Arch Biol Sci. 2023;75(3):287-297 https://doi.org/10.2298/ABS230314023S

Seselin promotes cisplatin-induced apoptosis of AGS gastric cancer cells by inhibiting β-catenin expression

Rong-Yaun Shyu[1], Chun-Hua Wang[2,3], Chang-Chieh Wu[4], Lu-Kai Wang[5] and Fu-Ming Tsai[6,*]

[1]*Department of Internal Medicine, Taipei Tzu Chi Hospital, The Buddhist Tzu Chi Medical Foundation*, 231 New Taipei City, Taiwan

[2]*Department of Dermatology, Taipei Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation*, 231 New Taipei City, Taiwan

[3]*School of Medicine, Tzu Chi University*, 970 Hualien, Taiwan

[4]*Department of Surgery, Tri-Service General Hospital Keelung Branch, National Defense Medical Center*, 202 Keelung, Taiwan

[5]*Department of Life Sciences, National Science and Technology Council*, 106 Taipei, Taiwan

[6]*Department of Research, Taipei Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation*, 231 New Taipei City, Taiwan

***Corresponding author**: afu2215@gmail.com

Received: March 14, 2023; **Revised**: April 19, 2023; **Accepted**: May 3, 2023; **Published online**: May 26, 2023

Abstract: Gastric cancer is a commonly diagnosed form of cancer, and cisplatin is commonly used as a chemotherapy drug for treating it. However, the side effects of cisplatin may reduce patients' willingness to use it. Seselin, a derivative of coumarin, has been found to have anticancer properties as well as anticoagulant effects. In this study, we investigated the effect of seselin on promoting cisplatin-induced gastric cancer cell death using the cell proliferation reagent WST-1, BrdU incorporation and lactate dehydrogenase release. The role of seselin and cisplatin in the apoptosis of gastric cancer cells was analyzed using a phospho-kinase array and Western blot analysis. Seselin did not affect G2/M stasis, but it promoted cell death in AGS cells treated with cisplatin. Phospho-kinase array analysis revealed that cisplatin regulates intracellular p53 phosphorylation, while seselin regulates intracellular β-catenin expression by affecting the phosphorylation of glycogen synthase kinase-3 beta (GSK-3β), extracellular-signal-regulated kinase (ERK) and Src tyrosine kinase. Seselin and cisplatin promote the apoptosis of gastric cancer cells by the synergistic effect of two distinct signaling pathways. These findings suggest that seselin may be used as a complementary therapy to reduce the clinical dose of chemotherapy.

Keywords: seselin, cisplatin, coumarin, apoptosis, complementary therapy

INTRODUCTION

Gastric cancer was the fifth most commonly diagnosed cancer worldwide in 2020 and has the fourth highest mortality rate among all cancers [1]. If gastric cancer is diagnosed at a very early stage and treated immediately, the five-year survival rate of patients is almost more than 70%; however, the survival rate declines rapidly once cancer cells spread [2]. The early symptoms of gastric cancer include heartburn, upper abdominal pain, nausea and loss of appetite, which are similar to the symptoms of peptic ulcer, leading to delayed medical treatment and a high mortality rate. Metastatic gastric cancer remains an incurable disease. In general, the five-year survival rate for patients with advanced gastric cancer is about 10% [2]. Chemotherapy has become one of the indispensable methods in the treatment of gastric cancer, and palliative chemotherapy has been proven to improve the survival rate or the quality of life in the final stage.

Cisplatin, an anticancer drug containing platinum metal compounds, was initially used as a chemotherapy drug for gastric cancer and was later applied to treat lung cancer, nasopharyngeal cancer, ovarian cancer, and other cancers [3]. The main function of cisplatin is to block the synthesis of DNA and inhibit the growth of tumor cells. Its side effects include nausea, vomiting, hair loss, and other symptoms. It may also cause ototoxicity or nephrotoxicity. Clinical studies have indicated that about one-third of patients will suffer acute kidney injury when receiving cisplatin treatment [4,5], and the

incidence of ototoxicity caused by cisplatin is 20-70%, which may cause irreversible hearing loss [6]. These side effects will discourage patients from taking cisplatin for longer, thus limiting its clinical efficacy.

Traditional Chinese medicine is often used as an adjuvant drug in the treatment of cancer, which can assist the efficacy of Western medicine or relieve the discomfort of Western medicine to patients [7,8]. Although traditional Chinese medicine cannot replace surgical treatment, chemotherapy, and radiotherapy for cancer, the discomfort and the side effects caused by Western medicine, such as insomnia, cancer fatigue, or pain caused by surgery, can be alleviated by traditional Chinese medicine treatment, and increase the efficacy of Western medicine to improve the quality of life of patients [9, 10].

Seselin is one of the coumarin derivatives, belonging to flavonoid compounds, which can be found in plants such as *Haplophyllum cappadocicum* or *Haplophyllum dshungaricum*. Coumarin was first used as an anticoagulant [11] and was later found to have the ability to inhibit the growth of cancer cells [12]. The effect of seselin has not been studied much in the past, although it has been reported to have anticancer and antiinflammatory effects [13,14]. The detailed mechanism of its inhibition of cancer cell growth remains unclear. In this study, we analyzed the effects of several potential coumarin derivatives on cisplatin in inhibiting the growth of gastric cancer cells and found that seselin can promote the toxicity of cisplatin in gastric cancer cells. Therefore, in this study, we further explored the intracellular target of seselin and its possible role in regulating cell death. It is hoped that seselin could be used as an auxiliary Chinese medicine for chemotherapy drugs.

MATERIALS AND METHODS

Ethics statement

This article does not contain any studies with human participants or animals.

Materials

Seselin was synthesized as described previously [15]. RPMI-1640 medium and fetal bovine serum (FBS) were obtained from Gibco (Thermo Fisher Scientific, USA). Cisplatin, WST-1 reagent, lactate dehydrogenase (LDH) activity with the Cytotoxicity Detection Kit, and caspase-3 substrate (Ac-DEVD-pNA) were purchased from Sigma (MO, USA). The cell proliferation ELISA kit, BrdU (colorimetric), was purchased from Roche Diagnostics (IN, USA). The Proteome Profiler™ Human Phospho-Kinase Array Kit was obtained from R&D Systems (MN, USA). Antibodies recognized for phospho-p53 (Ser15), phospho-p53 (Ser46), phospho-GSK3β (Ser9), phospho-Src (Tyr416), phospho-ERK, GSK-3β, Src, and β-catenin were purchased from Cell Signaling Technology (MA, USA). Antibodies for p53 and actin were purchased from Biogenex (CA, USA) and Sigma, respectively.

Cell culture and viability assay

Human gastric cancer AGS and SC-M1 cells were maintained at 37°C in humidified 95% air/5% CO_2 atmosphere in Roswell Park Memorial Institute (RPMI) Medium 1640 supplemented with 10% FBS, 2 mM glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin. For the cell viability assay, cells were initially incubated in triplicate with a cell density of 3×10^4 cells/per well in RPMI-1640 supplemented with 10% FBS for 24 h. Next, the cells were treated with seselin and 10 μM cisplatin at the indicated concentration, and then incubated in RPMI-1640 supplemented with 1% FBS for 48-72 h. After this incubation period, 100 μL of WST-1 reagent was added to each well, and the cells were incubated at 37°C for 4-6 h. Finally, the absorbance of the cells, which indicated cell viability was measured at 450 nm and 650 nm using an EIA reader (Infinite F200, Tecan, Durham, NC, USA).

Cell proliferation and cell death assay

AGS or SC-M1 cells were seeded at a density of 3×10^4 cells per well in 24-well plates. After incubating the cells in serum-free RPMI-1640 medium for 18 h, they were further cultured in medium containing 1% FBS supplemented with 100 nM seselin and 10 μM cisplatin for an additional 24 h. To assess cell proliferation, a 10 μM BrdU labeling solution was added to each well and incubated for 12 h. Following the removal of the BrdU labeling solution, the sample was fixed and denatured, and then incubated with peroxidase labeled anti-BrdU antibody for 90 min. Subsequently, a tetramethylbenzidine substrate was added to induce

color reaction, and the absorbance at 370 nm and 492 nm in each sample was measured using an EIA reader to reflect the level of cell proliferation. Alternatively, to detect the cytotoxic effect of seselin and cisplatin on the cells, the culture supernatant was harvested after treating the cells with 10 μM cisplatin and 100 nM seselin in 1% FBS/RPMI-1640 for 24 h. The amount of LDH released by the cells into the culture medium was determined using the Cytotoxicity Detection Kit, following the methods described in the literature [16].

Cell cycle distribution and sub-G1 phase analysis

To examine the effects of cisplatin and seselin on the AGS cell cycle, cells were stained with propidium iodide (PI) and then analyzed in a flow cytometer (Cytomics FC 500; Becton Dickinson, NJ, USA). The cell cycle distribution was calculated after gating for cell populations in the FL-2 area versus FL-2 width plot for PI fluorescence.

Analysis of phospho-kinase array

After the AGS cells were treated with seselin and cisplatin for 24 h, the cell lysate was collected with lysis buffer 6 provided in the kit, and the protein concentration was measured with Bio-Rad Protein Assay Reagent. A total of 300 μg of protein was loaded on each array and detected overnight. Then, the detection antibody was added for 1 h, streptavidin-horseradish peroxidase (HRP) was added for 30 min, and a luminometer (Bio-Rad ChemiDoc XRS+ System) was used for image development and film scanning after enzyme coloration and chemiluminescence.

Western blot

AGS cells were plated in 10-cm culture dishes at a cell number of 1×10^6 and cultured for 24 h. After treatment with seselin and cisplatin for 24 h, the cells were lysed with RIPA buffer containing protease inhibitor cocktail (Roche Diagnostics, Germany) and phosphatase inhibitors (2 mM NaF, 1 mM Na_3VO_4). The protein concentration in each cell lysate was measured with Bio-Rad Protein Assay Reagent, and 20-60 μg protein from each cell lysate was taken for immunoblot analysis. The operation method of immunoblot analysis was as described [17].

Measurement of caspase-3 activity

AGS cells were treated with seselin and cisplatin for 24 h and cell lysates were collected with RIPA buffer. Cell lysates were then incubated in buffer containing 25 mM HEPES [pH 7.5], 0.1% CHAPS, 5% sucrose, 5 mM DTT, 2 mM EDTA, and 2 mM caspase-3 substrate. Finally, the absorbance at 405 nm was measured in an EIA reader to reflect caspase-3 activity.

Statistical analysis

The data obtained from each experiment were analyzed at least three times and the results were expressed as the mean±standard deviation. Statistical analysis was conducted using one-way ANOVA with Dunnett's post hoc test. A P value of less than 0.05 was considered to indicate a statistically significant difference between groups.

RESULTS

Seselin promoted cisplatin to reduce the cell viability of gastric cancer cells

Several coumarin derivatives were examined for their effect on the viability of AGS gastric cancer cells following cisplatin treatment, including 7-((2-methylbut-3-yn-2-yl)oxy)-2H-chromen-2-one, 8,8-dimethyl-2H,8H-pyrano[2,3-f]chromen-2-one (also known as seselin, Fig. 1A), 8,8-dimethyl-2H,8H-pyrano[3,2-g]chromen-2-one, 7-hydroxy-3-phenyl-4H-chromen-4-one (another 7-hydroxyflavone), and 2-(2,2-dimethyl-2H-chromen-7-yl)-6-hydroxychroman-4-one. Only seselin was found to promote the death of cisplatin-treated gastric cancer cell lines (Supplementary Fig. S1), and therefore it was selected for subsequent experiments. When the AGS gastric cancer cell line was treated with 10 μM cisplatin for 48 h or 72 h, the cell viability decreased to 81.3% and 54.6% (Fig. 1B). When AGS cells were treated with seselin for 48-72 h, only higher doses (100-400 nM) of seselin could slightly reduce cell viability to 75.6%-93.1%. However, high doses of seselin promoted cisplatin to reduce cell viability in AGS cells to 32.4-57% (Fig. 1B). In addition to AGS cells, seselin also promoted the ability of cisplatin to reduce cell viability in SC-M1 gastric cancer

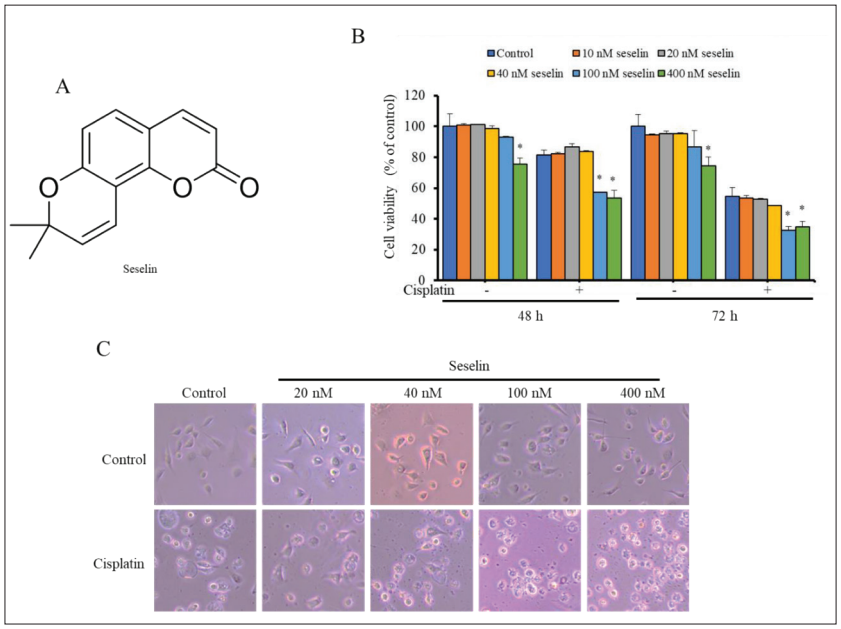


Fig. 1. The effects of seselin and cisplatin on the viability of AGS cells were determined using the WST-1 reagent. **A** – Chemical structure of seselin. **B** – The cell viability detected with different concentrations of seselin and 10 μM cisplatin for 48-72 h. **C** – Morphological changes in AGS cells treated with different concentrations of seselin and 10 μM cisplatin for 24 h observed by phase-contrast microscopy (100× magnification). *P<0.05 compared with the control group.

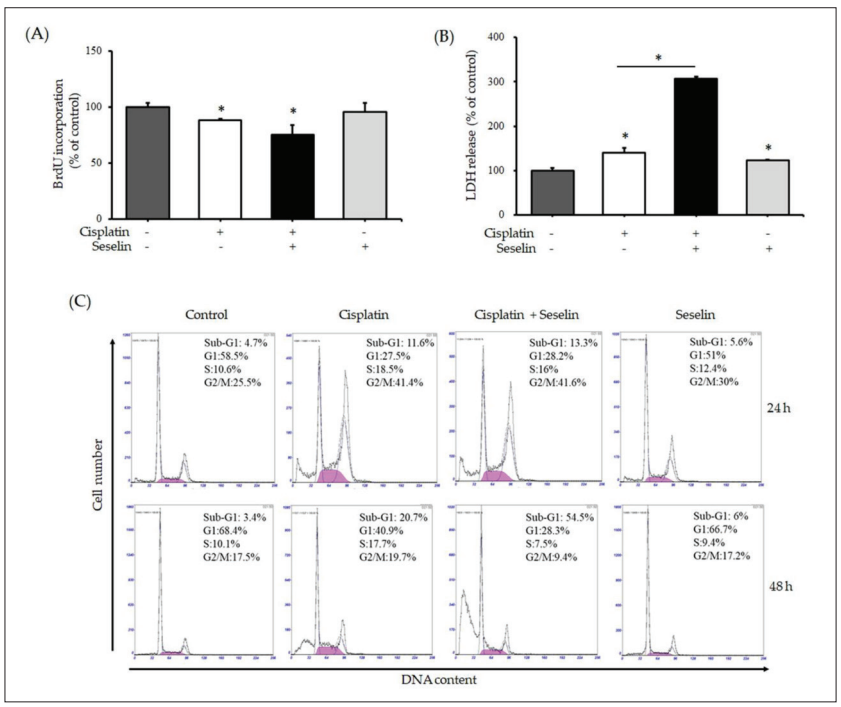


Fig. 2. The effects of seselin and cisplatin on the cell proliferation and death of AGS cells determined by the BrdU assay and LDH release. **A** – Cell proliferation detected after treatment with 100 nM seselin and 10 μM cisplatin for 24 h. **B** – Cell death detected after treatment with 100 nM seselin and 10 μM cisplatin for 24 h. **C** – Cell cycle distribution and sub-G1 cell population determined after treatment with 100 nM seselin and 10 μM cisplatin for 24-48 h. *P<0.05 compared with the control group or compared with the two groups.

cells (Supplementary Fig. S2). Direct observation of cell patterns under a microscope showed that no obvious changes in cell patterns were observed even after high doses of 400 nM seselin were treated for 24 h. However, after the treatment of cisplatin for 24 h, the cytoplasm of some cells began to shrink. When the cells were treated with a combination of cisplatin and higher dose of seselin (100-400 nM), more cells were rounded up and detached from the well, exhibiting a pattern of cell death (Fig. 1C). Since 100 nM dose of seselin had little effect on AGS cells, but significantly promoted cisplatin to reduce the cell viability of gastric cancer cells, the mechanism of seselin was investigated at 100 nM dose in subsequent experiments.

Seselin promoted cisplatin to induce cell death of AGS gastric cancer cells

To explore the possible factors by which seselin can promote cisplatin-induced reduction of cell viability in gastric cancer cells, we further analyzed the effects of seselin and cisplatin on cell proliferation and cell death. Fig. 2A shows that cisplatin inhibited the proliferation of AGS cells by 11.8%. When AGS cells were treated with cisplatin and seselin simultaneously, the proliferative ability was significantly inhibited by 23.1%. However, there was no significant change in the proliferative ability of AGS cells when treated with seselin alone. Fig. 2B shows that both cisplatin and seselin can significantly induce cell death, increasing it by 40.3% and 22.8%, respectively. When cells were treated with cisplatin and seselin at the same time, cell death increased dramatically by 206.1%. These results suggest that seselin does not affect cisplatin-inhibited cell proliferation but promotes cisplatin-induced cell death. Similar synergistic effects of seselin and cisplatin on cell death were also found in SC-M1 gastric cancer cell lines (Supplementary Fig. S3). LDH activity in the culture medium is a reliable indicator

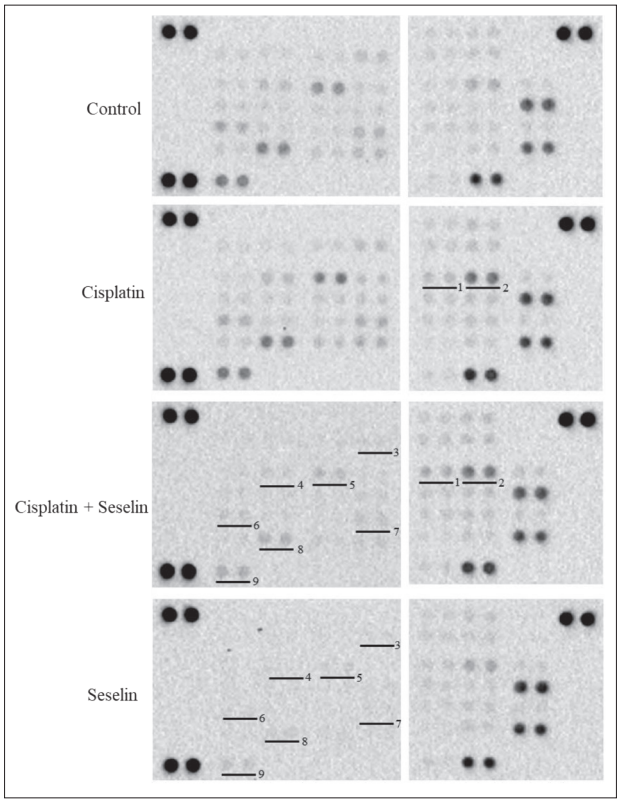


Fig. 3. The effects of seselin and cisplatin on the phospho-protein expression of AGS cells were determined using a human phospho-kinase array. Each dot represents 1. p53 (S15), 2. p53 (S46), 3. ERK (T202/Y204/T185/Y187), 4. GSK-3a/b (S21/S9), 5. GSK-3β (S9), 6. p38α (T180/Y182), 7. Src (Y419), 8. STAT5a/b (Y694/Y699), 9. β-catenin.

of cell membrane integrity and can be used to assess drug toxicity to cells. To determine whether seselin causes cell necrosis, we analyzed the release of LDH by seselin and cisplatin at different time points. The study results showed that LDH was released into the culture medium from AGS cells after treatment with seselin and cisplatin for 24 h and continued until 48 h. However, during the first 2-12 h of treatment with seselin and cisplatin, there was no significant increase in LDH activity in the medium, suggesting that seselin and cisplatin did not cause necrosis in the early stages of treatment (Supplementary Fig. 4). We also analyzed the effects of seselin and cisplatin on the cell cycle and apoptosis using the DNA dye PI reagent. Cisplatin resulted in G2/M cell cycle arrest in AGS cells at 24 h, while seselin did not affect cisplatin-induced G2/M cell cycle arrest (Fig. 2C). The analysis of the sub-G1 phase indicated that seselin slightly increased the number of sub-G1 cells induced by cisplatin (Fig. 2C). When the cells were treated with cisplatin for 48 h, the number of cells in the sub-G1 phase increased to 20.7%, while seselin promoted an increase in sub-G1 cells induced by cisplatin by 54.5% (Fig. 2C). Since seselin alone did not promote the accumulation of hypodiploid cells after an additional 24 h, the dramatic increase in the sub-G1 compartment after the combined treatment clearly demonstrated that seselin exhibits a synergistic effect with cisplatin.

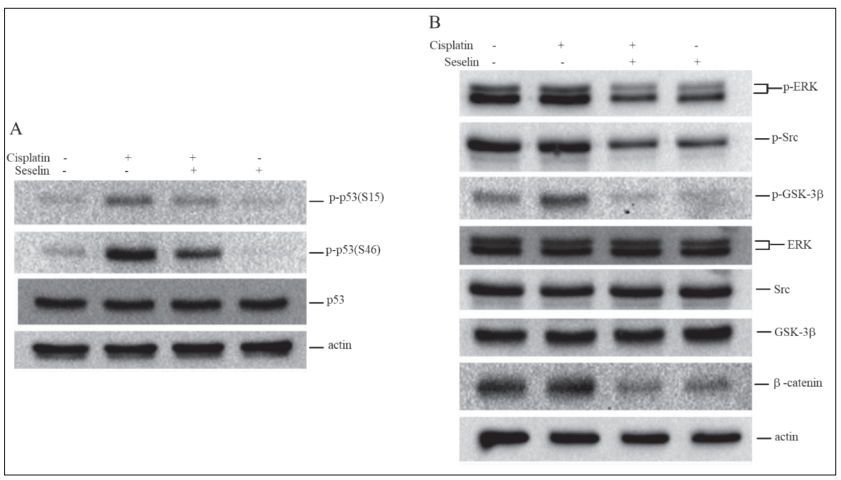


Fig. 4. The effects of seselin and cisplatin on the levels of phospho-p53 and β-catenin-related proteins in AGS cells as determined by Western blot analysis. The levels of p53 (A) and β-catenin-related proteins (B) detected after treatment with 100 nM seselin and 10 μM cisplatin for 24 h.

Analysis of the targets of seselin and cisplatin in cells by the phospho-kinase array

To further explore the target of seselin and cisplatin in inhibiting the cell viability of AGS gastric cells, human phospho-kinase array was used for analysis. According to the results of the array (Fig. 3), cisplatin increased the phosphorylation of p53 in AGS cells, implying that the p53 signaling pathway is involved in regulating the blocking of DNA synthesis caused by cisplatin. Phosphorylation of many proteins was affected when seselin was added to cells, such as ERK, GSK-3β, Src, and signal transducers and activators of transcription 5

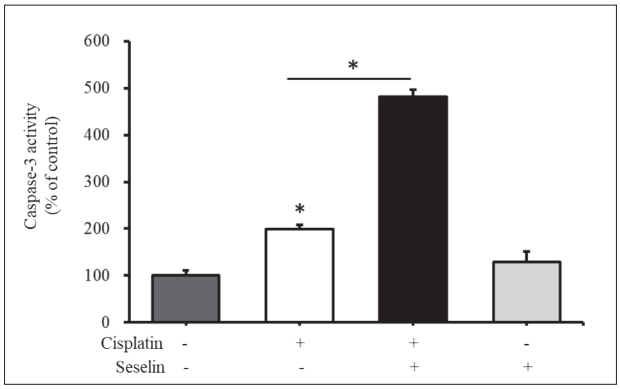


Fig. 5. The effects of seselin and cisplatin on caspase-3 activity in AGS cells as determined using a colorimetric substrate specific to caspase-3. *P<0.05 compared with the control group or compared with the two groups.

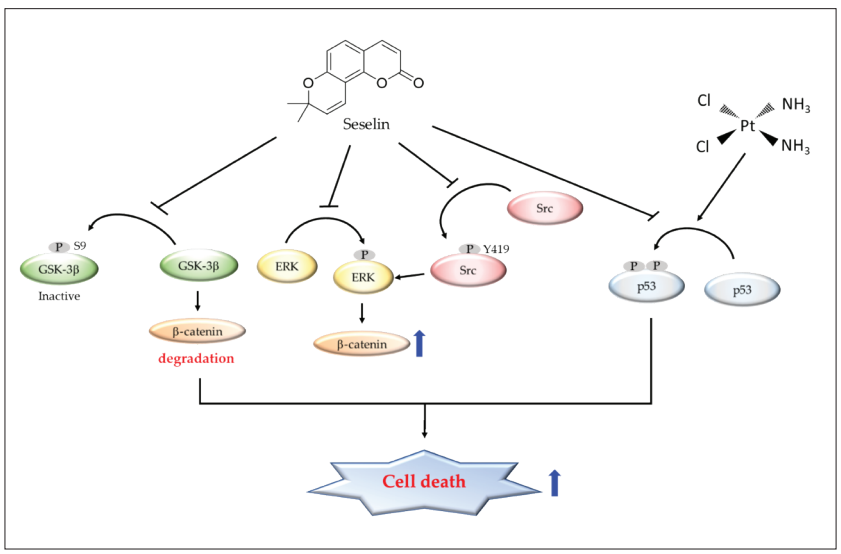


Fig. 6. Schematic diagram of seselin promoting cisplatin to cause death of AGS gastric cancer cells.

(STAT5a and STAT5b) (Fig. 3). Seselin also inhibited intracellular β-catenin expression. Seselin and cisplatin did not show an additive effect on the phosphorylation of p53 and other proteins when added together compared to when added alone. This suggests that seselin and cisplatin may synergistically regulate cell growth or death through different pathways.

Analysis of the effects of seselin and cisplatin on the expression of p53 and β-catenin related proteins

To verify the results of the phospho-kinase arrays, the effects of seselin and cisplatin on the expression of various proteins in cells were determined using Western blotting. Fig. 4A shows that cisplatin increased intracellular p53 phosphorylation at Ser15 and Ser46, while the simultaneous addition of seselin decreased cisplatin-induced p53 phosphorylation. The effects of cisplatin and seselin on the phosphorylation of other proteins were also analyzed (Fig. 4B). Seselin inhibited intracellular phosphorylation of ERK, Src, and GSK-3β. The levels of phosphorylated STAT5a/b and p38 were not detected in any group (data not shown). Treatment with cisplatin slightly increased intracellular β-catenin, but co-treatment with seselin decreased β-catenin levels.

Analysis of the effects of seselin and cisplatin on caspase-3 activity

Since seselin collaborates with cisplatin to cause cell death, and both phosphorylated p53 expression and β-catenin are affected by seselin and cisplatin, we further analyzed the effects of seselin and cisplatin on caspase-3 activity, which is regulated by p53 and β-catenin [18]. As shown in Fig. 5, cisplatin increased intracellular caspase-3 activity by 99.6%. Simultaneous treatment with seselin and cisplatin increased intracellular caspase-3 activity by 382%, while treatment with seselin alone did not affect caspase-3 activity. These results suggest that seselin's promotion of cisplatin-induced cell death may be related to the activation of caspase-3, which plays a major role in cell apoptosis [19].

DISCUSSION

The Wnt signaling pathway is implicated in numerous physiological processes, including cell proliferation, differentiation, apoptosis, migration and invasion. β-catenin, a transcription factor, plays a critical role in the Wnt signaling pathway, and its abnormal regulation leads to the expression of genes such as c-Myc, cyclin D1 and cyclin dependent kinase inhibitor 1A (CDKN1A) in cells underlying the early events of carcinogenesis [20]. GSK-3β is a multifunctional serine and threonine kinase that is involved in various cellular activities, including glycogen metabolism, cell proliferation, differentiation and apoptosis. Additionally, GSK-3β

can phosphorylate β-catenin, which triggers its ubiquitination and subsequent proteasomal degradation [21]. Src is a hub for several signals involved in many of these biological processes and is also a key regulator of cellular metabolism, including metabolic pathways like glucose uptake, glycolysis, pentose-phosphate pathway and oxidative phosphorylation [22]. The dysregulation of these signaling pathways is closely linked to the occurrence and progression of malignant tumors [23,24]. Similarly, in gastric cancer, the Wnt/β-catenin, GSK-3β, and Src signaling pathways are also abnormally regulated [25-27].

This study pointed out that seselin can promote the death of gastric cancer cells induced by cisplatin. According to the results of the phospho-kinase array and Western blotting, seselin can inhibit the levels of many phosphorylated proteins, while cisplatin can increase the expression of phosphorylated p53 in AGS gastric cells. Phosphorylation of Src and ERK leads to protein activation and induces the expression of β-catenin [28,29], while phosphorylated GSK-3β loses its ability to degrade β-catenin [24, 30]. Our study suggests that the addition of seselin can inhibit the phosphorylation of ERK, Src, and GSK-3β in cells, indicating that seselin reduces the content of β-catenin in cells through the ERK, Src, and GSK-3β pathway. Studies have shown that p53 is phosphorylated at Ser15 and Ser46, leading to cell death [31,32]. Cisplatin can induce the phosphorylation of p53 in AGS gastric cancer cells, suggesting that cisplatin may cause cell death through a p53-dependent pathway. Although seselin reduces cisplatin-induced phosphorylation of p53, there still remains a sufficient amount of phosphorylated p53 that does not seem to affect cisplatin-induced cell death. Conversely, β-catenin is involved in cell survival and resistance to cell death [33]. Seselin reduces intracellular β-catenin level through the ERK, Src, and GSK-3β pathways. Cisplatin increases intracellular phosphorylated p53. Therefore, the combination of these two signaling pathways may result in seselin promoting cisplatin toxicity in gastric cancer cells (Fig. 6). Although seselin or cisplatin can activate single signaling pathways, their effects on gastric cancer cell death are not significant, requiring either a longer treatment duration or higher dose. Our study suggests that the addition of seselin can reduce the dose of cisplatin in the treatment of gastric cancer, and at the same time reduce the side effects.

Cisplatin is a widely used chemotherapeutic agent for various types of cancer, but its mechanism of action in killing cancer cells is still being investigated. It is known that cisplatin induces the production of reactive oxygen species (ROS) through DNA damage or increased mitochondrial pressure, which activates p53 and downstream caspase cascades, leading to cell death [34,35]. Even in the presence of p53 mutations, cisplatin can still induce cell cycle arrest or apoptosis through p53-independent pathways [34,36]. In our study, both AGS and SC-M1 gastric cancer cell lines had wild-type p53 genes without any genetic variation, thus indicating that the observed phenomenon was biased toward the toxic effect of cisplatin on cancer cells. Further investigation is needed to determine whether seselin also assists cisplatin in inducing cell cycle arrest in p53 mutant cells.

The mechanism of cisplatin toxicity on cancer cells described above also contributes to the damage of cisplatin on normal tissues or organs, such as kidney injury or auditory nerve toxicity [37,38]. Currently, traditional Chinese medicine is used as an adjuvant to chemotherapy drugs, primarily aimed at reducing the adverse side effects of chemotherapy on the body and improving patient outcomes during chemotherapy. For instance, the Tuhuo & Taxillus Combination is often used in traditional Chinese medicine to alleviate kidney or nerve damage caused by chemotherapy drugs [39], while Ba Zhen Tang is used to mitigate immune system damage caused by chemotherapy drugs [40]. Moreover, some traditional Chinese medicines, such as *Solanum nigrum*, when combined with chemotherapy drugs, can increase the toxicity of chemotherapy drugs to cancer cells [41,42]. This synergistic effect may also allow for a lower dosage of chemotherapy drugs, thereby minimizing their side effects.

Recently, there has been an increase in the number of studies suggesting that natural products can be used as adjuvant drugs for chemotherapy, in addition to compounds used in traditional Chinese medicine. Flavonoids, which constitute the largest group of phytonutrients, have been shown to reduce chemotherapy-induced nephrotoxicity by inhibiting p53, mitogen-activated protein kinase, and AKT signaling pathways [43,44]. On the other hand, flavonoids can enhance the toxic effect of cisplatin on cancer cells by blocking intracellular glutathione [45]. Alkaloids, which are naturally occurring organic compounds, can increase the sensitivity of ovarian cancer

cells to cisplatin by regulating the AKT-Kβ and c-Jun N-terminal kinase pathway [46]. Polyphenols are also effective antitumor agents and can be used as natural products to promote cisplatin-induced cell damage through the AKT pathway [47]. Moreover, many other natural products have also been developed to serve as adjuvant drugs for chemotherapy [48]. Although the natural products mentioned above can promote the toxic effect of cisplatin on cancer cells by inhibiting specific signal transduction pathways, it has also been suggested that aloe emodin can cause cell death of non-small-cell lung cancer cells by inhibiting Akt and ERK signaling pathways [49]. This may lead to a reduction in the toxic ability of cisplatin to glioma cells [50]. Therefore, more verification is needed to understand the curative effect of these natural products in clinical practice.

When patients are treated with chemotherapeutic drugs, they often lose their effectiveness because the cancer cells develop resistance to them. The mechanisms of drug resistance in cells include increased drug metabolism, reduced drug entry into cells or accelerated drug expulsion, and mutations in DNA repair systems leading to drug inactivity [51]. In addition to mutations in the direct target of the drug, the expression of β-catenin can also increase the resistance of cancer cells to cisplatin [52,53]. In our study, β-catenin was one of the main targets of seselin. Therefore, by inhibiting the level of β-catenin in cells, seselin not only inhibits cell survival, but also appears to reduce the resistance of cancer cells to cisplatin. However, further verification of this hypothesis in cisplatin-resistant cell lines is necessary.

CONCLUSIONS

In summary, seselin can promote cisplatin-induced apoptosis in gastric cancer cells. Seselin can affect the activity of GSK-3β, ERK and Src in cells, interact with cisplatin to affect the phosphorylation of p53 in cells, and then promote the activity of caspass-3 in cells and cause cell death. Therefore, our study suggests that seselin can be used as an adjuvant drug for cisplatin treatment of cancer cells, which can reduce the dose of clinical chemotherapy drugs and thus reduce the side effects caused by chemotherapy drugs. Our study suggests that seselin can reduce the therapeutic dose of cisplatin and attenuate the side effects caused by the treatment. All in all, it can be concluded that this natural agent is worthy of further preclinical investigation, with the goal of including it in protocols as an adjunct to treatment.

Funding: This study was supported by the Taipei Tzu Chi Hospital through grants from the Buddhist Tzu Chi Medical Foundation under the numbers TCRD-TPE-110-13 and TCRD-TPE-111-23, Taipei, Taiwan.

Acknowledgments: The authors thank the Core Laboratory of the Buddhist Tzu Chi General Hospital for support.

Author contributions: Conceptualization, RYS and FMT; methodology, FMT; validation, CHW and LKW; formal analysis, CHW; investigation, LKW and FMT; resources, RYS; writing – original draft preparation, RYS and FMT; writing – review and editing, FMT; supervision, FMT; project administration, RYS; funding acquisition, CHW and FMT. All authors have read and agreed to the published version of the manuscript.

Conflict of interest disclosure: The authors declare no conflict of interest.

Data availability: The data underlying the findings presented in this study are available in the Supplementary Material.

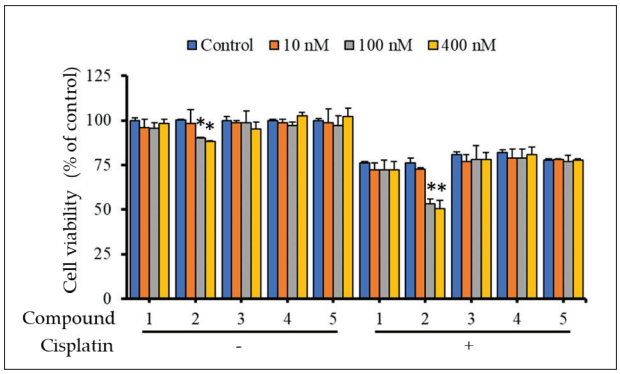
REFERENCES

1. Morgan E, Arnold M, Camargo MC, Gini A, Kunzmann AT, Matsuda T, Meheus F, Verhoeven RHA, Vignat J, Laversanne M, Ferlay, J, Soerjomataram, I. The current and future incidence and mortality of gastric cancer in 185 countries, 2020-40: A population-based modelling study. EClinicalMedicine. 2022;47:101404. https://doi.org/10.1016/j.eclinm.2022.101404
2. Li Y, Feng A, Zheng S, Chen C, Lyu J. Recent Estimates and Predictions of 5-Year Survival in Patients with Gastric Cancer: A Model-Based Period Analysis. Cancer Control. 2022:29:10732748221099227. https://doi.org/10.1177/10732748221099227
3. Petrelli F, Zaniboni A, Coinu A, Cabiddu M, Ghilardi M, Sgroi G, Barni S. Cisplatin or not in advanced gastric cancer: a systematic review and meta-analysis. PLoS One. 2013;8(12):e83022. https://doi.org/10.1371/journal.pone.0083022
4. Arany I, Safirstein RL. Cisplatin nephrotoxicity. Semin Nephrol. 2003;23(5):460-4. https://doi.org/10.1016/s0270-9295(03)00089-5
5. Tang C, Livingston MJ, Safirstein R, Dong Z. Cisplatin nephrotoxicity: new insights and therapeutic implications. Nat Rev Nephrol. 2023;19(1):53-72. https://doi.org/10.1038/s41581-022-00631-7
6. Tang Q, Wang X, Jin H, Mi Y, Liu L, Dong M, Chen Y, Zou Z. Cisplatin-induced ototoxicity: Updates on molecular mechanisms and otoprotective strategies. Eur J Pharm Biopharm. 2021;163:60-71. https://doi.org/10.1016/j.ejpb.2021.03.008
7. Zhang X, Qiu H, Li C, Cai P, Qi F. The positive role of traditional Chinese medicine as an adjunctive therapy for cancer. Biosci Trends. 2021;15(5):283-98. https://doi.org/10.5582/bst.2021.01318
8. Jiao L, Dong C, Liu J, Chen Z, Zhang L, Xu J, Shen X, Che J, Yang Y, Huang H Li, H, Sun J, Jiang Y, Mao Z, Chen P, Gong

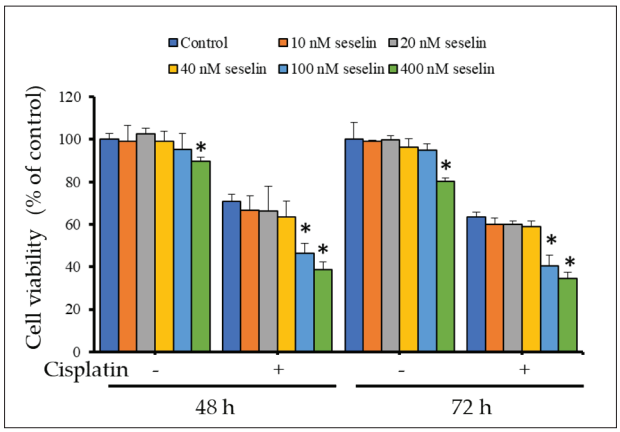
Y, Jin X, Xu L.Effects of Chinese Medicine as Adjunct Medication for Adjuvant Chemotherapy Treatments of Non-Small Cell Lung Cancer Patients. Sci Rep. 2017;7:46524. https://doi.org/10.1038/srep46524
9. Ling Y. Traditional Chinese medicine in the treatment of symptoms in patients with advanced cancer. Ann Palliat Med. 2013;2(3):141-52. https://doi.org/10.3978/j.issn.2224-5820.2013.04.05
10. Xiang Y, Guo Z, Zhu P, Chen J, Huang Y. Traditional Chinese medicine as a cancer treatment: Modern perspectives of ancient but advanced science. Cancer Med. 2019;8(5):1958-75. https://doi.org/10.1002/cam4.2108
11. Kasperkiewicz K, Ponczek MB, Owczarek J, Guga P, Budzisz E. Antagonists of Vitamin K-Popular Coumarin Drugs and New Synthetic and Natural Coumarin Derivatives. Molecules. 2020;25(6):1465. https://doi.org/10.3390/molecules25061465
12. 12. Banikazemi Z, Mirazimi SM, Dashti F, Mazandaranian MR, Akbari M, Morshedi K, Aslanbeigi F, Rashidian A, Chamanara M, Hamblin MR, Taghizadeh M, Mirzaei H. Coumarins and Gastrointestinal Cancer: A New Therapeutic Option? Front Oncol. 2021;11:752784. https://doi.org/10.3389/fonc.2021.752784
13. Nishino H, Okuyama T, Takata M, Shibata S, Tokuda H, Takayasu J, Hasegawa T, Nishino A, Ueyama H, Iwashima A. Studies on the anti-tumor-promoting activity of naturally occurring substances. IV. Pd-II [(+)anomalin, (+)praeruptorin B], a seselin-type coumarin, inhibits the promotion of skin tumor formation by 12-O-tetradecanoylphorbol-13-acetate in 7,12-dimethylbenz[a]anthracene-initiated mice. Carcinogenesis. 1990;11(9):1557-61. https://doi.org/10.1093/carcin/11.9.1557
14. Feng L, Sun Y, Song P, Xu L, Wu X, Wu X, Shen Y, Sun Y, Kong L, Wu X, Xu Q. Seselin ameliorates inflammation via targeting Jak2 to suppress the proinflammatory phenotype of macrophages. Br J Pharmacol. 2019;176(2):317-33. https://doi.org/10.1111/bph.14521
15. Lu PH, Liao TH, Chen YH, Hsu YL, Kuo CY, Chan CC, Wang LK, Chern CY, Tsai FM. Coumarin Derivatives Inhibit ADP-Induced Platelet Activation and Aggregation. Molecules. 2022;27(13): 4054. https://doi.org/10.3390/molecules27134054
16. Liu CJ, Wang LK, Kuo CY, Chen ML, Tzeng IS, Tsai FM. Tournefortia sarmentosa Inhibits the Hydrogen Peroxide-Induced Death of H9c2 Cardiomyocytes. Evid Based Complement Alternat Med. 2021;2021:8219141. https://doi.org/10.1155/2021/8219141
17. Wang CH, Lu TJ, Wang LK, Wu CC, Chen ML, Kuo CY, Shyu RY, Tsai FM. Tazarotene-induced gene 1 interacts with Polo-like kinase 2 and inhibits cell proliferation in HCT116 colorectal cancer cells. Cell Biol Int. 2021;45(11):2347-56. https://doi.org/10.1002/cbin.11681
18. Trejo-Solis C, Escamilla-Ramirez A, Jimenez-Farfan D, Castillo-Rodriguez RA, Flores-Najera A, Cruz-Salgado A. Crosstalk of the Wnt/beta-Catenin Signaling Pathway in the Induction of Apoptosis on Cancer Cells. Pharmaceuticals (Basel). 2021;14(9):871. https://doi.org/10.3390/ph14090871
19. Shalini S, Dorstyn L, Dawar S, Kumar S. Old, new and emerging functions of caspases. Cell Death Differ. 2015;22(4): 526-39. https://doi.org/10.1038/cdd.2014.216
20. Li YJ, Wei ZM, Meng YX, Ji XR. Beta-catenin up-regulates the expression of cyclinD1, c-myc and MMP-7 in human pancreatic cancer: relationships with carcinogenesis and metastasis. World J Gastroenterol. 2005;11(14):2117-23. https://doi.org/10.3748/wjg.v11.i14.2117
21. Nusse R, Clevers H. Wnt/beta-Catenin Signaling, Disease, and Emerging Therapeutic Modalities. Cell. 2017;169(6): 985-99. https://doi.org/10.1016/j.cell.2017.05.016
22. Pelaz SG, Tabernero A. Src: coordinating metabolism in cancer. Oncogene. 2022;41(45):4917-28. https://doi.org/10.1038/s41388-022-02487-4
23. Zhang Y, Wang X. Targeting the Wnt/beta-catenin signaling pathway in cancer. J Hematol Oncol. 2020;13(1):165. https://doi.org/10.1186/s13045-020-00990-3
24. He R, Du S, Lei T, Xie X, Wang Y. Glycogen synthase kinase 3beta in tumorigenesis and oncotherapy (Review). Oncol Rep. 2020;44(6):2373-85. https://doi.org/10.3892/or.2020.7817
25. Chiurillo MA. Role of the Wnt/beta-catenin pathway in gastric cancer: An in-depth literature review. World J Exp Med. 2015;5(2):84-102. https://doi.org/10.5493/wjem.v5.i2.84
26. Mello AA, Leal MF, Rey JA, Pinto GR, Lamarao LM, Montenegro RC, Alves AP, Assumpcao PP, Borges Bdo N, Smith MC, Burbano, RR. Deregulated Expression of SRC, LYN and CKB Kinases by DNA Methylation and Its Potential Role in Gastric Cancer Invasiveness and Metastasis. PLoS One. 2015;10(10):e0140492. https://doi.org/10.1371/journal.pone.0140492
27. DE Fátima Ferreira Borges DA Costa J, DE Castro Sant' Anna C, Muniz JAPC, DA Rocha CAM, Lamarão LM, DE Fátima Aquino Moreira Nunes C, DE Assumpção PP, Burbano RR.. Deregulation of the SRC Family Tyrosine Kinases in Gastric Carcinogenesis in Non-human Primates. Anticancer Res. 2018;38(11):6317-20. https://doi.org/10.21873/anticanres.12988
28. Lee SK, Hwang JH, Choi KY. Interaction of the Wnt/beta-catenin and RAS-ERK pathways involving co-stabilization of both beta-catenin and RAS plays important roles in the colorectal tumorigenesis. Adv Biol Regul. 2018;68:46-54. https://doi.org/10.1016/j.jbior.2018.01.001
29. Ryu WJ, Han G, Lee SH, Choi KY. Suppression of Wnt/beta-catenin and RAS/ERK pathways provides a therapeutic strategy for gemcitabine-resistant pancreatic cancer. Biochem Biophys Res Commun. 2021;549:40-6. https://doi.org/10.1016/j.bbrc.2021.02.076
30. Glibo M, Serman A, Karin-Kujundzic V, Bekavac Vlatkovic I, Miskovic B, Vranic S, Serman L. The role of glycogen synthase kinase 3 (GSK3) in cancer with emphasis on ovarian cancer development and progression: A comprehensive review. Bosn J Basic Med Sci. 2021;21(1):5-18. https://doi.org/10.17305/bjbms.2020.5036
31. Liebl MC, Hofmann TG. Cell Fate Regulation upon DNA Damage: p53 Serine 46 Kinases Pave the Cell Death Road. Bioessays. 2019;41(12):e1900127. https://doi.org/10.1002/bies.201900127

32. Yogosawa S, Yoshida K. Tumor suppressive role for kinases phosphorylating p53 in DNA damage-induced apoptosis. Cancer Sci. 2018;109(11):3376-82. https://doi.org/10.1111/cas.13792
33. Liu J, Xiao Q, Xiao J, Niu C, Li Y, Zhang X, Zhou Z, Shu G, Yin G. Wnt/beta-catenin signalling: function, biological mechanisms, and therapeutic opportunities. Signal Transduct Target Ther. 2022;7(1):3. https://doi.org/10.1038/s41392-021-00762-6
34. Ali S, Tahir M, Khan AA, Chen XC, Ling M, Huang Y. Cisplatin Synergistically Enhances Antitumor Potency of Conditionally Replicating Adenovirus via p53 Dependent or Independent Pathways in Human Lung Carcinoma. Int J Mol Sci. 2019;20(5):1125. https://doi.org/10.3390/ijms20051125
35. Kleih M, Bopple K, Dong M, Gaissler A, Heine S, Olayioye MA, Aulitzky WE, Essmann F. Direct impact of cisplatin on mitochondria induces ROS production that dictates cell fate of ovarian cancer cells. Cell Death Dis. 2019;10(11):851. https://doi.org/10.1038/s41419-019-2081-4
36. Zamble DB, Jacks T, Lippard SJ. p53-Dependent and -independent responses to cisplatin in mouse testicular teratocarcinoma cells. Proc Natl Acad Sci U S A. 1998;95(11):6163-68. https://doi.org/10.1073/pnas.95.11.6163
37. McSweeney KR, Gadanec LK, Qaradakhi T, Ali BA, Zulli A, Apostolopoulos V. Mechanisms of Cisplatin-Induced Acute Kidney Injury: Pathological Mechanisms, Pharmacological Interventions, and Genetic Mitigations. Cancers (Basel). 2021;13(7):1572. https://doi.org/10.3390/cancers13071572
38. Waissbluth S, Maass JC, Sanchez HA, Martinez AD. Supporting Cells and Their Potential Roles in Cisplatin-Induced Ototoxicity. Front Neurosci. 2022;16:867034. https://doi.org/10.3389/fnins.2022.867034
39. Qin M, Huang Q, Yang X, Yu L, Tang Y, Zhang C, Qin D, Zou W, Deng J, Liu J, Hu H, Wang L, Wu, A, Wu J. Taxillus chinensis (DC.) Danser: a comprehensive review on botany, traditional uses, phytochemistry, pharmacology, and toxicology. Chin Med. 2022;17(1):136. https://doi.org/10.1186/s13020-022-00694-5
40. Meyer-Hamme G, Beckmann K, Radtke J, Efferth T, Greten HJ, Rostock M, Schroder S. A survey of chinese medicinal herbal treatment for chemotherapy-induced oral mucositis. Evid Based Complement Alternat Med. 2013;2013:284959. https://doi.org/10.1155/2013/284959
41. Lai YJ, Tai CJ, Wang CW, Choong CY, Lee BH, Shi YC, Tai CJ. Anti-Cancer Activity of Solanum nigrum (AESN) through Suppression of Mitochondrial Function and Epithelial-Mesenchymal Transition (EMT) in Breast Cancer Cells. Molecules. 2016;21(5):553. https://doi.org/10.3390/molecules21050553
42. Nawaz A, Jamal A, Arif A, Parveen Z. In vitro cytotoxic potential of Solanum nigrum against human cancer cell lines. Saudi J Biol Sci. 2021;28(8):4786-92. https://doi.org/10.1016/j.sjbs.2021.05.004
43. Ju SM, Kang JG, Bae JS, Pae HO, Lyu YS, Jeon BH. The Flavonoid Apigenin Ameliorates Cisplatin-Induced Nephrotoxicity through Reduction of p53 Activation and Promotion of PI3K/Akt Pathway in Human Renal Proximal Tubular Epithelial Cells. Evid Based Complement Alternat Med. 2015;2015:186436. https://doi.org/10.1155/2015/186436
44. Wang SW, Xu Y, Weng YY, Fan XY, Bai YF, Zheng XY, Lou LJ, Zhang F. Astilbin ameliorates cisplatin-induced nephrotoxicity through reducing oxidative stress and inflammation. Food Chem Toxicol. 2018;114:227-36. https://doi.org/10.1016/j.fct.2018.02.041
45. Kachadourian R, Leitner HM, Day BJ. Selected flavonoids potentiate the toxicity of cisplatin in human lung adenocarcinoma cells: a role for glutathione depletion. Int J Oncol. 2007;31(1):161-8. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3983955
46. Mon MT, Yodkeeree S, Punfa W, Pompimon W, Limtrakul P. Alkaloids from Stephania venosa as Chemo-Sensitizers in SKOV3 Ovarian Cancer Cells via Akt/NF-kappaB Signaling. Chem Pharm Bull (Tokyo). 2018;66(2):162-9. https://doi.org/10.1248/cpb.c17-00687
47. Zhang Y, Chen S, Wei C, Rankin GO, Rojanasakul Y, Ren N, Ye X, Chen YC. Dietary Compound Proanthocyanidins from Chinese bayberry (Myrica rubra Sieb. et Zucc.) leaves inhibit angiogenesis and regulate cell cycle of cisplatin-resistant ovarian cancer cells via targeting Akt pathway. J Funct Foods. 2018;40:573-81. https://doi.org/10.1016/j.jff.2017.11.045
48. Dasari S, Njiki S, Mbemi A, Yedjou CG, Tchounwou PB. Pharmacological Effects of Cisplatin Combination with Natural Products in Cancer Chemotherapy. Int J Mol Sci. 2022;23(3):1532. https://doi.org/10.3390/ijms23031532
49. Kim HJ, Choi JW, Ree J, Lim JS, Lee J, Kim JI, Thapa SB, Sohng JK, Park YI. Aloe emodin 3-O-glucoside inhibits cell growth and migration and induces apoptosis of non-small-cell lung cancer cells via suppressing MEK/ERK and Akt signalling pathways. Life Sci. 2022;300:120495. https://doi.org/10.1016/j.lfs.2022.120495
50. Mijatovic S, Maksimovic-Ivanic D, Radovic J, Miljkovic D, Kaludjerovic GN, Sabo TJ, Trajkovic V. Aloe emodin decreases the ERK-dependent anticancer activity of cisplatin. Cell Mol Life Sci. 2005;62(11):1275-82. https://doi.org/10.1007/s00018-005-5041-3
51. Chen SH, Chang JY. New Insights into Mechanisms of Cisplatin Resistance: From Tumor Cell to Microenvironment. Int J Mol Sci. 2019;20(17):4136. https://doi.org/10.3390/ijms20174136
52. Li L, Liu HC, Wang C, Liu X, Hu FC, Xie N, Lu L, Chen X, Huang HZ. Overexpression of beta-Catenin Induces Cisplatin Resistance in Oral Squamous Cell Carcinoma. Biomed Res Int. 2016;2016:5378567. https://doi.org/10.1155/2016/5378567
53. Zhang J, Liu J, Li H, Wang J. beta-Catenin signaling pathway regulates cisplatin resistance in lung adenocarcinoma cells by upregulating Bcl-xl. Mol Med Rep. 2016;13(3):2543-51. https://doi.org/10.3892/mmr.2016.4882

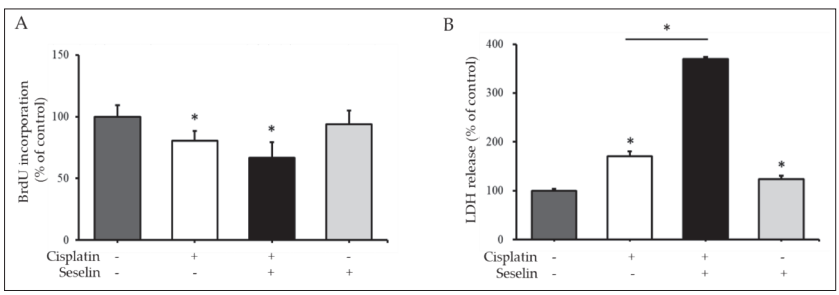
SUPPLEMENTARY MATERIAL



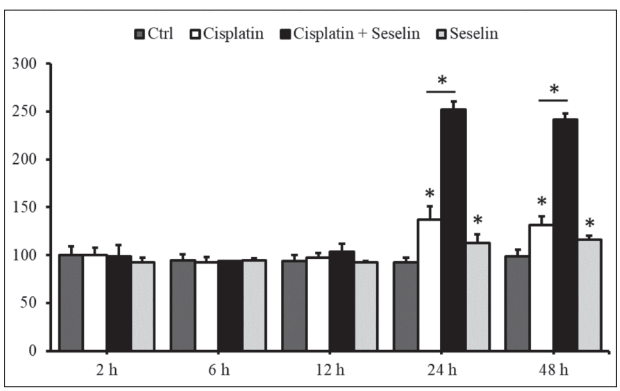
Supplementary Fig. S1. The effects of coumarin derivatives and cisplatin on the viability of AGS cells determined by the WST-1 reagent. Cell viability was detected with different concentrations of seselin and 10 μM cisplatin for 48-72 h. *P<0.05 compared with the control group. Compound 1: 7-((2-methylbut-3-yn-2-yl) oxy)-2H-chromen-2-one; compound 2: 8,8-dimethyl-2H,8H-pyrano[2,3-f]chromen-2-one (seselin); compound 3: 8,8-dimethyl-2H,8H-pyrano[3,2-g]chromen-2-one; compound 4: 7-hydroxy-3-phenyl-4H-chromen-4-one (7-hydroxyflavone); compound 5: 2-(2,2-dimethyl-2H-chromen-7-yl)-6-hydroxychroman-4-one).



Supplementary Fig. S2. The effects of seselin and cisplatin on the viability of SC-M1 cells determined by the WST-1 reagent. Cell viability was detected with different concentrations of seselin and 10 μM cisplatin for 48-72 h. *P<0.05 compared with the control group.



Supplementary Fig. S3. The effects of seselin and cisplatin on cell proliferation and death of SC-M1 cells determined by the BrdU assay and LDH release. **A** – cell proliferation was detected after treatment with 100 nM seselin and 10 μM cisplatin for 24 h. **B** – Cell death was detected after treatment with 100 nM seselin and 10 μM cisplatin for 24 h. *P<0.05 compared with the control group or compared with the two groups.



Supplementary Fig. S4. The effects of seselin and cisplatin on AGS cell death determined by LDH release. Cell death was detected after treatment with 100 nM seselin and 10 μM cisplatin for 2-48 h. *P<0.05 compared with the control group or compared with the two groups.