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Tangeretin Sensitizes *SGS1*-Deficient Cells by Inducing DNA Damage

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Supporting Information

ABSTRACT: Tangeretin, a polymethoxyflavone found in citrus peel, has been shown to have antiatherogenic, anti-inflammatory, and anticarcinogenic properties. However, the underlying target pathways are not fully characterized. We investigated the tangeretin sensitivity of yeast (*Saccharomyces cerevisiae*) mutants for DNA damage response or repair pathways. We found that tangeretin treatment significantly reduced ($p < 0.05$) survival rate, induced preferential G1 phase accumulation, and elevated the DNA double-strand break (DSB) signal γH2A in DNA repair-defective *sgs1*Δ cells, but had no obvious effects on wild-type cells or mutants of the DNA damage checkpoint (including *tel1*Δ, *sml1*Δ *mec1*Δ, *sml1*Δ *mec1*Δ *tel1*Δ, and *rad9*Δ mutants). Additionally, microarray data indicated that tangeretin treatment up-regulates genes involved in nutritional processing and down-regulates genes related to RNA processing in *sgs1*Δ mutants. These results suggest tangeretin may sensitize *SGS1*-deficient cells by increasing a marker of DNA damage and by inducing G1 arrest and possibly metabolic stress. Thus, tangeretin may be suitable for chemosensitization of cancer cells lacking DSB-repair ability.

KEYWORDS: *tangeretin, DNA damage, DNA repair, SGS1, Saccharomyces cerevisiae*

INTRODUCTION

Bioactive compounds are the subject of intense study, on account of their valuable effects on human health. However, these compounds often trigger complex interactions in cells, making their targets difficult to identify. Chemical−genetic screening is a powerful tool for identifying the putative gene targets of bioactive compounds.[1,2] One of the most efficient model organisms for chemical−genetic screening is the budding yeast *Saccharomyces cerevisiae*, due to its genetically tractable properties.[3,4]

The DNA damage response and DNA repair pathways are highly conserved between yeast and higher eukaryotes, such as humans.[5] Mutations in DNA repair or cell cycle checkpoint genes result in genomic instability, and this in turn may give rise to tumorigenesis.[6] Patients with Ataxia telangiectasia, Werner syndrome, and Bloom syndrome are at increased risk of cancer, attributable to defects in certain DNA damage response or repair genes (*ATM*, *WRN* and *BLM*, respectively).[7−9] The *ATM* gene, like the related *ATR* gene, encodes a protein kinase that activates the DNA damage checkpoint.[7,10] The *WRN* and *BLM* genes encode helicases involved in DNA double-strand break (DSB) repair.[11] The corresponding genes in yeast have been extensively studied, and the gene functions have been found to be highly conserved between yeast and human.[5,12] The yeast homologues of *ATM* and *ATR* are *TEL1* and *MEC1*; these genes also encode kinases with important roles in the DNA damage checkpoint pathway, required for activating the DNA repair response and cell cycle arrest.[12] The yeast homologue of human *WRN* and *BLM* is *SGS1*, which encodes a RecQ-like helicase crucial for repair of DNA double-strand breaks (DSBs); such repair is necessary to prevent chromosome mitotic crossover and thus maintain genome stability.[13−15]

Tangeretin is a polymethoxyflavone (PMF) found in citrus peel. Tangeretin (4′,5,6,7,8-pentamethoxyflavone; Figure 1a) contains five fully methylated hydroxyl groups; these confer high molecular hydrophobicity, which in turn results in a higher cellular uptake rate compared to nonmethylated polyhydroxylated flavonoids.[16,17] It has been demonstrated that tangeretin

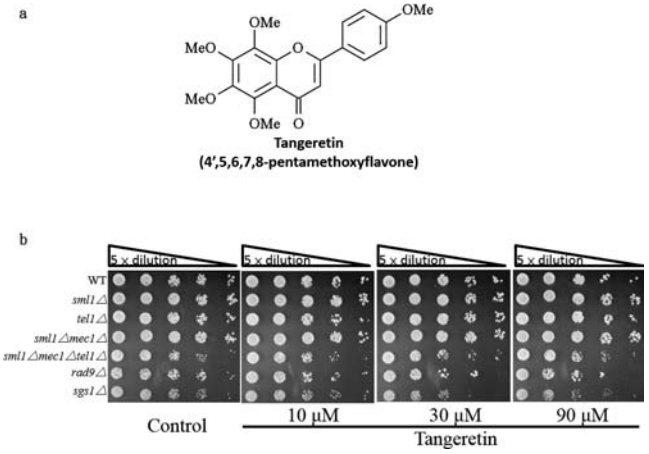

Figure 1. Sensitivity of mutant cells to tangeretin. (a) The structure of tangeretin. (b) Serially diluted mutant cells were spotted onto plates with or without tangeretin. The plates were incubated at 30 °C for 3 days. The number of colonies that grew on each spot was compared to that of the control group to estimate the survival rate at each dosage. WT stands for wild-type cells.

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has anti-inflammatory and antiatherogenic properties.[18−21] In addition, anticarcinogenic or antiproliferative effects of tangeretin have been observed in various cancer cells.[22−26] Several studies have indicated that tangeretin induces G1 arrest in breast and colon cancer cells.[24,26] This effect was postulated to arise from modulation of the activities of key G1 regulatory proteins, including CDK2 and CDK4, and the CDK regulators *p*21 and *p*27.[24] Recent studies have also shown that tangeretin can sensitize cispatin-resistant ovarian cancer cells through down-regulation of phosphoinositide 3-kinase/Akt signaling.[27−30] However, the molecular mechanisms and cellular targets underlying the anticancer activity of tangeretin are not fully understood.

In this study, we used chemical−genetic screening to investigate whether tangeretin inhibits growth of yeast mutants of DNA damage repair or cell cycle control. We report that tangeretin inhibits growth of cells deficient for the RecQ helicase gene *SGS1* but not of cells with defects in DNA damage checkpoint control. Tangeretin appears to increase DNA damage in yeast cells deficient for *SGS1* and may also affect nutrient and RNA processing in these cells. These results suggest that tangeretin may exacerbate DNA damage induced by *SGS1* deficiency, resulting in increased cell death.

MATERIALS AND METHODS

Yeast Strains, Cell Growth, and Cell Survival. The *Saccharomyces cerevisiae* strain used in this study was derived from EUROSCARF strain BY4741. Cells were cultured in synthetic complete media with or without tangeretin at 30 °C with shaking. Cell growth was determined by measuring optical density at 600 nm using a spectrophotometer. For the spotting assay, serially diluted cultures were spotted onto tangeretin-containing plates and incubated for 3 days. For the survival test, exponential-phase cultures were diluted to an appropriate concentration, and 200 cells were seeded onto plates containing various concentrations (10, 30, or 90 μM) of tangeretin; plates were then incubated for 3 days. The concentrations used were based on doses reported to be effective at treating cancer cells.[31,32] Relative survival rates of each experimental group were compared with the control group. Statistically significant differences between groups were analyzed by Student's *t*-test, and differences were considered significant at $p < 0.05$.

Flow Cytometry. YCL1131 and YCL1149 (*BAR1* gene deletion strains), which exhibit greater synchronization efficiency to α-factor, were used in this test (Table 1).[33] Exponential cells were inoculated into media containing 100 ng/mL of α-factor for 4 h to synchronize cells at G1 phase. Cells then were washed and released into media containing 0.1 mg/mL of Pronase, with or without 30 μM of tangeretin. Cells were collected and stained with SYTOX green (Invitrogen, CA) at the indicated times for cell cycle analysis by FACSCanto II (BD Biosciences, NJ).

Table 1. *Saccharomyces cerevisiae* Strains Used in This Study

strain	genotype	source
YCL1071	*MATα his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 trp1Δ::HPH*	this study
YCL1072	*MATα his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 trp1Δ::HPH sml1Δ::kanMX4*	this study
YCL1073	*MATα his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 trp1Δ::HPH tel1Δ::kanMX4*	this study
YCL1074	*MATα his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 trp1Δ::HPH sml1Δ::kanMX4 mec1Δ::HIS3*	this study
YCL1075	*MATα his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 trp1Δ::HPH sml1Δ::kanMX4 mec1Δ::HIS3 tel1Δ::LEU2*	this study
YCL1096	*MATα his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 trp1Δ::HPH rad9Δ::kanMX4*	this study
YCL1098	*MATα his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 trp1Δ::HPH sgs1Δ::kanMX4*	this study
YCL1131	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 bar1Δ::HIS3*	this study
YCL1149	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 sgs1Δ::kanMX4 bar1Δ::HIS3*	this study

Western Blot Analysis. Synchronized YCL1131 and YCL1149 cells were released from G1 phase and cultured in yeast media with or without tangeretin and were then collected for Western blot analysis. Total proteins were extracted by SUME buffer (0.1 M MOPS, 1% SDS, 4 M urea, 0.1 M EDTA). Total cellular proteins were resolved using electrophoresis on a 15% SDS-polyacrylamide gel. Resolved proteins were transferred onto polyvinylidene difluoride membranes (Immobilon-P, Millipore, Billerica, MA) and then probed with primary antibodies against γH2A (Abcam, Cambridge, UK) or glucose-6-phosphate dehydrogenase (G6PDH; Sigma, St. Louis, MO; used as a loading control), followed by secondary antibodies (antirabbit for both γH2A and G6PDH). The proteins were visualized using a chemiluminescent kit (Immobilon Western, Millipore) and imaged using the BioSpectrum Imaging System (UVP, Upland, CA). Band intensity was analyzed using VisionWorksLS (UVP) software. Cells were incubated with 0.2% MMS for 4 h as a positive control for DNA damage marker-γH2A.

Microarray Analysis. Exponential cells were incubated with or without 30 μM tangeretin overnight before being harvested. Sodium azide was added to terminate cellular biological function. Total RNA was extracted using the hot-phenol method and converted into cDNA for microarray analysis (Phalanx Biotech Group, Taiwan). Triplicate data were tested by the Pearson correlation coefficient calculation to confirm the reproducibility (R value >0.95). Normalized spot intensities were transformed to gene expression $\log_2$ ratios between the control and treatment groups. Spots with p value < 0.01 were selected for further analysis. Up- or down-regulated genes were identified by positive or negative $\log_2$ ratios, respectively. Affected genes were analyzed using The Database for Annotation, Visualization and Integrated Discovery (DAVID, http://david.abcc.ncifcrf.gov/).

RESULTS

Tangeretin Suppresses the Growth of *sgs1*Δ Cells but Not Wild-Type or Cells Defective in the DNA Damage Checkpoint. PMFs have been shown to have anticarcinogenic properties. However, the underlying pathways have not been fully elucidated. To determine whether tangeretin interacts with DNA damage and repair pathways, we examined the effect of tangeretin on the viability of wild-type yeast and mutants of DNA-damage-induced cell cycle checkpoint control (*tel1*Δ, *mec1*Δ *sml1*Δ, *mec1*Δ *tel1*Δ *sml1*Δ, and *rad9*Δ) or DNA double-strand break (DSB) repair (*sgs1*Δ) using a qualitative spotting assay. Serial dilutions of cells (from 1.25×10^4 cells/spot to 20 cells/spot) were spotted onto plates containing 10, 30, or 90 μM of tangeretin. The doses were modified from those used previously.[31,32] Our qualitative data indicate that wild-type cells and DNA damage checkpoint mutants were able to tolerate tangeretin at the doses tested (Figure 1b). However, cells lacking Sgs1 (*sgs1*Δ) were approximately five times more sensitive to tangeretin treatment than wild-type cells (Figure 1b).

Subsequently, we quantitatively examined growth and survival of *sgs1*Δ cells under various doses of tangeretin. The results confirmed that tangeretin markedly ($p < 0.05$) suppressed growth of *sgs1*Δ cells (Figure 2a; right panel) but did not affect ($p > 0.05$) the growth rate of wild-type cells (Figure 2a; left panel). Moreover, tangeretin significantly ($p < 0.05$) reduced the viability of *sgs1*Δ cells in a dose-dependent manner, as assessed by observing colony formation on plates containing various doses of tangeretin (Figure 2b). These

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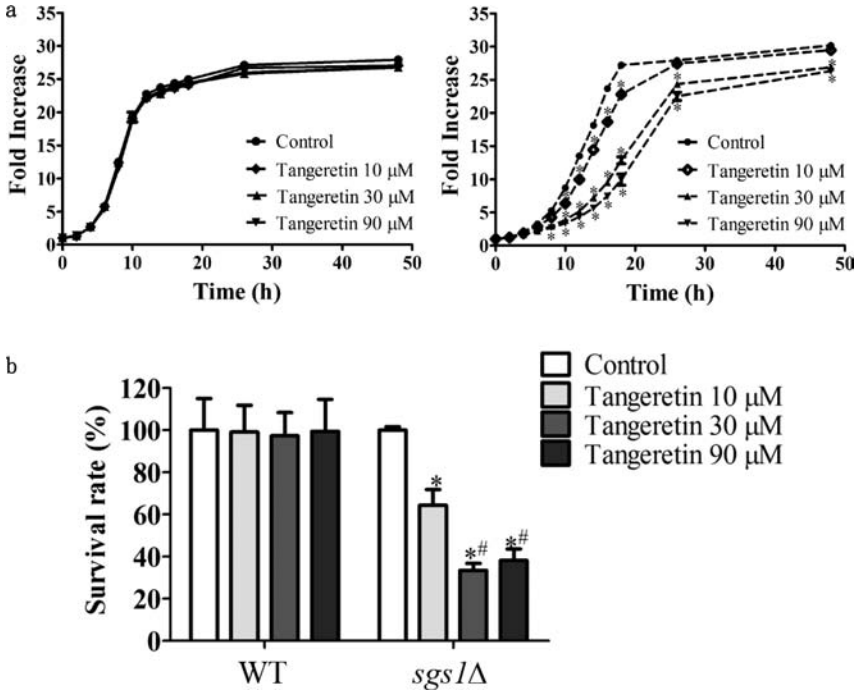

Figure 2. Yeast strains lacking *SGS1* were sensitive to tangeretin treatment. (a) Growth of wild-type (left panel) cells and *sgs1*Δ cells (right panel) under different doses of tangeretin. (b) Survival rates of wild-type and *sgs1*Δ cells under different doses of tangeretin. Data are presented as means ± SD from three independent experiments. Statistical significance was analyzed using Student's *t*-test at $p < 0.05$. * indicates a significant difference as compared with the control group, and # represents a significant difference as compared with the tangeretin-10 μM group. WT stands for wild-type cells.

Table 2. Biological Processes Affected by Tangeretin Treatment in Yeast Cells

wild-type					
gene ontology term (up-regulated)	genes count	*P* value	gene ontology term (down-regulated)	genes count	*P* value
ncRNA metabolic process	14	2.30×10^{-2}	ribonucleoprotein complex disassembly	2	6.40×10^{-2}
maturation of 5.8S rRNA from tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA)	5	3.50×10^{-2}	spliceosome disassembly	2	6.40×10^{-2}
maturation of 5.8S rRNA	5	5.30×10^{-2}	ribosomal large subunit biogenesis	4	6.60×10^{-2}
response to cycloalkane	2	5.30×10^{-2}	translational elongation	9	7.70×10^{-2}
response to cycloheximide	2	5.90×10^{-2}	response to abiotic stimulus	9	7.90×10^{-2}
*sgs1*Δ					
gene ontology term (up-regulated)	genes count	*P* value	gene ontology term (down-regulated)	genes count	*P* value
nitrogen compound biosynthetic process	19	8.20×10^{-8}	ncRNA processing	21	5.30×10^{-5}
cellular amino acid biosynthetic process	12	1.10×10^{-6}	RNA processing	27	6.20×10^{-5}
amine biosynthetic process	12	2.00×10^{-6}	ribonucleoprotein complex biogenesis	23	6.60×10^{-5}
arginine biosynthetic process	5	5.70×10^{-6}	ribosome biogenesis	21	1.00×10^{-4}
organic acid biosynthetic process	12	1.30×10^{-5}	ncRNA metabolic process	21	4.70×10^{-4}
carboxylic acid biosynthetic process	12	1.30×10^{-5}	tRNA methylation	5	6.10×10^{-4}
arginine metabolic process	5	4.70×10^{-5}	rRNA procession	15	9.50×10^{-4}
aspartate family amino acid biosynthetic process	7	5.00×10^{-5}	ribosomal large subunit biogenesis	8	1.10×10^{-3}
ornithine metabolic process	4	7.60×10^{-5}	biopolymer methylation	7	1.20×10^{-3}
glutamine family amino acid metabolic process	7	1.40×10^{-4}	methylation	7	1.30×10^{-3}
aspartate family amino acid metabolic process	7	1.70×10^{-4}	rRNA metabolic process	15	1.40×10^{-3}
translational elongation	14	4.70×10^{-4}	RNA methylation	5	1.60×10^{-3}
glutamine family amino acid biosynthetic process	5	4.70×10^{-4}	one-carbon metabolic process	7	9.90×10^{-3}
methionine biosynthetic process	5	7.90×10^{-4}	macromolecular complex assembly	8	1.80×10^{-2}
ornithine biosynthetic process	3	1.00×10^{-2}	RNA modification	5	3.20×10^{-2}
response to organic substance	9	1.30×10^{-3}	ribosomal small subunit biogenesis	4	3.30×10^{-2}
sulfur amino acid biosynthetic process	5	1.40×10^{-3}	transcription from RNA polymerase I promoter	13	4.60×10^{-2}

observations suggest that tangeretin may affect a molecular pathway that is in parallel with the functions of Sgs1.

Tangeretin Affects Expression of Genes Related to Metabolism and mRNA Processes in *sgs1*Δ Cells. To investigate the effects of tangeretin in *sgs1*Δ, we examined changes in gene expression upon tangeretin treatment using a DNA microarray. The microarray data have been deposited to the Gene Expression Omnibus (GEO) of the National Center for Biotechnology Information (NCBI) with the series number GSE43799. Genes differentially expressed between the control and the tangeretin-treated group were functionally annotated by DAVID. Examination of significantly enriched GO terms revealed that tangeretin treatment down-regulated pathways related to RNA processing; these include *DPB8*, *DPB9*, *ROK1*, and *PNO1*, which are involved in rRNA processing. However, most up-regulated genes in tangeretin-treated *sgs1*Δ cells were associated with nitrogen compound and amino acid biosynthetic processing (Table 2). Moreover, various single amino acid biosynthetic processes, such as arginine, asparagine, and methionine, were triggered through up-regulation of *ARG8*, *ARG2*, *AAT1*, *MET3*, *MET13*, and *MET28* genes (supplementary Table 1, Supporting Information). The results suggest that tangeretin may suppress rRNA processing or rDNA replication in *sgs1*Δ cells and induce metabolic stress, particularly of amino acid metabolism.

Tangeretin-Induced Preferential G1 Phase Accumulation in *sgs1*Δ Cells. To determine the mechanism by which tangeretin affects the growth of *sgs1*Δ cells, we examined cell cycle progression profiles through flow cytometry. We deleted the *BAR1* gene from wild-type and *sgs1*Δ cells to synchronize cells at G1 phase with high efficiency using α-factor.[32] Cells synchronized at G1 were released into media with or without tangeretin, and DNA content (indicating cell cycle progression) was measured every 10 min. Wild-type cells started to enter G2/M phase 50 min after release from G1, and this was unaffected by tangeretin treatment (supplementary Figure 1a, Supporting Information). On the other hand, tangeretin treatment resulted in accumulation of G1 cells 120 min postrelease from G1 phase (supplementary Figure 1). We further tested whether the tangeretin effects were persistent in *sgs1*Δ cells. Consistently, cell cycle distribution in wild-type cells was not affected during 6 h of tangeretin treatment (Figure 3a). However, a noticeable increase in the G1 population was observed in *sgs1*Δ cells after 2 h of treatment (Figure 3b). This result indicates that tangeretin may target a molecular pathway redundant with Sgs1 function.

Tangeretin Elevated γH2A in *sgs1*Δ Cells. Sgs1 is involved in the maintenance of genome stability and the suppression of DSB-induced illegitimate recombination repair.[14,15] As we observed that tangeretin suppressed growth and delayed cell cycle progression in *sgs1*Δ cells, we hypothesized that tangeretin treatment may inhibit a cellular function that is redundant to that of Sgs1, resulting in increased DNA damage, reduced cell viability (Figure 2b), and inhibition of cell proliferation (Figure 2a). To test this hypothesis, we examined the levels of γH2A by Western blot. Gamma H2A (H2A Ser129 phosphorylation) is induced by DNA damage.[34] The levels of G6PDH were examined as a loading control. In the absence of tangeretin, the level of γH2A in wild-type was very low during G1 arrest (Figure 4a; 0 h time point in left panel). The levels rose postrelease, peaking at 2 h and declining thereafter. In the presence of tangeretin, the levels of γH2A increased at a faster rate during the first two hours postrelease, but the overall

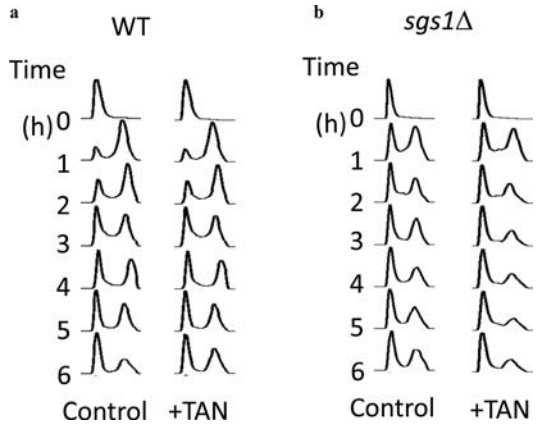

Figure 3. Tangeretin treatment causes *sgs1*Δ cells to accumulate at G1 phase. Cell cycle progression was analyzed by flow cytometry. (a) Wild-type cells and (b) *sgs1*Δ mutants were released from G1 arrest into control (containing DMSO) or + Tan (containing 30 μM of tangeretin) media. Samples at the 0 h time point are cells arrested at G1 phase. Cells were collected at 1 h intervals after release from G1 arrest, and DNA content was analyzed by flow cytometry. WT stands for wild-type cells. TAN stands for tangeretin treatment.

pattern was similar with that of cells growing in media without tangeretin (Figure 4a; compare left and right). The levels of γH2A in *sgs1*Δ cells were significantly higher than in wild-type during G1 arrest (compare 0 h time points in the left panels of Figure 4a and 4b), which may be a consequence of internal DNA damage.[34] Upon release from G1 arrest, the levels of γH2A increased in *sgs1*Δ cells, and the signal persisted until 4 h postrelease (Figure 4b; left panel). The γH2A signal increased more rapidly in *sgs1*Δ cells in the presence of tangeretin and remained at a high level until 6 h postrelease (Figure 4b; right panel). The persistent elevation of γH2A in tangeretin-treated *sgs1*Δ cells was consistent with the previously observed delay in cell cycle progression (Figure 3). Collectively, these results indicate that tangeretin aggravates chronic DNA DSBs in *sgs1*Δ, resulting in inhibition of cell growth and cell cycle progression. Taken together, these results imply that the detrimental effects of tangeretin on cells lacking Sgs1 may arise from a combination of chronic DNA damage induction and metabolic stress, thereby reducing cell survival.

■ DISCUSSION

In the present study, we have demonstrated that tangeretin suppresses cell growth and induces DNA damage and amino acid metabolic stress in DNA repair-defective *sgs1*Δ cells, ultimately resulting in cell death. Critically, we found that wild-type cells were able to endure tangeretin-induced stress.

When DSBs occur, phosphorylation of serine 129 of histone H2A (γH2A) is essential for activating the DNA repair process and checkpoint response.[34,35] Sgs1, in complex with Top3 and Rmi1, is involved in processing DSB-induced homologous recombination (HR) intermediates, restarting blocked or collapsed replication forks, and activating S-phase checkpoint arrest.[36−38] Replication fork damage occurs frequently during DNA replication, and the repair of stalled replication forks (including HR repair) is important for cell viability.[39] Thus, Sgs1 is crucial for these repair processes. Consistent with this hypothesis, our data demonstrate that tangeretin induces replication stress and activates γH2A in both wild-type and *sgs1*Δ cells. The finding that the DSB damage signal-γH2A was

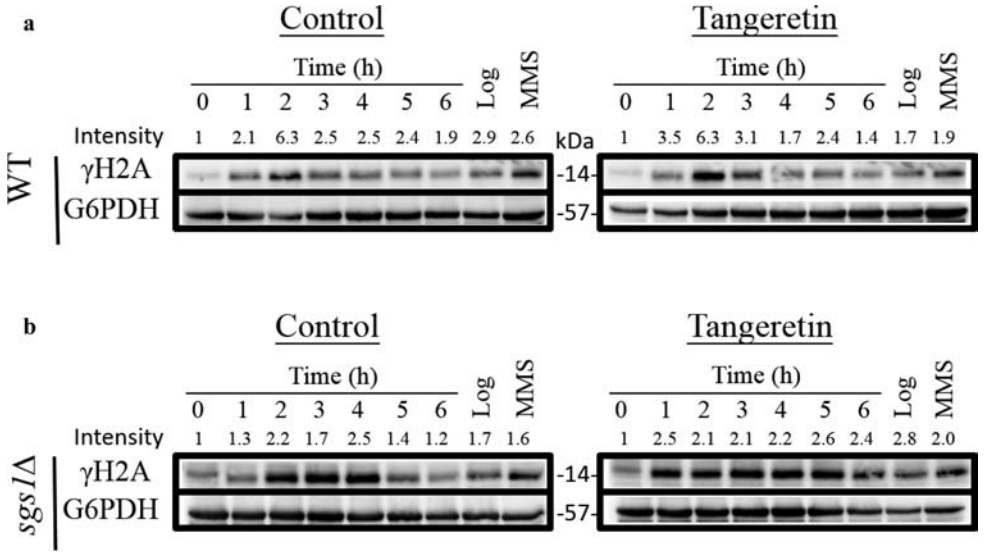

Figure 4. Tangeretin treatment elevated γH2A in *sgs1*Δ cells. Wild-type cells and *sgs1*Δ were released from G1 arrest into media with or without 30 μM tangeretin. Levels of γH2A in WT (a) and *sgs1*Δ (b) were determined by Western blots. Samples at the 0 min time point are arrested at G1; cells were collected at 1 h intervals after release from G1. Log phase and MMS-treated cells were collected as positive controls for γH2A. The values listed above each blot ("intensity") indicate the fold-change in γH2A as compared to the 0 h time point for each group. WT stands for wild-type cells. Log stands for log phase cells. MMS stands for MMS-treated cells.

reduced to basal levels within 4 h of treatment indicates that the damage is efficiently repaired in wild type cells (Figure 4). In contrast, DNA damage and G1 arrest are prolonged in cells lacking Sgs1 (5 h post-tangeretin treatment), indicating enhanced sensitivity toward tangeretin-induced replication stress. These findings may explain our observation that tangeretin significantly ($p < 0.05$) reduced the viability of *sgs1*Δ, but not wild-type cells, in a 3-day survival test. However, the underlying mechanisms by which tangeretin induces DNA damage in cells remain obscure. One possibility is that tangeretin directly interacts with DNA to form adducts, which may lead to breaks during replication. Indeed, DNA adducts are caused by several bioactive compounds, particularly compounds with a phenolic structure, such as quercetin.[40,41] Although tangeretin is a polymethoxyflavone containing several fully methylated hydroxyl groups, it possesses high hydrophobicity that results in it being taken up by cells at a higher rate than nonmethylated polyhydroxylated flavonoids.[16,17,30] It is unknown whether tangeretin metabolites in cells form hydroxyl groups and/or bind to DNA. A second possibility is that tangeretin may target other enzymes or proteins, which could affect DNA replication and induce DNA damage indirectly.

Several studies have also demonstrated that tangeretin inhibits growth of human breast and colon cancer cells by inducing G1 arrest.[24,29] Remarkably, epigenetic repression of the mammalian Sgs1 orthologue, WRN, has been observed in several cancer cell lines, including the colon cancer cell lines HCT-116, COLO-205, and MDA-MB-231, and human sporadic neoplasms.[42] Furthermore, these WRN-deficient cells are sensitive to DNA-damaging agents, such as topoisomerase I inhibitors.[42] Thus, it is possible that tangeretin is a potential candidate for chemosensitization of cancer cells lacking DSB-repair ability.

Our microarray data showed that tangeretin specifically up-regulated genes related to amino acid biosynthesis processes and down-regulated genes related to rRNA biological processes in *sgs1*Δ cells but not in wild-type cells. As Sgs1 is crucial for the transcription and maintenance of rDNA structure during replication[43−45] and tangeretin may target pathways redundant with Sgs1 function (Figure 1), we suggest that the suppression of RNA biological processes by tangeretin may lead to defective protein translation in *sgs1*Δ cells, on account of the lack of functional translation components. Consequently, genes involved in amino acid biosynthesis may be up-regulated by a feedback mechanism. Whether tangeretin (or its bioderivatives) is involved in these processes is currently under investigation. It is known that simultaneous deletion of Sgs1 and Srs2 helicase causes cell death in yeast.[46] Moreover, Sgs1 is essential for RNA polymerase I transcription in the absence of the Srs2 helicase.[43] A recent study showed that BLM helicase, a human Sgs1 orthologue, facilitates RNA polymerase I-mediated rRNA transcription.[47] These results suggest that Sgs1 is likely involved in rRNA processing and rDNA transcription. Consistent with this hypothesis, we found that deletion of *sgs1* affected expression of genes related to rRNA transcription (microarray data). Interestingly, tangeretin treatment significantly down-regulated rRNA processing in *sgs1*Δ cells. Whether these effects are correlated with the induction of γH2A in *sgs1*Δ cells will require further investigation.

We used a yeast-based genetic system to identify that tangeretin sensitizes *SGS1*-deficient cells by increasing a marker of DNA damage, inducing cell cycle G1 arrest and possibly metabolic stress. Tangeretin is thus a potential candidate for chemosensitization of cancer cells lacking DSB-repair function.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

These files contain supplementary Figure 1 (PDF) and supplementary Table 1 (Excel datasheet) for this study. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interests.

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