



Research Article

Ginsenoside compound K inhibits nuclear factor-kappa B by targeting Annexin A2

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ABSTRACT

Background: Ginsenoside compound K(C-K), a major metabolite of ginsenoside, exhibits anticancer activity in various cancer cells and animal models. A cell signaling study has shown that C-K inhibited nuclear factor-kappa B (NF-κB) pathway in human astroglial cells and liver cancer cells. However, the molecular targets of C-K and the initiating events were not elucidated.

Methods: Interaction between C-K and Annexin A2 was determined by molecular docking and thermal shift assay. HepG2 cells were treated with C-K, followed by a luciferase reporter assay for NF-κB, immunofluorescence imaging for the subcellular localization of Annexin A2 and NF-κB p50 subunit, coimmunoprecipitation of Annexin A2 and NF-κB p50 subunit, and both cell viability assay and plate clone formation assay to determine the cell viability.

Results: Both molecular docking and thermal shift assay positively confirmed the interaction between Annexin A2 and C-K. This interaction prevented the interaction between Annexin A2 and NF-κB p50 subunit and their nuclear colocalization, which attenuated the activation of NF-κB and the expression of its downstream genes, followed by the activation of caspase 9 and 3. In addition, the overexpression of Annexin A2-K320A, a C-K binding-deficient mutant of Annexin A2, rendered cells to resist C-K treatment, indicating that C-K exerts its cytotoxic activity mainly by targeting Annexin A2.

Conclusion: This study for the first time revealed a cellular target of C-K and the molecular mechanism for its anticancer activity.

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1. Introduction

Ginseng is regarded as a most famous herb in eastern Asia as both pharmaceutical and food resource for over a thousand years, and ginsenosides are the main bioactive compounds in ginseng. Ginsenoside compound K (C-K) is a major metabolite when high-molecular weight ginsenosides were degraded in the intestinal tract with a remarkable human availability compared with other ginsenosides [1–3]. Recent reports have shown that C-K inhibits both proliferation and angiogenesis of cancer cells *in vitro* [4–7]. C-K inhibits growth of cancer cells primarily via apoptosis, and both mitochondrial- and death receptor-mediated apoptosis pathway were triggered by C-K [4,5,8–10]. Moreover, despite the extraordinary direct damage to cancer cells, C-K appears to sensitize cancer cells to anticancer reagents such as fluorouracil and

cisplatin and presents a synergistic effect under cotreatment with them, indicating that C-K probably functions via removing drug resistance in cancer cells [11,12]. A further study shows that C-K inhibits nuclear factor-kappa B (NF-κB) pathway and acts as an inhibitor of nuclear factor kappa-B kinase (IKK) in inflammatory response [13]. However, its exact cellular target and molecular acting mechanism remain elusive.

NF-κB is a highly conserved transcription factor involved in immune and inflammatory responses in mammals. Recent reports suggest the constitutive activation of NF-κB is necessarily required in multiple types of cancer [14], particularly contributing to the acquisition of chemical resistance by inducing inhibition of apoptosis proteins (IAPs) [15,16]. In liver tissue, NF-κB functions as a key regulator in the development of liver diseases and even cancer by promoting survival and inflammation of injured

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hepatocytes, leading to spontaneous liver damage, fibrosis, and hepatocellular carcinoma [17–20]. Thus, targeting NF- κ B may provide a new strategy for liver cancer prevention and treatment.

A couple of studies show that C-K inhibits NF- κ B activation and cell proliferation in cancer cells [21–23]. To explore the molecular mechanisms involved in NF- κ B suppression by C-K, we performed a thermal shift assay with proteins regulating NF- κ B. In the present study, we testified that C-K positively acted as an inhibitor to Annexin A2, a cancer-related protein, promoting NF- κ B activation by binding to the NF- κ B p50 subunit (p50) [24]. C-K prevented Annexin A2 binding to p50 and inhibited NF- κ B activation, followed by a downregulation of its downstream genes, IAPs. Moreover, overexpression of Annexin A2-K302A, a single-site mutant version of Annexin A2 failing to interact with C-K, rendered cells to resist C-K-induced NF- κ B deactivation and apoptosis. These results suggested that C-K exerts its anti-NF- κ B and subsequent pro-apoptosis effect mainly by inhibiting Annexin A2 in HepG2 cells.

2. Materials and method

2.1. Cell lines, reagents, and plasmid vectors

HepG2 cells were obtained from the American Type Culture Collection (ATCC, Rockville, MA, USA). Dulbecco's Modified Eagle's Medium and fetal bovine serum were obtained from Gibco BRL (Grand Island, NE, USA). Chemical reagents including C-K, phorbol myristate acetate (PMA), and etoposide were obtained from Sigma-Aldrich (St. Louis, MO, USA). C-K was dissolved to a final concentration of 12 mM in 75% alcohol. PMA (10 μ g/mL) and etoposide (25 mg/mL) were dissolved in dimethyl sulfoxide (DMSO). Primary antibodies, including mouse anti-Annexin A2 (sc-47696), mouse anti-p50 (sc-8414), rabbit anti-p50 (sc-7178), mouse anti-C myc (sc-49), rabbit anti-C myc (sc-789), mouse anti-X-linked inhibitor of apoptosis (X-IAP) (sc-55551), rabbit anti-cellular inhibitor of apoptosis (c-IAP1) (sc-7943), rabbit anti-c-IAP2 (sc-7944), mouse anti-survivin (sc-17779), and mouse anti- β -actin (sc-47778), were obtained from Santa Cruz Biotechnology (Dallas, Texas, USA). Secondary antibodies conjugated with horse radish peroxidase (HRP) for immune blotting were obtained from Pierce (Thermo Fisher Scientific, Rockford, IL, USA). Cy3 affipure donkey anti-mouse IgG and Alexa Fluor 488 affipure donkey anti-Rabbit IgG were obtained from Jackson (Jackson ImmunoResearch Inc., PA, USA).

Vectors were constructed as previously described [25] for Annexin A2 knockdown, luciferase reporter assay, prokaryotic expression of Annexin A2 and p50, and eukaryotic expression of Annexin A2 and K302A mutant of Annexin A2.

2.2. Molecular docking

Molecular docking was performed with C-K and Annexin A2 with structure resource of PubChem CID: 9852086 from the NCBI PubChem Compound database (<http://www.ncbi.nlm.nih.gov/pccompound>) and PDB ID: 2HYU from the RCSB Protein Data Bank (<http://www.rcsb.org/pdb>). Molecular docking was performed with AutoDock tools (version 4.2.6) and visualized with the Discovery Studio 4.0 Visualizer (BIOVIA, CA, USA).

2.3. Thermal shift assay

Cellular thermal shift assay, CTSA (*in vivo*), was performed with HepG2 cells and 12 μ M of C-K with procedure described previously [25].

In vitro thermal shift assay: Purified Annexin A2 protein with final concentration of 0.2 mM (\sim 7 μ g/mL) was mixed with C-K with a dose gradient from 0.2 μ M to 10 μ M with a final volume of 200 μ L then heated at 55°C for 3 min, with the control tube on ice for 3 min. Then, 10 μ L from each tube was used for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblot.

2.4. Immunoprecipitation

In vivo immunoprecipitation was carried out with HepG2 cells pretreated with C-K (6 μ M), PMA (100 ng/mL), and etoposide (25 μ g/mL) for 12 h. Cell lysate was precipitated by anti-Annexin A2, anti-p50, or anti-C-myc antibody and used for immunoblot.

In vitro immunoprecipitation was carried out with prokaryotic-expressed Annexin A2 and p50. Protein lysate was precipitated by anti-Annexin A2 or anti-p50 antibody and used for immunoblot.

2.5. Immunofluorescence

HepG2 cells were seeded onto glass cover slips and treated with C-K, PMA, and etoposide for 2 h. Then, cells were fixed,

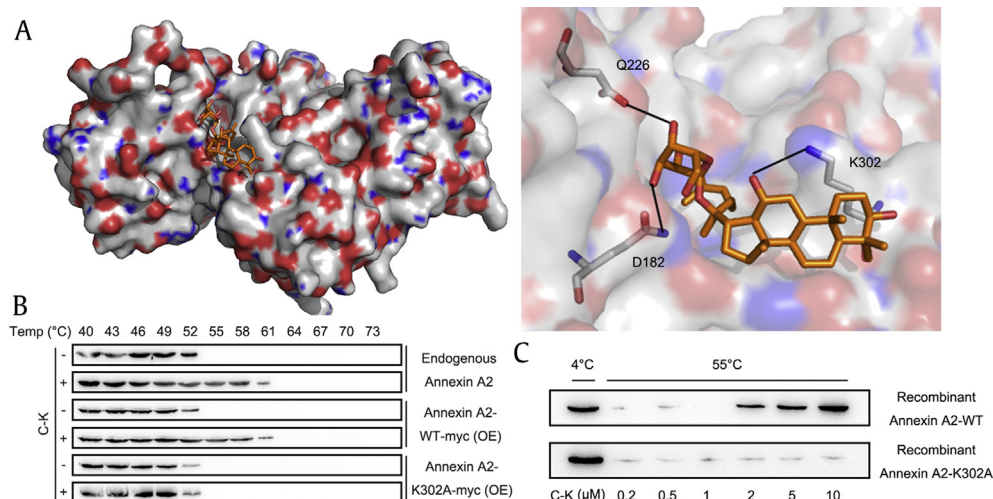


Fig. 1. C-K bound to Annexin A2. (A) Results of molecular docking shows the interaction between Annexin A2 and C-K. (B) Cellular thermal shift assay presented the thermal stability of Annexin A2 under treatment with C-K (12 μ M). (C) Thermal shift *in vitro* showed the thermal stability of purified Annexin A2 (0.2 μ M) under treatment with C-K.

permeabilized, immunolabeled, and stained as described [25]. Images were gained by fluorescence microscopy.

2.6. Quantitative real-time polymerase chain reaction

Total RNA was isolated using TRIzol (Invitrogen, Thermo Fisher Scientific, Rockford, IL, USA) and reverse transcribed with the EasyScript Reverse Transcriptase kit (Transgen, Beijing, China). Quantitative real-time polymerase chain reaction (qRT-PCR) was generated on Applied Biosystem 7500 Real-time PCR system (Applied Biosystems, Thermo Fisher Scientific, Rockford, IL, USA). The amplification was performed with a three-step program, one cycle at 95°C for 30 s, followed by 45 cycles at 95°C for 5 s, 50°C for 15 s, and 72°C for 10 s, with signal-collecting steps after annealing and extending for 34 s.

2.7. Statistical analysis

Data are repeated thrice and presented with the mean \pm standard deviation. Statistical significance was determined by the Student *t* test.

3. Results

3.1. C-K interacted with Annexin A2

Molecular docking was performed with C-K- and NF- κ B-associated proteins, among which Annexin A2 interacted with C-K, and Asp182, Gln226, and Lys302 appeared to be responsible for their interaction (Fig. 1A). A following CTSA in HepG2 cells showed evident increase of the thermal stability of Annexin A2 by C-K, indicating that C-K penetrated cells and bound to Annexin A2 (Fig. 1B). A further CTSA with overexpressed wild-type version of Annexin A2 (Annexin A2-WT) also showed a similar thermal stability shift under treatment of C-K, whereas that of the K302A mutant of Annexin A2 (Annexin A2-K302A) was only slightly affected by C-K, indicating Lys302 was responsible for the interaction between C-K and Annexin A2 (Fig. 1B). To eliminate the influence of other cellular component, a thermal shift *in vitro* was then performed. C-K enhanced the thermal stability of Annexin A2-WT, but not Annexin A2-K302A, in a dose-dependent manner, indicating that C-K upregulated Annexin A2 thermal stability by direct interaction and Lys302 was a dominant amino acid for their interaction (Fig. 1C).

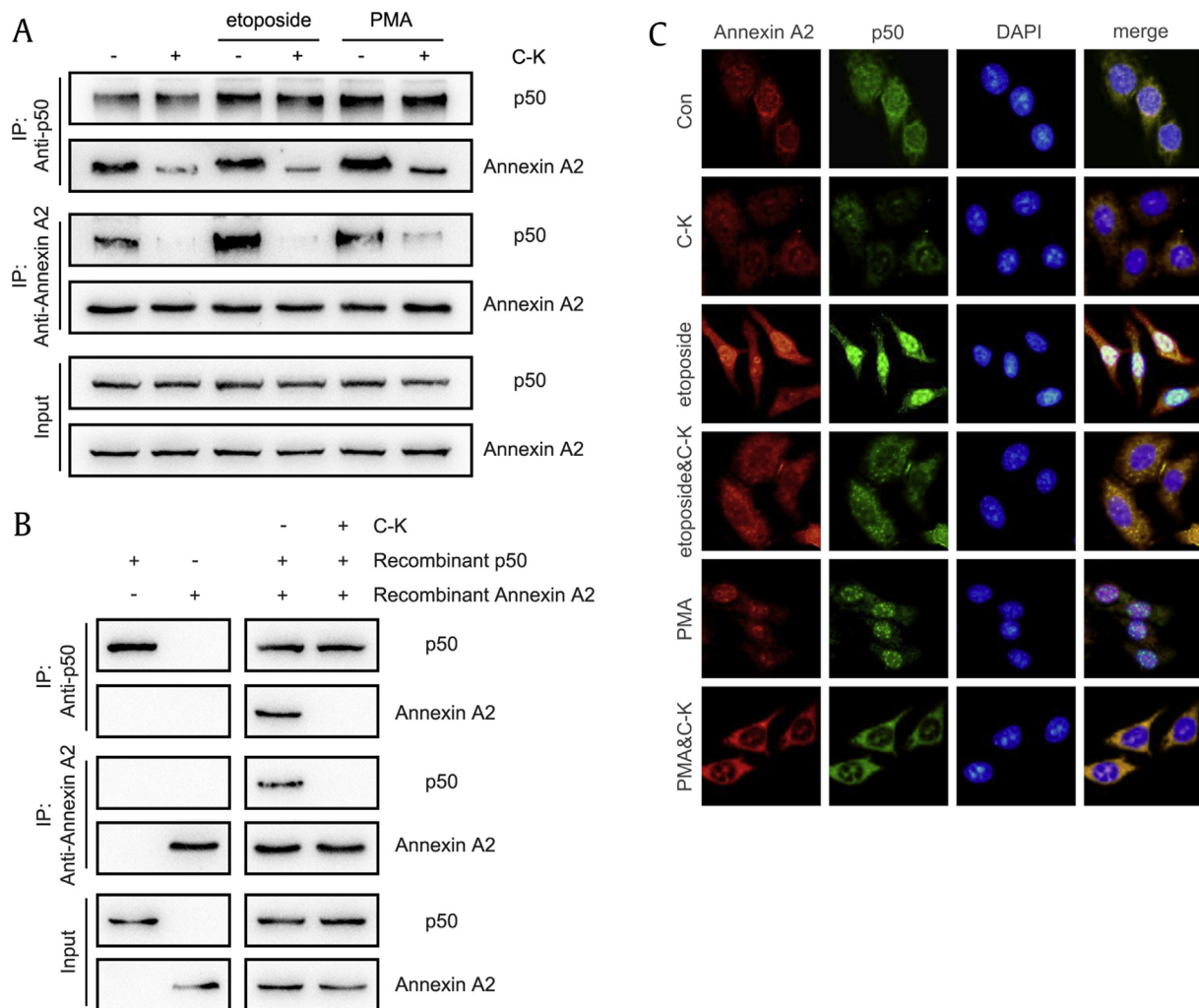


Fig. 2. C-K inhibited interaction between Annexin A2 and NF- κ B p50 subunit and their nuclear colocalization. (A) Immunoprecipitation was performed with whole-cell lysate under treatment with C-K (6 μ M), etoposide (25 μ g/mL), and PMA (100 ng/mL), and the interaction was analyzed by an immunoblot. (B) Immunoprecipitation was performed with prokaryotic cells—expressed Annexin A2 and NF- κ B p50 subunit under treatment with C-K (6 μ M), and the interaction was analyzed by an immunoblot. (C) The subcellular distribution of Annexin A2 and NF- κ B p50 subunit was examined by immunofluorescence under treatment with C-K (6 μ M), etoposide (25 μ g/mL), and PMA (100 ng/mL), and DAPI showed the nuclear region.

C-K, compound K; DAPI, 4',6-diamidino-2-phenylindole; NF- κ B, nuclear factor-kappa B; PMA, phorbol myristate acetate.

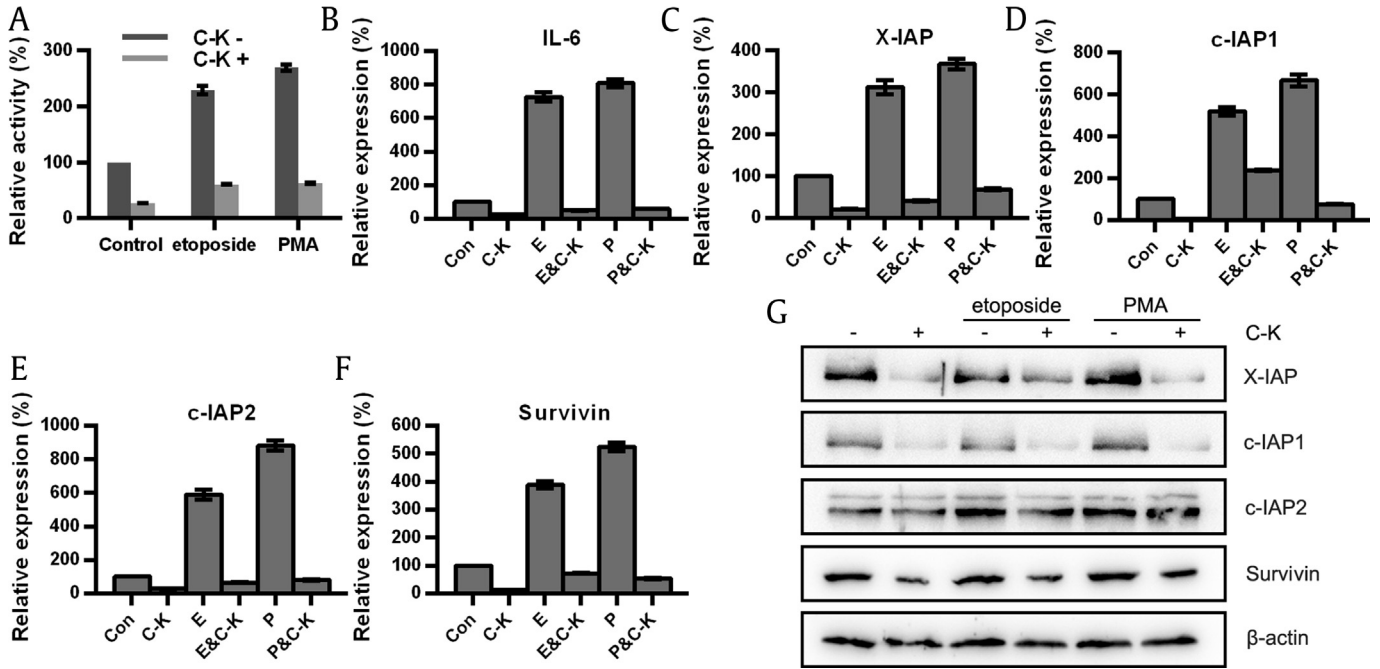


Fig. 3. C-K inhibited NF-κB activation and downregulated downstream antiapoptosis genes. (A) NF-κB activity was examined by luciferase reporter assay under treatment with C-K (6 μM), etoposide (25 μg/mL), and PMA (100 ng/mL). (B–F) Relative gene expression levels of (B) IL-6, (C) X-IAP, (D) c-IAP1, (E) c-IAP2, and (F) survivin were examined by qRT-PCR under treatment with C-K (6 μM), etoposide (25 μg/mL), and PMA (100 ng/mL). E represented etoposide and P represented PMA, with E&C-K and P&C-K representing etoposide and PMA under cotreatment with C-K, respectively. (G) Protein levels of X-IAP, c-IAP1, c-IAP2, and survivin were examined by immunoblot under treatment with C-K (6 μM), etoposide (25 μg/mL), and PMA (100 ng/mL), and β-actin was shown as a loading control. All data are shown as the mean ± SD, and the experimental points show the average of at least three replicates. All experiments were repeated at least three times. c-IAP, cellular inhibitor of apoptosis; C-K, compound K; IAP, inhibition of apoptosis protein; IL, interleukin; NF-κB, nuclear factor-kappa B; PMA, phorbol myristate acetate; qRT-PCR, quantitative real-time polymerase chain reaction; SD, standard deviation; X-IAP, X-linked inhibitor of apoptosis.

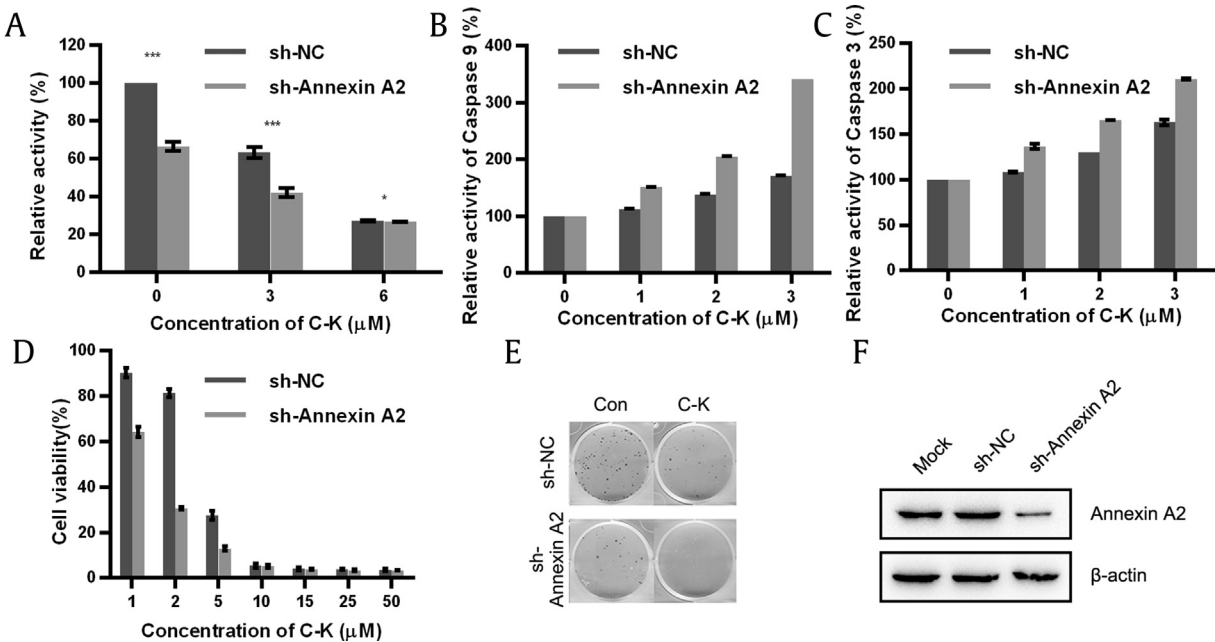


Fig. 4. Knockdown of Annexin A2 enhanced the cytotoxicity of C-K. (A) NF-κB activity was examined by luciferase reporter assay under treatment of C-K with (sh-Annexin A2) or without (sh-NC) knockdown of Annexin A2. Activity of (B) caspase 9 and (C) caspase 3 was examined under treatment of C-K with (sh-Annexin A2) or without (sh-NC) knockdown of Annexin A2. (D) Cell viability was examined by MTT for 48 h under treatment of C-K with (sh-Annexin A2) or without (sh-NC) knockdown of Annexin A2. (E) Plate clone formation assay was examined under treatment of C-K (6 μM) with (sh-Annexin A2) or without (sh-NC) knockdown of Annexin A2. (F) Protein level of Annexin A2 was examined by immunoblot with (sh-Annexin A2) or without (sh-NC) knockdown of Annexin A2, and β-actin was shown as a loading control. All data are shown as the mean ± SD. **p* < 0.05, ***p* < 0.01, and ****p* < 0.001, and the experimental points show the average of at least three replicates. All experiments were repeated at least three times. Statistical analyses were performed using Student *t* test. C-K, compound K; NF-κB, nuclear factor-kappa B; SD, standard deviation.

3.2. C-K inhibited the interaction and nuclear colocalization of Annexin A2 and p50

To assess whether C-K can exert its activity by targeting Annexin A2, the effect of C-K on the association between Annexin A2 and p50 was examined. Immunoprecipitation was performed with whole-cell lysate of HepG2 cells under treatment of 6 μ M of C-K, with or without NF- κ B activator, 100 ng/mL of PMA, or 25 μ g/mL of etoposide. C-K significantly reduced the interaction between Annexin A2 and p50 both in resting cells and NF- κ B activator-treated cells (Fig. 2A). Immunoprecipitation with purified proteins also showed significant inhibition of C-K on this interaction, indicating that C-K inhibits the assembly of Annexin A2-p50 complex by directly targeting Annexin A2 (Fig. 2B). Then, immunofluorescence was engaged to examine the subcellular distribution of

Annexin A2 and p50 under C-K treatment. The colocalization of Annexin A2 and p50 in the nucleus was significantly reduced under C-K treatment in NF- κ B activator-stimulating and resting states (Fig. 2C). Taken together, these data suggested that C-K prevented Annexin A2 binding to p50 and inhibited their nuclear colocalization by targeting Annexin A2.

3.3. C-K suppressed NF- κ B activation and downregulated the expression of downstream antiapoptosis gene

HepG2 cells were cultured with C-K (6 μ M) and etoposide (25 μ g/mL) or PMA (100 ng/mL) followed by a dual luciferase reporter assay to determine the activity of NF- κ B. The results showed that NF- κ B activation was narrowed by C-K both in resting state and under treatment with etoposide or PMA, which almost tripled NF-

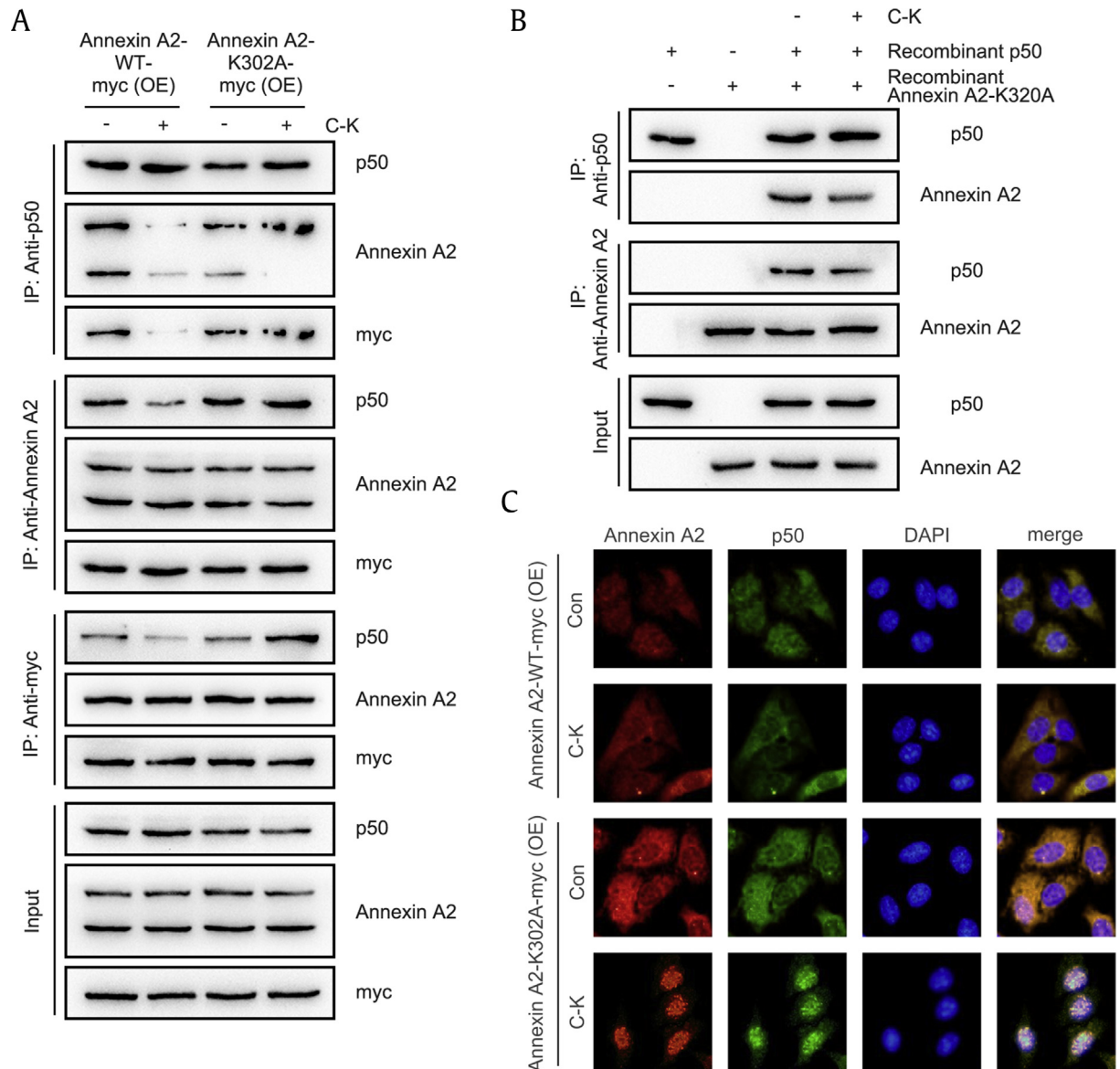


Fig. 5. Annexin A2-K302A blocked the inhibitory effect of C-K on the interaction between Annexin A2 and NF- κ B p50 subunit and their nuclear colocalization. (A) HepG2 cells were transfected with Annexin A2-WT-myc and Annexin A2-K302A-myc, and immunoprecipitation was performed with whole-cell lysate under treatment with C-K (6 μ M) and examined by an immunoblot. (B) Immunoprecipitation was performed with prokaryotic cells-expressed Annexin A2-K302A and NF- κ B p50 subunit under treatment with C-K (6 μ M), and the interaction was analyzed by an immunoblot. (C) HepG2 cells were transfected with Annexin A2-WT-myc and Annexin A2-K302A-myc, and the subcellular distribution of Annexin A2 and NF- κ B p50 subunit was examined by immunofluorescence under treatment with C-K (6 μ M), and DAPI showed the nuclear region. C-K, compound K; IP, immunoprecipitation; NF- κ B, nuclear factor-kappa B; OE, overexpression.

κ B activation individually (Fig. 3A). Interestingly, the well-known NF- κ B-regulated genes, interleukin-6 (Fig. 2B), and antiapoptosis genes from IAPs superfamily including X-IAP, c-IAP1, c-IAP2, and survivin were transcriptionally downregulated under C-K treatment (Figs. 3C–3F). As expected, the protein levels of these genes were also downregulated by C-K in both resting and NF- κ B activator-stimulated cells (Fig. 3G), indicating that the pro-apoptosis effect of C-K might be mediated by inhibiting the expression of IAPs

3.4. Annexin A2 knockdown enhanced anticancer activity of C-K

To examine the role of Annexin A2 in NF- κ B activation, NF- κ B activity was determined in HepG2 cells with Annexin A2 knockdown via shRNA. Both Annexin A2 knockdown and C-K treatment effectively reduced NF- κ B activity respectively and presented a synergistic effect when combined treatment (Fig. 4A). In addition, caspase 3 and 9 were activated under both C-K or Annexin A2 knockdown treatment, and the activity was also enhanced under cotreatment with C-K and Annexin A2 knockdown (Figs. 4B, 4C). MTT assay also showed a significant reduction of cell viability under C-K treatment, which was further enhanced by Annexin A2 knockdown (Fig. 4D). Then, a plate clone formation assay also showed that the clone number was markedly decreased by either C-K or Annexin A2 knockdown. As expected, the clone-forming activity was much more significantly impaired by cotreatment with these two steps (Fig. 4E).

3.5. K302A mutant of Annexin A2 protected cells from anticancer effect induced by C-K

As C-K failed to bind to Annexin A2-K302A (Figs. 1B, 1C), functional studies were carried out with Annexin A2-K302A- or Annexin A2-WT-overexpressed HepG2 cells. Both Annexin A2-K302A and Annexin A2-WT interacted with p53 *in vivo*, and only

the interaction between p53 and Annexin A2-WT (both endogenous and ectopically expressed) was decreased under C-K treatment (Fig. 5A). Interestingly, C-K failed to dissociate Annexin A2-K302A and p53 (Fig. 5A), and further immunoprecipitation *in vitro* demonstrated similar results with prokaryotic cells-expressed Annexin A2-K302A and p53 (Fig. 5B). Then, the subcellular distribution was detected by immunofluorescence. The nuclear colocalization of Annexin A2-WT and p53 disappeared under C-K treatment, whereas p53 colocalized with Annexin A2-K302A in the nucleus under C-K treatment (Fig. 5C). The NF- κ B activity was enhanced when Annexin A2-WT was overexpressed and downregulated by C-K in a dose-dependent manner. Interestingly, C-K could not inhibit the NF- κ B activation in Annexin A2-K302A-overexpressed cells (Fig. 6A). A cell viability assay showed that overexpression of both Annexin A2-WT and Annexin A2-K302A desensitized HepG2 cells to cytotoxicity of C-K and Annexin A2-K302A-expressing cells presented a better resistance to C-K (Fig. 6B). Activation of caspase 9 and 3 was less evident in Annexin A2-WT-overexpressing cells than in mock transfectant cells, and only slight activation was detected in Annexin-K302A-expressing cells (Figs. 6C, 6D). A following plate clone formation showed that C-K presented weaker cytotoxicity in Annexin A2-WT-overexpressing cells and little effect was detected in Annexin A2-K302A-overexpressing cells (Fig. 6E). Taken together, overexpression of Annexin A2-WT and Annexin A2-K302A rendered cells resistance to C-K treatment, and Annexin A2-K302A showed more potent resistance to the inhibition of NF- κ B and subsequent caspase activation.

4. Discussion

Antiproliferation activity is one of the major effects of C-K [4–13]. A number of study showed that C-K inhibits NF- κ B activation [13,21–23]; however, the underlying mechanism was not fully revealed. Previous reports have shown that Annexin family

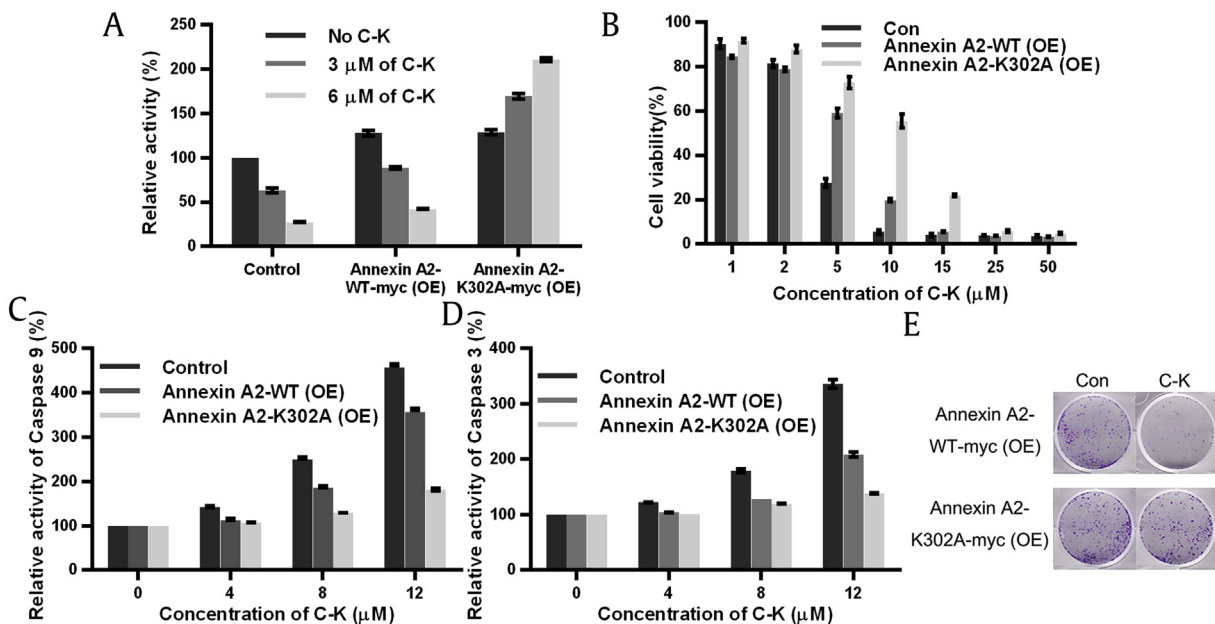


Fig. 6. Annexin A2-K302A protected cells from the cytotoxicity of C-K. (A) NF- κ B activity was examined by luciferase reporter assay under treatment of C-K with Annexin A2-WT-myc and Annexin A2-K302A-myc overexpressed in HepG2 cells. (B) Cell viability was examined by MTT for 48 h under treatment of C-K with Annexin A2-WT-myc and Annexin A2-K302A-myc overexpressed in HepG2 cells. Activity of (C) caspase 9 and (D) caspase 3 was examined under treatment of C-K with Annexin A2-WT-myc and Annexin A2-K302A-myc overexpressed in HepG2 cells. (E) Plate clone formation assay was examined under treatment C-K (6 μ M) with Annexin A2-WT-myc and Annexin A2-K302A-myc overexpressed in HepG2 cells. All data are shown as the mean \pm SD, and the experimental points show the average of at least triplicates. All experiments were repeated at least three times. C-K, compound K; NF- κ B, nuclear factor-kappa B; SD, standard deviation.

proteins including Annexin A1 [26], A2 [24], A4 [27], and A6 [28] interact with NF- κ B members and regulate NF- κ B pathway. Molecular docking analysis and CTSA displayed that Annexin A2, but not the other members, interacted with C-K (Figs. 1A–1C). To confirm the interaction, single-site mutants of D182A, Q226A, and K302A were constructed, with only K032A mutant successfully expressed. As Lys302 was shown responsible for the conformational stability of the N-terminus of Annexin A2 and interacted with p50 [29], a single-point mutant was generated for further research, and Annexin A2-K302A was demonstrated as the binding-deficient mutant of Annexin A2 (Figs. 1B,1C).

Annexin A2 interacts with p50 and facilitates their nuclear colocalization, which promotes NF- κ B transcriptional activity [24]. The treatment of C-K broke the interaction between Annexin A2 and p50 (Figs. 2A,2B) and their colocalization in the nucleus (Fig. 2C). As a consequence, NF- κ B failed to get activated; accordingly, the expression of downstream antiapoptosis proteins was reduced under C-K treatment (Fig. 3), resulting in the inhibition of cell viability (Fig. 4B–4E).

Knockdown of Annexin A2 induced the loss of NF- κ B activation and cell viability, indicating the essential role of Annexin A2 on NF- κ B activation and cell survival in HepG2 cells. Gene knockdown of Annexin A2 showed a synergistic effect with C-K in NF- κ B activation (Fig. 4A). On the other hand, the overexpression of Annexin A2-WT reduced the cytotoxicity of C-K but did not change the pattern as C-K inhibited NF- κ B (Figs. 5A–5C, 6A). However, the overexpression of Annexin A2-K302A totally relieved NF- κ B suppression by C-K and transformed C-K to an activator of NF- κ B pathway (Figs. 5A–5C, 6A).

The following series of analysis on the proliferation capability under modulation of Annexin A2 well coincided with those of NF- κ B activity. Knockdown of Annexin A2 presented a synergetic effect coupled with C-K cytotoxicity (Fig. 4), and the overexpression of Annexin A2 markedly attenuated the cytotoxicity of C-K (Fig. 6). The overexpression of Annexin A2-K302A further protected HepG2 cells from C-K-induced cell death (Fig. 6). Thus, it was possible that C-K-induced downregulation of NF- κ B activity was mainly mediated by Annexin A2. We have previously indicated that (20S)G-Rh2, G-Rg5, and G-Rk1 inhibited NF- κ B activation targeting Annexin A2; however, a similar mechanism was not obtained to other ginsenosides such as (20S/R)G-Rg3, (20R)G-Rh2, protopanaxadiol (PPD), and protopanaxatriol (PPT) (data not shown) [25,30]. These researches suggested that C-K acted as a specific binding molecule to Annexin A2.

Upregulation of NF- κ B is a frequent and origin event during hepatocarcinogenesis, which may trigger the acquisition of transformed phenotype [31], and may also contribute to the spreading of liver tumor cells via proliferative and antiapoptosis effect [32,33]. Targeting NF- κ B may provide a new strategy for liver cancer prevention and treatment. The anti-liver cancer function of C-K will be examined in future studies.

In summary, we demonstrated that C-K bound to Annexin A2 and inhibited the interaction between Annexin A2 and p50, which resulted in the impairment of NF- κ B activity and downstream antiapoptosis genes expression. On the contrary, Annexin A2-K302A, a binding-deficient mutant of Annexin A2, relieved the inactivation of NF- κ B driven by C-K and protected cells from cytotoxicity of C-K. In this study, we described the cellular target of C-K for the first time and explained how C-K inhibited NF- κ B activation and cancer cell proliferation in human hepatoma cell lines.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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Yu-Shi Wang contributed to thermal shift assay in Fig. 1, immunoprecipitation in Figs. 2 and 5, NF- κ B activity analysis in Figs. 3, 4, and 6, and caspase activity analysis in Figs. 4 and 6, as well as the writing of the article.

Hongyan Zhu contributed to the immunofluorescence in Figs. 2 and 5.

He Li contributed to the construction of the vectors and purification of Annexin A2.

Yang Li contributed to the cell viability assay in Figs. 4 and 6.

Bing Zhao contributed to molecular docking analysis in Fig. 1.

Ying-Hua Jin was responsible for the arrangement of the project and contributed to the writing of the article.

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Further reading

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