Original Article
Cistanche phenylethanoid glycosides induce apoptosis and pyroptosis in T-cell lymphoma

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Received November 21, 2023; Accepted March 13, 2024; Epub March 15, 2024; Published March 30, 2024

Abstract: Cistanche deserticola, known for its extensive history in Traditional Chinese Medicine (TCM), is valued for its therapeutic properties. Recent studies have identified its anticancer capabilities, yet the mechanisms underlying these properties remain to be fully elucidated. In this study, we determined that a mixture of four cistanche-derived phenylethanoid glycosides (CPhGs), echinacoside, acteoside, 2-acetylacteoside, and cistanoside A, which are among the main bioactive compounds in C. deserticola, eliminated T-cell lymphoma (TCL) cells by inducing apoptosis and pyroptosis in vitro and attenuated tumor growth in vivo in a xenograft mouse model. At the molecular level, these CPhGs elevated P53 by inhibiting the SIRT2-MDM2/P300 and PI3K/AKT carcinogenic axes and activating PTEN-Bax tumor-suppressing signaling. Moreover, CPhGs activated noncanonical and alternative pathways to trigger pyroptosis. Interestingly, CPhGs did not activate canonical NLRP3-caspase-1 pyroptotic signaling pathway; instead, CPhGs suppressed the inflammasome factor NLRP3 and the maturation of IL-1β. Treatment with a caspase-1/4 inhibitor and silencing of Gasdermin D (GSDMD) or Gasdermin E (GSDME) partially rescued CPhG-induced cell death. Conversely, forced expression of NLRP3 restored cell proliferation. In summary, our results indicate that CPhGs modulate multiple signaling pathways to achieve their anticancer properties and perform dual roles in pyroptosis and NLRP3-driven proliferation. This study offers experimental support for the potential application of CPhGs in the treatment of TCL.

Keywords: Cistanche phenylethanoid glycosides, apoptosis, pyroptosis, NLRP3

Introduction

T-cell lymphoma (TCL) represents a spectrum of malignancies classified under non-Hodgkin lymphoma. While some TCL variants show slow progression and respond well to treatment, others exhibit resistance to current therapeutic strategies. In consideration of developing new pharmaceutical agents for cancers, the active ingredients of Traditional Chinese Medicine (TCM) have garnered increasing attention [1]. Cistanche deserticola, a member of the Cistanche genus which predominantly includes Cistanche deserticola and Cistanche tubulosa, is recognized as an authentic herb in TCM, with a lengthy history of application in various formulations aimed at treating a broad spectrum of diseases, including cancer [2, 3]. Extensive research has confirmed the safety of Cistanche species for human consumption at typical doses in TCM formulation [3, 4]. Cistanche-derived phenylethanoid are the primary bioactive components of cistanche varieties. The four main bioactive ingredients in C. deserticola comprise echinacoside, acteoside, 2-Acetylaceoside, and cistanoside A, accounting for approximately 0.22-0.85%, 0.04%-0.93%, 0.05-0.63%, and 0.04-0.13% of the total weights of the dried whole herb, respectively [5, 6]. Studies have shown that individual or mixtures of Cistanche-derived phenylethanoid glycosides have anti-tumor activities, in addition to their anti-inflammatory, anti-aging, anti-fatigue, liver-protective, and immunomodulatory properties [7-9]. Whole extracts of C. deserticola or its purified phenylethanoid glycosides have been found to inhibit various types of cancers such as breast, ovarian, hepatocellular, glioblasto-
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ma, and pancreatic cancers, both in vitro and in vivo by influencing cellular signaling, including Wnt/β catenin, PI3K/AKT, miR-503-3p, and SKP2 [10-15].

Phenylethanoid glycosides have been shown to induce apoptosis in several cancer models [8, 14, 16]. Recent studies have also indicated that the role of pyroptosis, an inflammatory signal-mediated cell death, in the action of phenylethanoid glycosides [17]. Induction of pyroptosis, with or without apoptosis, represents a crucial strategy for eliminating cancer cells [18]. In TCL, the involvement of critical pro-apoptotic and anti-apoptotic proteins, including P53, PTEN, SIRT2, and PI3K/AKT, in apoptosis or pyroptosis induced by phenylethanoid glycosides, and the activation of pyroptosis, remain underexplored.

In this study, we explored a mixture of the four most prevalent phenylethanoid glycosides, echinacoside, acteoside, 2-acetylacteoside, and cistanoside A (referred to as CPhGs), to study the synergistic tumor-suppressing effects of TCM on TCL. We discovered that CPhGs significantly killed and inhibited TCL in vitro and in vivo. Our study found that CPhGs predominantly induce cell death in T lymphoma cells through apoptosis and pyroptosis, via the regulation of multiple interconnected pathways involving key proteins such as P53 and caspase-3. Moreover, we also identified a novel mechanism by which CPhGs suppress NLRP3 inflammatory signaling while activating pyroptosis and apoptosis, thus maximizing their anticancer effect in TCL.

**Materials and methods**

**Cell lines and reagents**

Human TCL lines Jurkat (ATCC), HH, Hut-78 (ATCC), and MyLa (Sigma-Aldrich) were cultured as described previously [19, 20]. All cell lines were authenticated by short tandem repeat (STR) markers, and tested for mycobacterial contamination by mycoplasma agar culture. Echinacoside, Acteoside, Cistanoside A, and 2-Acetylacteoside (> 98% purity for all) were purchased from Yuanye Bio-Technology (Shanghai, China), and used as an equal concentration mixture (CPhGs), as indicated in the experiments. Specific inhibitors Z-VAD-FMK (Z-VAD) and VX765 were purchased from Selleckchem (Houston, TX).

**Proliferation assays and apoptosis assays**

Proliferation assays were performed using a CCK-8 kit from Meilunbio (Dalian, China). Briefly, cells were cultured and then treated with indicated concentrations of CPhGs, with or without inhibitors, for 3 days before the addition of CCK-8 solution, and absorbance was measured following the manufacturer’s instructions. For apoptosis analysis, cells were treated with CPhGs at indicated concentrations for 48 h. An Annexin V apoptosis detection kit and flow cytometry were utilized for staining and detection, respectively, with FlowJo software used for data analysis.

**GSDMD and GSDME knockdown**

GSDMD and GSDME knockdown was achieved using siRNA. siRNA oligos targeting GSDMD (#SR312602), GSDME (#SR301192), or non-specific siRNAs were purchased from OriGene (Rockville, MD). Cells were transfected with siRNAs through electroporation using an Amaza Nucleofector II Device from Lonza (Basel, Switzerland). Briefly, 1×10^6 cells were centrifuged and resuspended in 100 μl of transfection solution L, and siRNA was added to a final concentration of 1 mM. Transfection was carried out using the Nucleofector program X-001. Cells were then cultured for 24 h before further experimentation.

**Expressing plasmid vector construction and transfection**

The NLRP3 gene (cDNA) was reverse-transcribed from mRNA, PCR-amplified, purified, and inserted into the pcDNA3.1-GFP plasmid. Following clone selection in *Escherichia coli* bacterial and Ampicillin screening, the insert was confirmed by sequencing. The plasmid DNA was then extracted, purified, and transfected into cells through electroporation using a Nucleofector. Transfection efficiency was assessed by detecting GFP expression using flow cytometry, and transfected cells were used for functional assays.

**Western blotting**

Cells were washed with PBS, collected, and lysed for total protein extraction. Proteins were then resolved by SDS-PAGE, transferred to membranes, and probed with primary and secondary antibodies. Detection was perfor-
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Students’ t-test. Experiments were conducted in triplicate. Data are presented as mean ± SD, with statistical significance defined as $P < 0.05$.

**Results**

*CPhGs induced cell death, inhibited cell proliferation, and triggered intrinsic and extrinsic apoptosis in TCL*

Initially, to explore the cytotoxic effects of CPhGs, we treated the TCL cell lines HH, Hut78, MyLa, and Jurkat with various concentrations of CPhGs for 3 d and assessed live/dead rates using the Cell-Counting Kit-8 (CCK-8) cell viability assay. Treatment with CPhGs resulted in dose-dependent cell death (*Figure 1A*) and significantly reduced proliferation at concentrations of 40 µM and 80 µM compared to the control group (*Figure 1B*).

Given evidence that phenylethanoid glycosides may induce pyroptosis, we explored whether CPhGs triggered pyroptosis in HH and Hut78 cells. CPhGs facilitated the cleavage of GSDMD, a marker of the non-canonical pyroptotic pathway, and GSDME, a marker of an alternative pyroptotic pathway marker (*Figure 3A*). Lactate dehydrogenase (LDH) is a cytosolic enzyme typically retained within the cell cytosol, is released upon loss of membrane integrity. To further validate CPhGs-induced pyroptosis in TCL cells, we measured LDH levels in cell culture supernatants before and after treatment, observing elevated LDH levels following CPhGs...
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Figure 1. CPhGs inhibited cell proliferation and triggered apoptosis in TCL. Cell lines HH, Hut78, Myla, and Jurkat were treated with different concentrations of CPhGs. A. Changes in the percentage of living cells as measured by CCK8 assay. B. Cell growth curves of the four cell lines treated with CPhGs over 3 d. C. Representative flow cytometry analysis of four cell lines treated with indicated concentrations of CPhGs for 48 h. D. Quantitative analysis of apoptosis detected by Annexin V/PI staining and flow cytometry. E. Representative images of H&E staining of xenograft tumor tissues of mice treated with indicated doses of CPhGs (left) and quantification of living cells (right). Data are presented as mean ± SD. *P < 0.05, **P < 0.01. Scale: 100 μm.

Figure 2. CPhGs activated both intrinsic and extrinsic apoptotic pathways. The intrinsic pathway marker cleaved caspase-9, the extrinsic marker cleaved caspase-8, and the apoptosis executor cleaved caspase-3 were detected by Western blotting (upper) and quantified (lower). Data are represented as the mean ± SD. Results are from three individual replicates. One-way ANOVA was used for statistical analysis. *P < 0.05, **P < 0.01 vs. the control groups.

treatment (Figure 3B). Despite GSDMD serving as a shared executor of both canonical and non-canonical pyroptotic pathways, the canonical pathway appeared inactive in CPhGs-treated cells, as evidenced by the absence of cleaved caspase-1, even though caspase-4, a marker of the non-canonical pyroptotic pathway, was cleaved (Figure 3C). These findings suggest that the activation of non-canonical and alternative signaling pathways is responsible for CPhGs-induced pyroptosis in TCL cells.

As pyroptosis is mediated by caspase-4, we investigated the rescue effects of the cas-
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**Figure 3.** CPhGs induced pyroptosis. HH and Hut78 cells were treated with indicated concentrations of CPhGs. A. Western blotting of cleaved GSDMD and GSDME (upper) with quantitative analysis (lower). B. Changes in LDH levels. C. Western blotting analysis of cleaved caspase-4 and caspase-1 (left) and the quantitative analysis (right).

pase-4 inhibitor VX765 after CPhGs treatment along with the apoptosis inhibitor ZVAD-FMK (ZVAD) to examine changes in pyroptosis and apoptosis ratios. HH and Hut78 cells were treated with CPhGs alone, in combination with VX765, ZVAD, or VX765 plus ZVAD. Both VX765 and ZVAD significantly decreased CPhGs-induced cell death, with ZVAD exhibiting a greater reduction than VX765. The percentage of viable cells increased from 52% to 74% and...
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58% to 78% in HH and Hut78, respectively, after co-treatment with CPhGs and VX765, whereas co-treatment with CPhGs and ZVAD increased the percentage of living cells from 52% to 82% and 58% to 80% in the same cell lines. Combined treatment with VX765 and ZVAD completely blocked cell death induced by CPhGs (Figure 4A). Treatment with VX765 partially blocked CPhGs-induced LDH release and caspase-4 cleavage or activation (Figure 4B and 4C). To further confirm that pyroptosis is a mechanism through which CPhGs induce cell death in malignant T cells, we determined the role of pyroptosis executors, GSDMD and GSDME, in CPhGs-induced killing of TCL through siRNA silencing. As hypothesized, silencing GSDMD and GSDME partially rescued cell viability (Figure 4D).

CPhGs inhibited NLRP3 expression and IL-1β maturation

Given that CPhGs did not induce caspase-1 activation, we explored whether other factors in the canonical pyroptotic pathway were activated. Interestingly, the expression of NLRP3, an initiating factor of the NLRP3 inflammasome complex, was down-regulated after CPhGs treatment (Figure 5A). As NLRP3 inflammasome signaling facilitates both canonical pyroptosis and IL-1β-dependent proliferation, we speculate that CPhGs not only fail to activate the canonical pyroptosis pathway but also actively suppress proliferation signaling. Consequently, we assessed IL-1β levels before and after CPhGs treatment in HH and Hut78 cells. The protein level of IL-1β decreased proportionally with NLRP3 downregulation in a dose-dependent manner following CPhGs treatment (Figure 5A). Similarly, in situ immunohistochemical staining of tumor tissue sections from the TCL xenograft mouse model also displayed reduced NLRP3 expression after CPhGs treatment juxtaposed with the control (Figure 5B). To further confirm the role of NLRP3 in cell proliferation, we induced exogenous NLRP3 expression by plasmid transfection into HH and Hut78 cells pre-treated with CPhGs, resulting in a partial restoration of proliferation in both CPhGs-treated cell lines (Figure 5C).

CPhGs regulates SIRT2-MDM2/P300 and PI3K/AKT pathways

P53, a crucial tumor suppressor, is inhibited by the SIRT2-P300/MDM2 signaling axis. The PI3K/AKT pathway promotes proliferation and is involved in the carcinogenesis of various cancer types. To further investigate the influence of CPhGs on the major pathways influencing cancer development, we examined SIRT2/P53, PI3K/AKT, and other pro- and anti-apoptotic signaling pathways. As shown in Figure 6, CPhGs treatment significantly reduced the expression of SIRT2, P300, MDM2, and Bcl2; inhibited PI3K and AKT activation; and upregulated P53, PTEN, and BAX expression in a dose-dependent manner, as compared to controls. In situ immunohistochemical staining of tumor tissues from the TCL xenograft mouse model also indicated increased expression of P53 after CPhGs treatment (Figure 6A-D).

CPhGs inhibited TCL tumor growth in xenograft mouse model

To investigate the effects of CPhGs on TCL tumor growth in vivo, we established a xenograft TCL tumor model. Tumor cells were subcutaneously injected into BALB/c female nude mice (6-8 weeks old). Mice bearing TCL cell-derived tumors were then intraperitoneally injected with saline, 20 mg/kg CPhGs, or 60 mg/kg CPhGs. Tumors were surgically removed after 12 days of continuous dosing. CPhGs significantly inhibited tumor growth in a dose-dependent manner (Figure 7A). Tumor growth curves, where tumor volumes measured every 3 days, were plotted (Figure 7B), and showed an increase in the body weight of mice treated with CPhGs (Figure 7C), indicating that CPhGs were well-tolerated at the administered doses, and might even improve the physical conditions of tumor-bearing mice. The tumor inhibition rates on day 18 were also calculated (Figure 7D).

Discussion

The efficacy of TCM relies on the combined functions of many compounds. It has the advantage of additive or synergistic effects; however, this complexity also poses challenges for analyzing the functions of each compound individually. One practical method is to use a few of the main bioactive ingredients of one herb or formula as the study target to obtain the most representative data [21]. In this study, we used a mixture of the four most abundant CPhGs in C. deserticola to replicate the natural synergistic effects of CPhGs observed in TCM formulas incorporating C. deserticola.
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Studies on single or a mixture of multiple CPhGs have revealed that their anti-cancer mechanisms include the induction of apoptosis by regulating multiple signaling pathways [10, 11, 22]. In the present study, CPhGs triggered both intrinsic and extrinsic apoptosis. Pro-apoptosis
CPhGs induce apoptosis and pyroptosis in TCL factors such as P53, PTEN, and BAX were upregulated, whereas anti-apoptosis factors including Bcl-2, PI3K, and AKT were inhibited, suggesting that the anti-cancer function of CPhGs is derived through regulating these pathways. Silent information regulator 2 (SIRT2) is a histone deacetylase that regulates multiple cellular functions including cell cycle, proliferation, and apoptosis [23]. The role of SIRT2 in malignant cells remains unclear. It may func-

Figure 5. CPhGs inhibited the expression of NLRP3 and maturation of IL-1β. HH and Hut78 cells were treated with CPhGs. A. The expression of NLRP3, pro-IL-1β, and matured IL-1β were detected by Western Blotting. Quantitative results are displayed by histograms. B. Representative images of NLRP3-specific immunohistochemical staining of xenograft tumor tissues with results quantified by histograms. C. Upper panel: Rescue effect of forced expression of NLRP3 on cell death. The long dotted line, solid line, and short dotted lines represent the non-treatment group, CPhGs treated group and CPhGs-treated + NLRP3 forced expression group, respectively. Lower panel: The transfection and expression efficiencies of forced expression of NLRP3 by pcDNA3.1-GFP plasmid transfection. X-axis shows isotype antibody, anti-NLRP3 antibody, and GFP signals. White, gray, and black columns indicate untransfected, empty vector-transfected, and NLRP3 vector-transfected cells respectively. Data are presented as mean ± SD and compared control group, *P < 0.05, **P < 0.01. Scale: 100 μm.
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Am J Cancer Res 2024;14(3):1338-1352

tion as either a tumor suppressor or oncogenic factor, depending on cell type and physiological conditions, by influencing downstream proteins such as P53, P300, NF-kB, and MDM2 (murine double minute 2) [24]. P53 is a tumor-suppressor gene that can be negatively regulated by its upstream SIRT2-P300/MDM2 signal axis [24, 25]. In addition, evidence shows that PI3K/AKT

Figure 6. CPhGs modulate multiple cellular signaling pathways contributing to apoptosis. (A-C) Western blotting detection of the expression of key proteins in the SIRT2/P53 (A), PI3K/AKT (B), and pro-/anti-apoptosis pathways (C). (D) Representative images of P53-specific immunohistochemical staining of xenograft tumor tissues, with data quantified by histogram (right). Data are presented as mean ± SD. *P < 0.05, **P < 0.01. Scale: 100 μm.
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signaling enhances MDM2 expression, which targets P53 for degradation [26]. In this study, P53 up-regulation was accompanied by the inhibition of the SIRT2-P300/MDM2 axes and PI3K/AKT signaling. Therefore, CPhGs positively modulate P53 by directly and indirectly blocking SIRT2-P300/MDM2 axes. The proposed mechanisms of CPhGs-induced apoptosis are summarized in Figure 8 (left section).

The activation of pyroptosis can be summarized in three pathways: 1. the canonical pathway which encompasses the sequential steps of NLRP3 inflammasome formation, caspase-1 activation, and cleavage of Gasdermin D (GSDMD); 2. the non-canonical pyroptotic pathway, with activation of caspase-4/5/11 and GSDMD cleavage; and 3. an alternative pathway, mediated by caspase-3 and Gasdermin E (GSDME). Given the caspase-3 is also the executor of apoptosis it serves as a key component of both the apoptosis and pyroptosis pathways, facilitating the switch between apoptosis-pyroptosis under certain conditions. The cleaved N-terminal fragments of GSDMD and GSDME (GSDMD-N and GSDME-N), acting as executive proteins of pyroptosis, form pores in the cell membrane, leading to K+ efflux, H₂O influx, and subsequent osmotic cell swelling and death [18, 27, 28].

The role of CPhGs-induced pyroptosis in disease treatment remains controversial. CPhGs have demonstrated the ability to either trigger or inhibit pyroptosis, depending on the disease or cell type. For example, echinacoside, a principal component of the CPhGs family, induces pyroptosis in lung cancer cells through mitochondria-mediated Raf/MEK/ERK signaling, yet inhibits pyroptosis in cardiomyocytes and neurons in non-cancer diseases [17, 29, 30]. Thus, pyroptosis may be beneficial or detrimental, contingent on the tissue or disease context. In this study, we determined that CPhGs induced significant pyroptosis, exerting a synergistic cytotoxic effect alongside apoptosis in malignant T cells.

Additionally, related signaling pathways may exhibit dual effects, even within the context of cancer treatment alone. The NLRP3 inflammasome, a complex comprising NLRP3, ASC, and caspase-1, plays a pivotal role in the canonical pyroptotic pathway. However, the NLRP3 inflammasome also promotes tumor proliferation, metastasis, and invasion through cas-

Figure 7. CPhGs inhibited tumor growth in a xenograft TCL mouse model. A. Image representing tumors surgically dissected from six groups of mice (columns 1-6) treated with doses of 0, 20, and 60 CPhGs (mg/kg body weight). B. Tumor growth curves plotted at the end of the observation period (18 d). C. Body weight changes of mice over 18 d. D. Tumor inhibition rates were calculated using the formula: (mean tumor weight of the control group - treatment group)/control group * 100%.
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Figure 8. The molecular mechanisms of the anti-tumor properties of CPhGs. A schematic figure illustrates that CPhGs: A. Induce apoptosis by activating both intrinsic and extrinsic apoptotic pathways; B. Up-regulate pro-apoptotic factors PTEN and BAX, increase tumor-suppressor P53 by inhibiting SIRT2/MDM2 signaling, and suppress anti-apoptotic PI3K/AKT signaling; C. Induce pyroptosis by activating both caspase-4 (non-canonical pyroptosis) and caspase-3 (alternative pyroptosis) pathways; D. Inhibit proliferation by down-regulating NLRP3 and downstream IL-1β. Red lines denote inhibitory effects, and ‘x’ indicates blockade of functions. Italicized text in red shows three pyroptosis pathways. (+) and (-) denote activation and non-activation of the pathways, respectively. The figure is divided into two parts by a dotted line in the middle. The left part outlines the key components of apoptosis and their relationships. Both caspase-8 and -9 are activated to cleave the executor caspase-3, resulting in apoptosis and possibly an apoptosis-pyroptosis switch. The right part shows the mechanism of pyroptosis: GSDMD and GSDME are pyroptosis executors that form pores in the cell membrane, leading to K⁺ efflux, cell swelling, and death. VX765 is identified as a caspase 4 inhibitor, blocking non-canonical pyroptosis.

Studies have shown that inhibition of NLRP3 leads to the regression of cancer cells both in vitro and in vivo [32-35]. Consequently, activating NLRP3 inflammasome signaling represents a “double-edged sword” in cancer therapy, beneficial for inducing pyroptosis in cancer cells but potentially contributing to cancer pathogenesis. Since agents that induce pyroptosis via the canonical pathway will inevitably activate NLRP3 inflammasome signaling, identifying agents that can separately modulate pyroptosis and NLRP3 inflammasome signaling will emerge as valuable drug candidates for achieving optimal anti-cancer effects. In this study, CPHGs effectively induced pyroptosis by activating caspase-4 and -5 as well as caspase-3 signaling, but not caspase-1 signaling. Conversely, CPHGs downregulated NLRP3 expression and inhibited NLRP3/caspase-1 inflammasome signaling and subsequent IL-1β maturation. Therefore, CPHGs simultaneously promotes pyroptosis and inhibits IL-1β-driven proliferation.

Caspase-3, known as an apoptosis executor, may also play a crucial role in alternative pyroptotic signaling by cleaving GSDME. Like GSDMD, GSDME, a gasdermin superfamily member, facilitates pore formation in the cell membrane. Activated caspase-3 can initiate pyroptosis, transitioning the cell death type from apoptosis to pyroptosis [36, 37]. Given the activation of GSDME in our findings, caspase-3
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acts as a central junction of the two cell death pathways, inducing both apoptosis and pyroptosis in CPhGs-treated malignant T cells. The proposed mechanisms of CPhGs-induced pyroptosis and NLRP3 and IL-1β inhibition are shown in the right section of schematic Figure 8.

P53 plays a comprehensive role in cellular functions and contributes to the activation of NLRP3 and caspase-1 [38]. However, our data showed that elevated P53 doses did not correlate with the activation of NLRP3/caspase-1 by CPhGs treatment. Numerous studies have demonstrated that P53 promotes caspase-3 activation. Therefore, P53 can engage in pyroptosis by supporting the caspase-3-GSDME dependent alternative pyroptotic pathway. Similarly, inhibition of PI3K/AKT and increased levels of PTEN and BAX can elevate caspase-3 levels, thereby triggering both pyroptosis and apoptosis.

As stated above, our research utilized a mixture of four CPhGs to investigate the integrated function of the herb C. deserticola. While further research is necessary to elucidate each function of CPhGs, at this study stage, we have determined that CPhGs induce apoptosis and pyroptosis by modulating multiple signaling pathways, significantly inhibiting TCL growth in vitro and in vivo. We reveal a novel mechanism that CPhGs plays dual functions in promoting pyroptosis and inhibiting IL-1β dependent proliferation. As summarized in Figure 8, CPhGs comprehensively influence the network of intrinsic and extrinsic apoptotic pathways, non-canonical and alternative pyroptotic pathways, tumor-suppressing and -promoting pathways, along with their interactions. Our study suggests that CPhGs may be effective for the treatment of TCL.

Acknowledgements

This work was supported by the Inner Mongolia Science & Technology Project Plan (2019-GG037) and the Key Project of Inner Mongolia Medical University (YKD2022-ZD016).

Disclosure of conflict of interest

None.

Abbreviations

TCL, T-cell lymphoma; TCM, Traditional Chinese Medicine; C. deserticola, Cistanche deserticola Y. C. Ma; CPhGs, Cistanche-derived phenylethanoid glycosides; SIRT2, Silent information regulatory 2; BAX, BCL-2 Associated X; Bcl-2, B-cell lymphoma-2; MDM2, Murine double minute 2; PTEN, Phosphatase and tensin homolog deleted on chromosome ten; GSDMD, Gasdermin D; GSDME, Gasdermin E; NLRP3, NOD-, LRR- and pyrin domain-containing protein 3; PBS, Phosphate-buffered saline; CCK8, Cell Counting Kit-8; OD, Optical density; mRNA, Messenger RNA; PI, Propidium Iodide; RT-PCR, Real-time Polymerase chain reaction; SDS-PAGE, Sodium dodecyl sulfate polyacrylamide gel electrophoresis; HE, Hematoxylin-Eosin.

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