Sesamin Induces Cell Cycle Arrest and Apoptosis through the Inhibition of Signal Transducer and Activator of Transcription 3 Signalling in Human Hepatocellular Carcinoma Cell Line HepG2

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Sesamin, one of the most abundant lignans in sesame seeds, has been shown to exhibit various pharmacological effects. The aim of this study was to elucidate whether sesamin promotes cell cycle arrest and induces apoptosis in HepG2 cells and further to explore the underlying molecular mechanisms. Here, we found that sesamin inhibited HepG2 cell growth by inducing G2/M phase arrest and apoptosis. Furthermore, sesamin suppressed the constitutive and interleukin (IL)-6-induced signal transducer and activator of transcription 3 (STAT3) signalling pathway in HepG2 cells, leading to regulate the downstream genes, including p53, p21, cyclin proteins and the Bcl-2 protein family. Our studies showed that STAT3 signalling played a key role in sesamin-induced G2/M phase arrest and apoptosis in HepG2 cells. These findings provided a molecular basis for understanding of the effects of sesamin in hepatocellular carcinoma tumour cell proliferation. Therefore, sesamin may thus be a potential chemotherapy drug for liver cancer.

Key words sesamin; signal transducer and activator of transcription 3; HepG2 cell; cell cycle; apoptosis

Hepatocellular carcinoma (HCC) is a frequent malignancy with a high rate of metastasis that occurs worldwide. Although HCC has a variety of therapeutic method, including surgical resection, chemotherapy, radiofrequency and cryotherapy, the recurrence and metastasis rate is very high compared with other major tumours. Therefore, an urgent needs for the development of more effective drugs remains.1)

Signal transducers and activators of transcription (STATs) is a family of transcription factors which regulate the cell growth, survival, and differentiation. Among the seven STAT proteins, the hyperactivation of STAT3 has been shown to promote tumour growth directly by tumour-autonomous mechanisms and indirectly by regulating the anti-tumour response of the tumour-associated immune system.2) Suppressing STAT3 signalling has been shown to inhibit cell proliferation, induce apoptosis, stimulate immune responses and suppress angiogenesis in tumour cells.3) STAT3 is activated by phosphorylation on a specific tyrosine residue by Janus kinase 2 (JAK2).4) Once induced by cytokines, activated JAK2 phosphorylates monomeric STAT3. STAT3 then dimersises and is translocated to the nucleus, where it binds to DNA and regulate genes involved in cell growth and survival, including cyclin proteins and the Bcl-2 protein family.5) Interleukin-6 (IL-6) is a pluripotent cytokine in the inflammatory microenvironment surrounding the tumour, and promote malignant progression through regulating many biological processes, including proliferation, survival, and invasion.6) The cytokine receptor gpl30 is a common signalling subunit of receptors used by the IL-6 cytokine family. More specifically, IL-6, IL-11, oncostatin M, leukaemia inhibitory factor, and ciliary neurotropic factor belong to the IL-6 family cytokines that use gpl30 as a signalling subunit. IL-6 can bind to an IL-6-spe-

cific binding receptor (IL-6Ra) and cause the dimerization and phosphorylation of gpl30, leading to the phosphorylation of JAK2 and activation of the STAT3 signalling pathway.7,8) Previous studies in animal models and cultured cells have validated that STAT3 is a promising molecular target for cancer therapies.3)

Sesamin (Fig. 1) is the most abundant lignan in sesame (Sesamum indicum L.) seed oil and is also found in several other medicinal herbs, including Acanthopanax senticosus HARMS. Sesamin has been found to have various pharmaceutical functions, including protection against oxidative stress, anti-inflammation, anti-hypertension, and the reduction of chemical-induced tumours.9–12) Previous studies have demonstrated that the proliferation of a wide variety of tumour cells was suppressed by sesamin treatment, such as myeloma, leukaemia, and cancers of the prostate, colon, pancreas, breast, and lung.13) However, the underlying mechanism behind sesamin-induced inhibition of the proliferation of tumour cells

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**Fig. 1. Chemical Structure of Sesamin**
remains to be elucidated.

In the present study, we found that sesamin inhibited the IL-6-induced HepG2 cell growth, by inducing cell cycle arrest at the G2/M phase and apoptosis. For the first time, we demonstrated that sesamin exerts its anti-cancer activity through suppressing the gpl30-mediated STAT3 signalling pathway required for carcinogenesis by inhibiting transcriptional activities and suppressing downstream genes expression.

MATERIALS AND METHODS

Materials Sesamin was purchased from Shanghai Tauto Biotech Co., Ltd. (Shanghai, China). The product analysis report is available in Supplementary information.). It was dissolved in dimethylsulfoxide (DMSO) to create a stock solution for subsequent dilution and use. Dulbecco’s modified Eagle’s medium (DMEM), foetal bovine serum (FBS), and phosphate-buffered saline (PBS) were obtained from Gibco (Grand Island, NY, U.S.A.). DMSO, 3-(4,5-dimethyl-thiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), AG490, trypan blue, propidium iodide (PI), fluorescein isothiocyanate (FITC)-conjugated Annexin-V, and Hoechst 33258 were obtained from Sigma (St. Louis, MO, U.S.A.). Trizol Reagent kit and M-MLV reverse transcriptase were obtained from Invitrogen (Carlsbad, CA, U.S.A.). Cell Lysis Buffer for Western blotting and IP, Nuclear and Cytoplasmic Extraction Reagents Kit, and Cell Viability Assay Kit were obtained from Millipore (Billerica, MA, U.S.A.).

Cell Line and Culture Human hepatocellular carcinoma cell line (HepG2) and human normal liver cell line (L02) was obtained from the China Center for Type Culture Collection (Wuhan University, Wuhan, China). Enhanced BCA Protein Assay Kit and IP, Nuclear and Cytoplasmic Extraction Reagents Kit, and Enhanced BCA Protein Assay Kit were obtained from Beyo- time (Nanjing, China). Antibodies against Cyclin A, Cyclin B1, p21, p53, Bcl-2, Bcl-xL, Bax, JAK2, phospho-JAK2 (Tyr 1007/Tyr 1008), STAT3, phospho-STAT3 (Tyr 705), gpl30, phospho-gpl30 (Ser 782), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and β-actin were obtained from Santa Cruz (CA, U.S.A.). The secondary horseradish peroxidase (HRP)-linked antibodies were obtained from Boster (Wuhan, China). Enhanced chemiluminescence kit was obtained from Millipore (Billerica, MA, U.S.A.).

Cell Viability Assay Cell growth and viability were determined by the MTT assay. HepG2 and L02 cells were plated in 96-well plates at a density of 1×10^4 cells per well and cultured overnight. Cells were incubated with sesamin at the following concentrations: 0, 25, 50, 75, 100, or 125 µM for 24 and 48 h. A total of 20 µL of MTT was added to each well and the plate was incubated for 4 h at 37°C. After removal of the supernatant from each well, the insoluble product was dissolved by addition of 100 µL of DMSO. The product was quantified by measuring the absorbance at 496 nm using a microplate reader (Sunrise, Tecan, Switzerland). Cells incubated with DMSO alone served as a control.

Cell Cycle and Apoptosis Analysis Flow cytometry was used to determine the presence of cell cycle arrest. After treatment with sesamin at concentrations of 0, 25, 50, 75, 100 or 125 µM for 48 h, the cells were harvested by trypsinisation and washed twice with PBS, fixed in ice-cold 80% ethanol, and stored overnight at 4°C. For analysis, the cells were washed with PBS twice, suspended in 1 mL of a cold PI solution (50 µg/mL PI and 100 µg/mL RNase A). Next, the cells were incubated on ice for 30 min in the dark and then analysed with a Coulter Epics XL Flow Cytometer (Beckman, Miami, FL, U.S.A.).

After treatment with sesamin for 48 h, the extent of apoptosis and necrosis were determined. The cells were harvested and washed twice with cold PBS, suspended in a binding buffer (1×) and then stained with PI and FITC-conjugated annexin V for 15 min in dark. The stained cells were analysed directly by flow cytometry. Apoptosis of HepG2 cells was observed morphologically by nuclear staining with Hoechst 33258 dye. HepG2 cells were placed in the 6-well plates and seeded on sterile coverslips. After 24 h, the cells were treated with 75 µM DMSO or sesamin for 48 h. Next, the cells were washed with PBS and fixed with 4% paraformaldehyde for 10 min, and incubated with 50 µM Hoechst 33258 staining solution for 15 min in the dark, then washed with PBS for three times. The cells were viewed under a fluorescence microscope (Olympus, Tokyo, Japan).

Reverse Transcription-Polymerase Chain Reaction (RT-PCR) Analysis HepG2 cells were placed in the 6-well plates and treated with sesamin for 48 h. The total RNA was extracted using a Trizol Reagent kit, then reverse transcribed using M-MLV reverse transcriptase following the instructions. The cDNAs were amplified by PCR, with the primers showed in Table 1. The PCR reaction mixture contained 10 µM Tris–HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl2, 2.5 U of Taq DNA polymerase, 0.2 mM deoxynucleotide triphosphate (dNTPs), and 0.1 µM of each primer. PCR amplification was performed using the following conditions: 5 min at 94°C followed by 35 repetitive cycles of 30 s at 94°C, 30 s at 55°C, and 30 s at 72°C with a final extension.

Western Blotting Cells were harvested and lysed using Cell Lysis Buffer for Western blotting and IP. The nuclear protein was extracted according to the instructions provided with the Nuclear and Cytoplasmic Extraction Reagents Kit. Protein concentration was measured using an Enhanced BCA Protein Assay Kit. The Equal quantities of protein samples were loaded onto a 7.5% SDS-PAGE gel and transferred to a polyvinylidene difluoride membrane. The membrane was blocked in 5% nonfat milk in Tris–HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl2, 2.5 U of Taq DNA polymerase, 0.2 mM deoxynucleotide triphosphate (dNTPs), and 0.1 µM of each primer. PCR amplification was performed using the following conditions: 5 min at 94°C followed by 35 repetitive cycles of 30 s at 94°C, 30 s at 55°C, and 30 s at 72°C with a final extension.

<table>
<thead>
<tr>
<th>Primers</th>
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<td>Cyclin A</td>
<td>5'-GTACCCACACATACATGGACATG-3'</td>
</tr>
<tr>
<td>Antisense</td>
<td>5'-AAATTTTTCTCTCAGCAGCAGTACGAC-3'</td>
</tr>
<tr>
<td>Cyclin B1</td>
<td>5'-CAGTCAGACACAAAATACTCTGCT-3'</td>
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<tr>
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<td>5'-ACACAAACCGCAGCAGCAGTCTTT-3'</td>
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<td>Cdc2</td>
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</tr>
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<td>p21</td>
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</tr>
<tr>
<td>Antisense</td>
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<tr>
<td>p53</td>
<td>5'-GCTCAGACGAGCTAGAGAC-3'</td>
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<tr>
<td>Antisense</td>
<td>5'-ACACAAACCGCAGCAGCAGTCTTT-3'</td>
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<td>Bcl-2</td>
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<tr>
<td>Bax</td>
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<tr>
<td>Antisense</td>
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were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred by electroblotting to a nitrocellulose membrane. The membrane was blocked with 5% non-fat milk in TBST buffer (20 mmol Tris–HCl, pH 7.4, 150 mmol NaCl and 0.1% Tween 20) overnight at 4°C. Next, the membrane was incubated with specific primary antibodies for 2h and a secondary antibody (horseradish peroxidase-conjugated anti-mouse or anti-rabbit immunoglobulin G) for 1h. The antibodies are listed in Table 2. The signal was visualised with an enhanced chemiluminescence kit and Enhanced Chemiluminescence Plus (ECL Plus) detection system (Bio-Rad, Richmond, CA, U.S.A.).

**Statistical Analysis** All the experiments were repeated for three independent experiments and the data are expressed as the mean±S.D. Group results were analysed by Nosa5 software. A value of p<0.05 was considered to be statistically significant.

**RESULTS**

**Sesamin Inhibits HepG2 Cell Viability** We investigated the effects of sesamin on the proliferation of HepG2 cells using the MTT assay. Our results indicated that the proliferation of HepG2 cells was inhibited after 48h of treatment with sesamin in a concentration-dependent manner. However, no obvious toxic effects were observed at 24h, except when the concentration of sesamin reached 125 µM. The IC$_{50}$ value for sesamin was 98 µM. In contrast, sesamin was less cytotoxic to the hepatocyte L02 cells when compared with HepG2 cells (Fig. 2A). Morphological changes of HepG2 cells demonstrated that cell growth was inhibited by sesamin after a 48h-treatment (Fig. 2B). AG490, a JAK2 specific inhibitor, could inhibit the HepG2 cell growth, whereas was attenuated by IL-6. However, AG490 was less cytotoxic to L02 cells (Fig. 2C). These findings indicated that STAT3 signalling might play an important role in the decreased viability of HepG2 cells but not important in L02 cells. In addition, DMSO alone treated cells did not show any obvious inhibitory effects compared with untreated cells (data not shown).

**Sesamin Induces G2/M Arrest and Apoptosis of HepG2 Cells** To examine the effects of sesamin on cell cycle distribution, HepG2 cells were incubated for 48h with sesamin (0, 25, 50, 75, 100, or 125 µM) and examined by flow cytometry (Fig. 3A). Sesamin treatment leaded to a concentration-dependent increase in the G2/M phase cell population (Fig. 3B). These results indicated that sesamin could induce G2/M arrest of the HepG2 cell cycle and cause growth inhibition.

To determine whether sesamin-induced HepG2 cell death involves apoptosis, flow cytometry using Annexin V-PI staining was performed. HepG2 cells were treated with different concentrations of sesamin (0, 25, 50, 75, 100, or 125 µM) for 48h. As shown in Figs. 4A, B, the proportions of Annexin V-positive and PI-negative (Annexin V+/PI−) cells were markedly increased, indicating that sesamin induced early apoptosis of HepG2 cells. The proportion of apoptotic cells in treated cells increased in a dose-dependent manner. The apoptosis of HepG2 cells induced by sesamin was also observed by chromatin condensation and nuclear fragmentation in cells stained with Hoechst 33258 fluorescent dye (Fig. 4C).

**Sesamin Inhibits the Constitutive JAK2 andSTAT3 Activation of HepG2 Cells** Previous studies have shown that STAT3 is a key point of multiple oncogenic signalling pathways. In the present study, we investigated whether sesamin could modulate the constitutive STAT3 signalling pathway in HepG2 cells. As shown in Fig. 5A, Western blotting analysis with specific antibodies against JAK2, p-JAK2, STAT3, and p-STAT3 revealed that 48h-sesamin treatment resulted in a strong concentration-dependent decrease in the phosphorylation level of JAK2 and STAT3 in HepG2 cells. However, sesamin had no effect on the total protein level of JAK2 and STAT3. Moreover, sesamin treatment significantly reduced STAT3 level in the nuclear in a dose-dependent manner as well as the phosphorylation level of nuclear STAT3 (Fig. 5B). These results suggested that sesamin inhibited the STAT3 signalling pathway and STAT3 nuclear translocation in HepG2 cells.

**Sesamin Regulates the Expression of STAT3 Downstream Genes Related to the HepG2 Cell Cycle and Apoptosis at Transcriptional and Translational Levels**

The STAT3 signalling pathway participates in cell cycle and apoptosis by directly or indirectly regulating many genes, including cyclin A, cyclin B1, Cdc2, p53, p21, and the Bcl-2

**Table 2. Antibodies Used for Western Blotting**

<table>
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<tr>
<th>Primary antibody</th>
<th>Concentration</th>
<th>Source</th>
<th>Second antibody</th>
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<td>1:500</td>
<td>Rabbit</td>
<td>Goat anti-rabbit IgG</td>
</tr>
<tr>
<td>Cyclin B1 (H-433)</td>
<td>1:500</td>
<td>Rabbit</td>
<td>Goat anti-rabbit IgG</td>
</tr>
<tr>
<td>Cdc2 (PSTAIRE)</td>
<td>1:500</td>
<td>Rabbit</td>
<td>Goat anti-rabbit IgG</td>
</tr>
<tr>
<td>p21 (F-5)</td>
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<td>Mouse</td>
<td>Goat anti-mouse IgG</td>
</tr>
<tr>
<td>p53 (DO-1)</td>
<td>1:1000</td>
<td>Mouse</td>
<td>Goat anti-mouse IgG</td>
</tr>
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<td>Bel-2 (N-19)</td>
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<td>gpi130 (C-20)</td>
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<td>Goat anti-rabbit IgG</td>
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<tr>
<td>Phospho-gp130 (Ser 782)</td>
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<td>JAK2 (C-20)</td>
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<td>Phospho-JAK2 (Tyr 1007/Tyr 1008)</td>
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<td>STAT3 (C-20)</td>
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<td>Phospho-STAT3 (Tyr 705)</td>
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<td>β-Actin (AC-15)</td>
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protein family. The expression of these genes were investigated at transcriptional and translational levels by RT-PCR and Western blotting in HepG2 cells treated with 0, 25, 50, or 75 \( \mu \)M of sesamin for 24h or 48h. Viable cell numbers were determined using an MTT assay. (B) Morphological changes of HepG2 cells under treatment of sesamin. HepG2 cells were incubated with 125 \( \mu \)M sesamin or DMSO (control) for 48h, and then the cells were observed under the microscope. (C) Cell viability assay of HepG2 and L02 cells under treatment of AG490 and IL-6. The cells were pretreated with or without IL-6 (50ng/mL) 2h before AG490 (75 \( \mu \)M) treatment for an additional 48h. Viable cell numbers were determined using an MTT assay. The results are expressed as the mean±S.D. of three independent experiments.* \( p < 0.05 \) and ** \( p < 0.01 \) when compared with the control group (DMSO treatment without sesamin).

Sesamin Inhibits IL-6-Induced gp130/STAT3 Signalling
IL-6 is an important cytokine in the inflammatory microenvironment surrounding the tumour, which can induce cell growth and gp130/STAT3 signalling. To further study whether sesamin induces reduction of gp130, which is mediated to the STAT3 signalling pathway, we investigated the sesamin
effects on IL-6-induced HepG2 cells. HepG2 cells were pre-
treated without or with IL-6 (50 ng/mL) for 2 h, and then in-
cubated for 48 h in the presence or absence of sesamin. MTT
assay demonstrated that IL-6 attenuated the reduced viability
caused by sesamin (Fig. 8A). Next, levels of gp130, p-gp130,
STAT3 and p-STAT3 were assessed. We found that IL-6 up-
regulated the level of p-gp130 and p-STAT3 as well as nu-
clear STAT3. However, sesamin attenuated the IL-6-induced
changes in p-gp130 and p-STAT3 in a dose-dependent manner
(Fig. 8B). Furthermore, we examined whether sesamin modu-
lated the expression of genes which were directly regulated
by STAT3 following IL-6 treatment in HepG2 cells (Fig. 8C).
We found that IL-6 antagonised sesamin-induced changes in
HepG2 cells pretreated with IL-6.

DISCUSSION

Sesamin (Fig. 1) has been shown to have anti-cancer
effects. In the present study, the effects of sesamin on a
human hepatoma cell line (HepG2) were investigated. Our
results showed that sesamin inhibited the viability of HepG2
cells but exerted less cytotoxicity in normal human hepa-
tocyte (L02) cells (Fig. 2). Subsequent flow cytometric analysis
showed that the growth inhibition observed in response to
sesamin was due to G2/M cell cycle arrest and apoptosis
(Figs. 3, 4).

IL-6 is an important cytokine that maintains the inflam-
atory microenvironment surrounding a tumour, which is
a STAT3 signalling activator. Our results showed that the
growth of sesamin treated HepG2 cells was recovered by
IL-6 (Fig. 8A). Moreover, the JAK2 specific inhibitor AG490
could inhibit the HepG2 cell growth, whereas was attenuated
by IL-6 (Fig. 2C). These results indicated that sesamin in-
hibited tumour growth caused by constitutively activated
IL-6 induced STAT3 signalling. A large body of evidence has
shown that STAT3 is one of the few key regulatory signalling
molecules whose aberrant activation is invariably associated
with inflammation and cancer. Interestingly, sesamin stron-
ger inhibited HepG2 cell growth than AG490 at the same con-
centration, suggesting that there might be other mechanisms
(Fig. 2C). Harikumar et al. found that sesamin could suppress
the nuclear factor (NF)-kappa B signalling in tumour cells,
Fig. 4. The Effects of Sesamin on Cell Cycle and Apoptosis

(A) Annexin V/FITC and PI staining. HepG2 cells were grown for 48 h in the presence of 0, 25, 50, 75, 100, or 125 µM sesamin and then stained by Annexin V/FITC and PI. Next, living and apoptotic cell distribution was analysed by flow cytometry. (B) The percentage of cells in each quadrant. LL, living cells (Annexin V−/PI−); LR, early apoptotic cells (Annexin V+/PI−); UR, late apoptotic cells (Annexin V+/PI+); UL, necrotic cells (Annexin V−/PI+). An increase in early apoptotic cells was showed. (C) Hoechst 33258 staining. Nuclear morphology as determined by Hoechst staining in HepG2 cells incubated for 48 h with DMSO as control, or 75 µM sesamin. Apoptotic cells showed chromatin condensation and nuclei fragmentation (arrows).

Fig. 5. The Effects of Sesamin on the STAT3 Signalling Pathway

(A) After the cellular proteins were lysed, p-STAT3, STAT3, p-JAK2, and JAK2 were examined by Western blotting. GAPDH was used as an internal control. (B) Nuclear proteins were extracted, and the amount of both p-STAT3 and STAT3 were examined. β-Actin was used as an internal control for the nuclear fraction.
which confirmed our view.\textsuperscript{13} Both sesamin and AG490 were less cytotoxic to L02 cells, suggesting that STAT3 signalling might be not abnormal activated in normal cells (Fig. 2C). Sesamin might be useful and safe for targeted therapy, especially in malignancies which have aberrant IL-6 expression.

Cell growth is controlled by cell cycle and apoptosis. Many anti-cancer drugs play a therapeutic role through inducing cell cycle arrest and apoptotic.\textsuperscript{15} STAT3 plays a key role in cell cycle and apoptosis. Constitutively activated STAT3 signalling is widely found in cancer cells and results in tumour development by regulation of genes encoding apoptosis regulators, such as Bcl-2 and Bcl-xL, and cell cycle regulators, such as cyclin proteins.\textsuperscript{16,17} Here we found that sesamin inhibited IL-6-induced gp130 phosphorylation leading to block the JAK2 and STAT3 phosphorylation, and then suppressed STAT3 nuclear translocation in HepG2 cells (Figs. 5, 8). The suppression of STAT3 signalling by sesamin resulted in the regulation of its target genes, including p53, p21, cyclin A, cyclin B1, Cdc2, Bcl-xL, and Bcl-2, which was enhanced by co-treated with sesamin and AG490 (Fig. 7). However, the expression of these genes in sesamin treated HepG2 cells were recovered by the IL-6 treatment (Fig. 8C). Based on these observations, we might suggest that sesamin-induced G2/M arrest and apoptosis of HepG2 cells were carried out through the STAT3 signalling pathway.

A number of Cdk and cyclin proteins have been shown to control cell cycle events. The Cdc2-cyclin A complex and Cdc2-cyclin B1 complex are required for the G2/M phase.\textsuperscript{18} Our data suggested that the down-regulation of cyclin A and cyclin B1 by sesamin caused G2/M phase arrest in HepG2 cells. Cdc2 levels were markedly down-regulated by sesamin treatment at the protein level; however, the expression of Cdc2 was not affected by sesamin treatment at the mRNA level, suggesting that sesamin might induce Cdc2 protein degradation to suppress the Cdc2 complex (Fig. 6).

The results of our present study indicated that sesamin enhanced the expression of the p53 and p21 Cdk inhibitor at both transcriptional and translational levels through suppressing the activation of STAT3. There are multiple STAT-binding sites in the p53 promoter and STAT3 interacts with them.\textsuperscript{19} Previous studies have shown that STAT3 was used to down-regulate p53 gene expression in tumours.\textsuperscript{3} As a target gene of STAT3, the tumour suppressor p53 is an crucial point in every cell cycle checkpoints.\textsuperscript{20} Several p53 target genes have been found to play a role in p53-induced G2/M arrest, such as Cdc25C, GADD45, and p21.\textsuperscript{21–23} Activated p53 binds to DNA and activates expression of the cyclin-dependent kinase inhibitor p21 which plays an primary role in the DNA damage response by inducing cell cycle arrest and direct inhibition of DNA replication; in addition, p21 regulates fundamental processes, including apoptosis and transcription.\textsuperscript{21} p21 was also implicated in G2/M arrest by inhibiting cyclin A-Cdc2 complex and cyclin B1-Cdc2 complex activity through blocking of the interaction between Cdc25C and the complexes.\textsuperscript{20} We found that the activation of STAT3 was suppressed by sesamin, leading to up-regulated of downstream p53/p21, and then induced G2/M arrest in HepG2 cells. These results were demonstrated by treating cells with sesamin and AG490 or IL-6 in combination (Figs. 6–8). Previous research has shown that cell cycle arrest at G1/S phase occurs via STAT3 inhibition.\textsuperscript{24–27} however, our current study suggested that STAT3 also played an important role in drug-induced G2/M phase arrest, which was demonstrated by Weissenger et al.,\textsuperscript{28} Chetty et al.,\textsuperscript{29} and Huang et al.\textsuperscript{30} Interestingly, Yokota et al. found that sesamin induced growth arrest at the G1 phase of the cell cycle in a human breast cancer cell line MCF-7.\textsuperscript{31} These data implied that the anti-cancer mechanisms of sesamin might vary depending on the cancer type.

The STAT3 downstream genes, p53 and p21, are also considered to be the most widespread inducers of apoptosis because of their ability to integrate signals from various sources and activate a number of genes that are vital for apoptosis.\textsuperscript{3} The Bcl-2 protein family is another protein family which participates in apoptosis by exerting anti-apoptotic actions, such as Bcl-2 and Bcl-xL, or pro-apoptotic actions, such as

Fig. 6. The Effects of Sesamin on the Expression of Genes Involved in Cell Cycle Regulation and Apoptosis in HepG2 Cells

HepG2 cells were incubated for 48h with 0, 25, 50, or 75 µM sesamin. (A) The effects of sesamin on gene expression at the mRNA level were analysed by RT-PCR. GAPDH was used as an internal control. (B) The effects of sesamin on gene expression at the protein level were analysed by Western blotting. GAPDH was used as an internal control.

Fig. 7. The Effects of Sesamin and AG490 on the STAT3 Signalling and Downstream Genes in HepG2 Cells

HepG2 cells were incubated for 48h with or without 75 µM sesamin or 75 µM AG490. (A) The expression of STAT3 signalling downstream genes at the mRNA level were analysed by RT-PCR. GAPDH was used as an internal control. (B) STAT3, p-STAT3 and STAT3 signalling downstream genes were examined by Western blotting. GAPDH was used as an internal control.
The ratio of anti-apoptotic members and pro-apoptotic members of this family controls the permeability of mitochondrial membrane. Over-expression of anti-apoptotic members or under-expression of pro-apoptotic members can lead to the fail of cell death, which has been identified as a reason of various cancers and resistance to cancer treatments. Previous studies have shown that STAT3 could increase Bcl-2 and Bcl-xL promoter activity, and the inhibition of constitutively activated STAT3 with specific inhibitors or RNA interference (RNAi) correlates with altered Bcl-2 protein family expression, including Bcl-2 and Bcl-xL. IL-6 and AG490 are the activator and inhibitor of STAT3 signalling, respectively. Our results showed that the expression of Bcl-2 and Bcl-xL were suppressed by sesamin or AG490 but enhanced by IL-6 (Figs. 6–8), suggesting that sesamin could inhibit the STAT3 signalling in HepG2 cells, result in the up-regulation of p53, p21, and down-regulation of the Bcl-2/Bax and Bcl-xL/Bax ratio. When the ratio was down-regulated, the cytochrome c would be released from the intermembrane space into the cytosol, and then activated caspase-9 and caspase-3, leading to apoptosis.

In conclusion, we have provided evidence that sesamin has the ability to suppress the STAT3 signalling pathway in HepG2 cells, leading to the decrease of cell proliferation through G2/M phase arrest and inducing apoptotic. The results in this study also provide evidence for sesamin as a potential STAT3 inhibitor and therapeutic compound for the treatment of hepatocellular carcinoma.

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