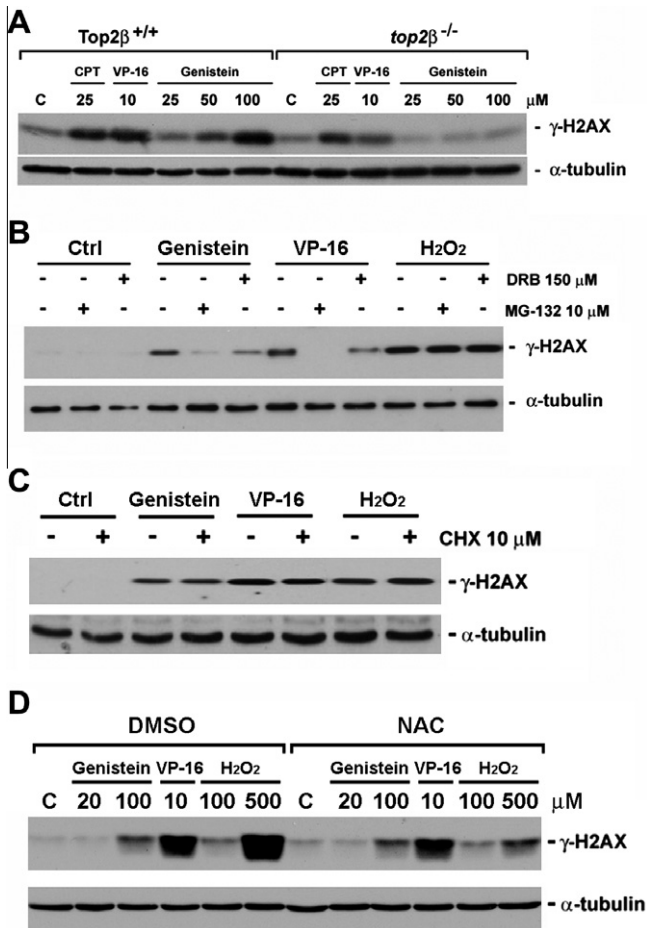


Fig. 2. Genistein-induced DNA damage signal is Top2 β - and proteasome-dependent in primary MEFs. (A) Primary Top2 $\beta^{+/+}$ and Top2 $\beta^{-/-}$ MEFs were treated with 25 μ M CPT, 10 μ M VP-16 and/or increasing concentrations of genistein (25, 50, 100 μ M) for 1 h, followed by lysis in 6 \times SDS sample buffer and immunoblotted with antibodies against γ -H2AX and α -tubulin. (B) The genistein-induced γ -H2AX signal requires proteasome activity and transcription. Primary Top2 $\beta^{+/+}$ MEFs were treated with MG132 (10 μ M) or DRB (150 μ M) for 30 min, followed by co-treatment with VP-16 (10 μ M), genistein (100 μ M) or hydrogen peroxide (H_2O_2 , 400 μ M) for 1 h. Western blotting was performed as described in (A). (C) Genistein-induced γ -H2AX does not require protein synthesis. Primary Top2 $\beta^{+/+}$ MEFs were treated with cycloheximide (CHX, 10 μ M) for 30 min, followed by co-treatment with VP-16 (10 μ M), genistein (100 μ M) or hydrogen peroxide (H_2O_2 , 400 μ M) for 1 h. Western blotting was performed as described in (A). (D) N-acetyl cysteine had no effect on the genistein-induced DNA damage. Primary Top2 $\beta^{+/+}$ MEFs were treated with 0.1% DMSO or 1 mM N-acetyl cysteine (NAC) for 30 min, followed by co-treatment with genistein (20 μ M, 100 μ M), VP-16 (10 μ M) or H_2O_2 (100 μ M, 500 μ M) for 2 h. Western blotting was performed as described in (A).

compared to Top2 $\beta^{+/+}$ cells. Together, these results suggest strongly that Top2 β plays an important role in genistein-, and VP-16-, induced DNA sequence rearrangements.

We next tested the role of proteasome in genistein-induced chromosome rearrangements using the plasmid integration assay. As shown in Fig. 4B, co-treatment with the proteasome inhibitor MG132 during transfection led to a greater than 2-fold decrease in genistein-stimulated plasmid integration frequency, similar to that observed for VP-16 (this study and also see Ref. [16]). These findings are in agreement with the model that proteasomal processing of Top2 β cleavage complexes exposes Top2 β -concealed DSBs, leading to chromosome rearrangements.

4. Discussion

Previous studies have demonstrated that genistein-induced chromosomal DNA cleavages as well as chromosome translocations

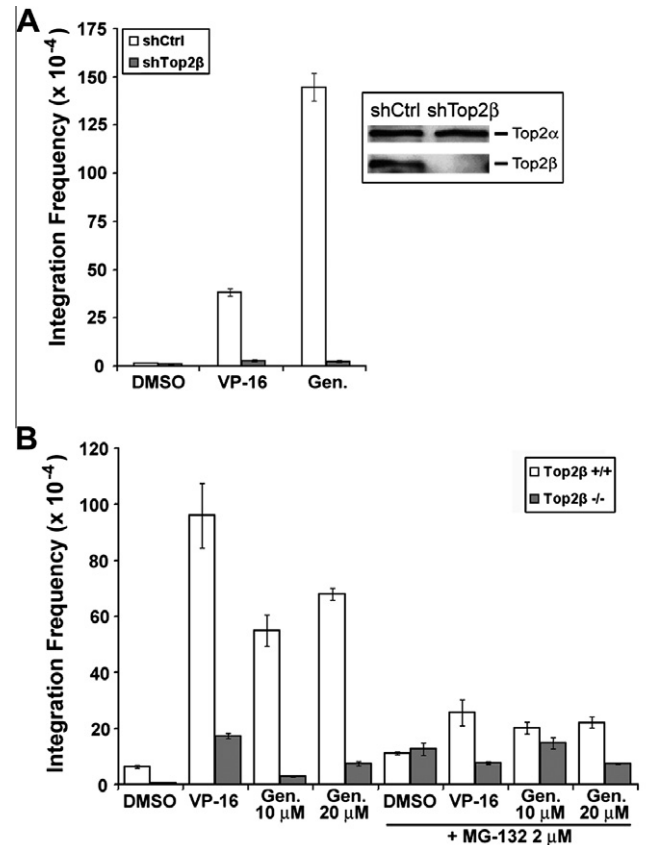


Fig. 3. Genistein-induced plasmid integration is Top2 β and proteasome-mediated. (A) Mouse 32Dc13 progenitor cells expressing control shRNA (shCtrl) or Top2 β shRNA (shTop2 β) were transfected with linearized pUCSV-BSD plasmid in the presence of DMSO (0.1%), VP-16 (0.5 μ M) or genistein (100 μ M) as indicated. Plasmid integration frequency was then determined and plotted as histograms. (B) SV40-transformed Top2 $\beta^{+/+}$ and Top2 $\beta^{-/-}$ MEFs were transfected with the linearized pUCSV-BSD plasmid in the presence of DMSO (0.1%), VP-16 (0.5 μ M) or genistein (10 or 20 μ M) as indicated. In addition, similar transfections were also performed in the presence of the proteasome inhibitor MG132 (2 μ M). Plasmid integration frequencies were then determined.

involve the MLL BCR [9,22]. These genistein-induced DNA cleavages and chromosome translocations have been suggested to result from genistein-trapped Top2 cleavage complexes within the MLL BCR [8,23,24]. However, the relative contribution of the two Top2 isozymes (i.e. Top2 α and Top2 β) as well as the mechanism for processing Top2 cleavage complexes into DSBs, in genistein-induced chromosome translocations is unknown. Studies of etoposide (VP-16)-induced carcinogenesis in the classical mouse skin carcinogenesis model have suggested that etoposide-induced skin carcinogenesis is primarily Top2 β -mediated [16]. It has been suggested that etoposide behaves as a stage II tumor promoter, presumably by promoting DNA sequence rearrangements [17]. The predominant role of the Top2 β isozyme in etoposide-induced carcinogenesis could suggest that the Top2 β isozyme may be involved in genistein-induced MLL translocations and infant leukemia.

In the current studies, we show that genistein-induced DNA sequence rearrangements in murine myeloid progenitor D32c13 cells and MEFs require both Top2 β and the proteasome activity. Although genistein can induce the formation of both Top2 α and Top2 β cleavage complexes in these cells, the strong dependence on Top2 β rather than Top2 α for genistein-induced DNA sequence rearrangements is most likely related to the differential cellular processing of Top2 β and Top2 α cleavage complexes. It is known that Top2 β cleavage complexes are more readily converted into DSBs than Top2 α cleavage complexes through a proteasome-dependent pathway [16,17]. Indeed, we have shown that

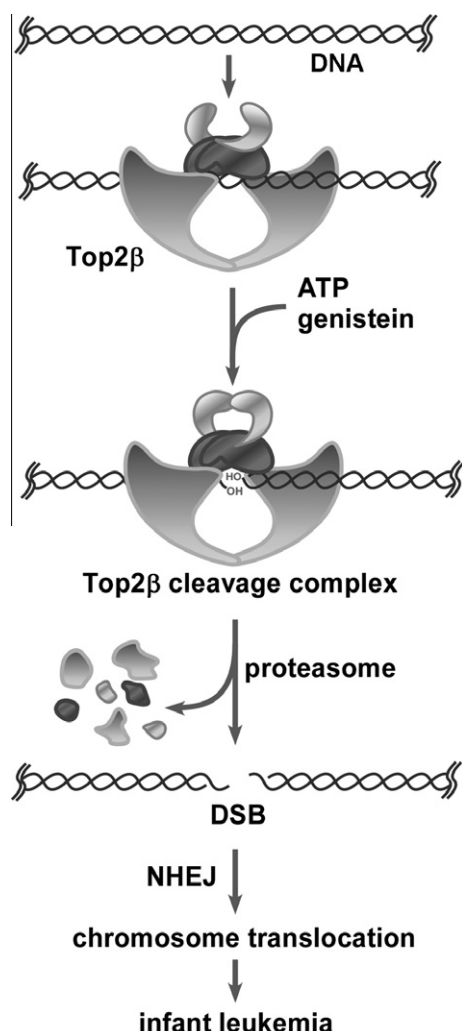


Fig. 4. A proposed model for genistein-induced DNA sequence rearrangements and infant leukemia. In this model, genistein stabilizes Top2 β -DNA covalent adducts (Top2 β cleavage complex) on chromosomal DNA within the transcribed regions. Top2 β cleavage complexes arrest transcription elongation, triggering Top2 β degradation through a proteasome-dependent pathway. Proteasomal degradation of Top2 β cleavage complexes expose Top2 β -concealed DSBs. Subsequent repair of the DSBs through the non-homologous end joining pathway (NHEJ) leads to DNA sequence rearrangements. DSBs located within MLL BCR can undergo NHEJ leading to MLL translocations and hence infant leukemias.

genistein-induced DSBs are both Top2 β - and proteasome-dependent. Consequently, while genistein induces both Top2 α and Top2 β cleavage complexes to a similar extent, genistein-induced DNA sequence rearrangements are Top2 β -dependent, presumably due to the propensity of Top2 β cleavage complexes to undergo proteasomal processing into DSBs (see Fig. 4 for a schematic model).

The difference between the two Top2 isozymes in mediating cellular responses to the Top2-targeting drugs is probably related to their differential regulation and distinct roles in various cellular functions. Top2 α is known to be a proliferation marker and participate in cell cycle events such as DNA replication, chromosome condensation and sister-chromatid segregation [11]. It seems possible that the chemopreventive and antitumor activities of genistein could at least in part result from its targeting of the Top2 α isozyme. Top2 α cleavage complexes induced by genistein could selectively kill tumor cells due to elevated levels of Top2 α and hence elevated Top2 α cleavage complexes in tumor cells. By contrast, the Top2 β isozyme is expressed more or less at a constant level in both proliferating and quiescent cells [11], and has been implicated in regulation of gene expression, presumably by controlling regional

chromatin condensation/decondensation [13,25]. Top2 β has been shown to localize in the transcribed regions of human rDNA repeats [26], as well as the promoter and transcribed regions of protein-coding genes [13]. As genistein-induced MLL translocations occur within the transcribed region of the MLL gene (i.e. the MLL BCR) and the transcribed regions of the various MLL partner genes, it seems plausible that the initial step may involve trapping of Top2 β into cleavage complexes within the transcribed regions of both MLL (e.g. BCR) and its partner genes. The Top2 β -DNA covalent adducts (cleavage complexes) then arrest the elongating RNA polymerase complexes, triggering proteasomal degradation of Top2 β -DNA covalent adducts and concomitant exposure of otherwise Top2 β -concealed DSBs. The initial formation of genistein-induced DSBs could have at least two outcomes; first, these DSBs may directly undergo NHEJ resulting in chromosomal translocations (e.g. MLL translocations). Second, these DSBs could induce apoptosis, resulting in activation of apoptotic nucleases (e.g. CAD) and the formation of secondary DSBs at nuclease hypersensitive sites (e.g. the MLL BCR). These DSBs formed at nuclease hypersensitive sites, which are reflected in the formation of high-molecular-weight DNA fragmentations [27–29], can also undergo NHEJ causing chromosomal translocations (e.g. MLL translocations at MLL BCR). Clearly, the possible involvement of CAD in genistein-induced MLL translocations and infant leukemia needs further investigation.

Although the concentration of genistein used in our studies seems to be much higher than the peak plasma concentration as determined previously (8 μ mol/L [30]), it is possible that the intracellular accumulation of genistein may reach much higher levels. In addition, our *in vitro* studies have shown that genistein could induce Top2 β -mediated DNA cleavages at concentrations as low as 3.13, 6.25 and 12.5 μ M (Fig. 1A, lanes 23–25). These results could suggest that genistein may induce low levels of chromosome breaks at physiologically achievable concentrations *in vivo*. Although undetectable by biochemical methods, these low levels of chromosome breaks at specific chromosomal regions (e.g. the MLL BCR) could contribute to chromosome translocations and infant leukemia.

5. Conclusions

In the present study, we have tested the role of Top2 β and proteasome in genistein-induced DSBs and chromosome rearrangements. We show that genistein, like other Top2 drugs, induces proteasomal degradation of Top2 β . In addition, we show that genistein-induced DNA damage and sequence rearrangements are Top2 β -mediated and can be prevented by co-treatment with the proteasome inhibitor MG132. These results suggest that proteasomal processing of genistein-induced Top2 β cleavage complexes results in DSB formation and DNA sequence rearrangements, thus implicating an important role of Top2 β and proteasome in genistein-induced MLL translocations and infant leukemia.

Acknowledgments

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Appendix A. Supplementary data

Supplementary Materials and Methods associated with this article can be found, in the online version, at [doi:10.1016/j.bbrc.2010.07.043](https://doi.org/10.1016/j.bbrc.2010.07.043).

References

- [1] T. Fotsis, M. Pepper, H. Adlercreutz, T. Hase, R. Montesano, L. Schweigerer, Genistein a dietary ingested isoflavonoid inhibits cell proliferation and in vitro angiogenesis, *J. Nutr.* 125 (1995) 790S–797S.
- [2] B. Halliwell, Are polyphenols antioxidants or pro-oxidants? What do we learn from cell culture and in vivo studies?, *Arch Biochem. Biophys.* 476 (2008) 107–112.
- [3] G. Peterson, Evaluation of the biochemical targets of genistein in tumor cells, *J. Nutr.* 125 (1995) 784S–789S.
- [4] J.A. Ross, Maternal diet and infant leukemia: a role for DNA topoisomerase II inhibitors, *Int. J. Cancer (Suppl. 11)* (1998) 26–28.
- [5] C.A. Felix, B.J. Lange, Leukemia in infants, *Oncologist* 4 (1999) 225–240.
- [6] L.G. Spector, Y. Xie, L.L. Robison, N.A. Heerema, J.M. Hilden, B. Lange, C.A. Felix, S.M. Davies, J. Slavin, J.D. Potter, C.K. Blair, G.H. Reaman, J.A. Ross, Maternal diet and infant leukemia: the DNA topoisomerase II inhibitor hypothesis: a report from the children's oncology group, *Cancer Epidemiol. Biomarkers Prev.* 14 (2005) 651–655.
- [7] E. Canaani, T. Nakamura, T. Rozovskaia, S.T. Smith, T. Mori, C.M. Croce, A. Mazo, ALL-1/MLL1 a homologue of *Drosophila* TRITHORAX modifies chromatin and is directly involved in infant acute leukaemia, *Br. J. Cancer* 90 (2004) 756–760.
- [8] Y. Zhang, J.D. Rowley, Chromatin structural elements and chromosomal translocations in leukemia, *DNA Repair (Amst.)* 5 (2006) 1282–1297.
- [9] R. Strick, P.L. Strissel, S. Borgers, S.L. Smith, J.D. Rowley, Dietary bioflavonoids induce cleavage in the MLL gene and may contribute to infant leukemia, *Proc. Natl. Acad. Sci. USA* 97 (2000) 4790–4795.
- [10] C.A. Austin, K.L. Marsh, Eukaryotic DNA topoisomerase II beta, *Bioessays* 20 (1998) 215–226.
- [11] J.C. Wang, Cellular roles of DNA topoisomerases: a molecular perspective, *Nat. Rev. Mol. Cell Biol.* 3 (2002) 430–440.
- [12] B.G. Ju, V.V. Lunyak, V. Perissi, I. Garcia-Bassets, D.W. Rose, C.K. Glass, M.G. Rosenfeld, A topoisomerase IIbeta-mediated dsDNA break required for regulated transcription, *Science* 312 (2006) 1798–1802.
- [13] Y.L. Lyu, C.P. Lin, A.M. Azarova, L. Cai, J.C. Wang, L.F. Liu, Role of topoisomerase IIbeta in the expression of developmentally regulated genes, *Mol. Cell. Biol.* 26 (2006) 7929–7941.
- [14] Y.L. Lyu, J.C. Wang, Aberrant lamination in the cerebral cortex of mouse embryos lacking DNA topoisomerase IIbeta, *Proc. Natl. Acad. Sci. USA* 100 (2003) 7123–7128.
- [15] K. Tsutsui, K. Sano, A. Kikuchi, A. Tokunaga, Involvement of DNA topoisomerase IIbeta in neuronal differentiation, *J. Biol. Chem.* 276 (2001) 5769–5778.
- [16] A.M. Azarova, Y.L. Lyu, C.P. Lin, Y.C. Tsai, J.Y. Lau, J.C. Wang, L.F. Liu, From the cover: roles of DNA topoisomerase II isozymes in chemotherapy and secondary malignancies, *Proc. Natl. Acad. Sci. USA* 104 (2007) 11014–11019.
- [17] A. Zhang, Y.L. Lyu, C.P. Lin, N. Zhou, A.M. Azarova, L.M. Wood, L.F. Liu, A protease pathway for the repair of topoisomerase II-DNA covalent complexes, *J. Biol. Chem.* 281 (2006) 35997–36003.
- [18] K.M. Tewey, T.C. Rowe, L. Yang, B.D. Halligan, L.F. Liu, Adriamycin-induced DNA damage mediated by mammalian DNA topoisomerase II, *Science* 226 (1984) 466–468.
- [19] E.S. Hars, Y.L. Lyu, C.P. Lin, L.F. Liu, Role of apoptotic nuclease caspase-activated DNase in etoposide-induced treatment-related acute myelogenous leukemia, *Cancer Res.* 66 (2006) 8975–8979.
- [20] Y. Mao, S.D. Desai, C.Y. Ting, J. Hwang, L.F. Liu, 26 S proteasome-mediated degradation of topoisomerase II cleavable complexes, *J. Biol. Chem.* 276 (2001) 10658–10652.
- [21] L.F. Liu, DNA topoisomerase poisons as antitumor drugs, *Annu. Rev. Biochem.* 58 (1989) 351–375.
- [22] S. Barjesteh van Waalwijk van Doorn-Khosrovani, J. Janssen, L.M. Maas, R.W. Godschalk, J.G. Nijhuis, F.J. van Schooten, Dietary flavonoids induce MLL translocations in primary human CD34⁺ cells, *Carcinogenesis* 28 (2007) 1703–1709.
- [23] O.L. Kantidze, S.V. Razin, Chemotherapy-related secondary leukemias: a role for DNA repair by error-prone non-homologous end joining in topoisomerase II – induced chromosomal rearrangements, *Gene* 391 (2007) 76–79.
- [24] M. Malik, K.C. Nitiss, V. Enriquez-Rios, J.L. Nitiss, Roles of nonhomologous end-joining pathways in surviving topoisomerase II-mediated DNA damage, *Mol. Cancer Ther.* 5 (2006) 1405–1414.
- [25] K. Sano, M. Miyaji-Yamaguchi, K.M. Tsutsui, K. Tsutsui, Topoisomerase IIbeta activates a subset of neuronal genes that are repressed in AT-rich genomic environment, *PLoS One* 3 (2008) e4103.
- [26] M. Govoni, S. Neri, T. Labella, J.E. Sylvester, F. Novello, A. Pession, Topoisomerase-II-mediated DNA cleavage within the human ribosomal genes, *Biochem. Biophys. Res. Commun.* 213 (1995) 282–288.
- [27] M. Stanulla, J. Wang, D.S. Chervinsky, S. Thandla, P.D. Aplan, DNA cleavage within the MLL breakpoint cluster region is a specific event which occurs as part of higher-order chromatin fragmentation during the initial stages of apoptosis, *Mol. Cell. Biol.* 17 (1997) 4070–4079.
- [28] S.P. Sim, L.F. Liu, Nucleolytic cleavage of the mixed lineage leukemia breakpoint cluster region during apoptosis, *J. Biol. Chem.* 276 (2001) 31590–31595.
- [29] C.J. Betti, M.J. Villalobos, Q. Jiang, E. Cline, M.O. Diaz, G. Loreda, A.T. Vaughan, Cleavage of the MLL gene by activators of apoptosis is independent of topoisomerase II activity, *Leukemia* 19 (2005) 2289–2295.
- [30] R.A. King, D.B. Bursill, Plasma and urinary kinetics of the isoflavones daidzein and genistein after a single soy meal in humans, *Am. J. Clin. Nutr.* 67 (1998) 867–872.