# Abrogation of STAT3 activation cascade by Ginkgolide C mitigates tumorigenesis in lung cancer preclinical model

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**Abstract**

Background and purpose: Ginkgolide C (GGC) isolated form Ginko biloba (Ginkgoaceae) leaf can demonstrate pleiotropic pharmacological actions although. its anti-oncogenic impact in non-small cell lung cancer (NSCLC) model has not been reconnoitered. As signal transducer and activator of transcription 3 (STAT3) cascade can promote tumor growth and survival, we contemplated that GGC may interrupt this signaling cascade to expend its anti-cancer actions in NSCLC. Experimental approach: The effect of GGC on STAT3 activation, associated protein kinases, STAT3-regulated gene products, cellular proliferation, and apoptosis was examined. The in vivo effect of GGC on the growth of human NSCLC xenograft tumors in athymic nu/nu female mice was also investigated. Key results: GGC attenuated the phosphorylation of STAT3 and varying upstream kinases effectively. Exposure to pervanadate modulated GGC-induced down-regulation of STAT3 activation and promoted an elevation in the level of PTP? protein. Indeed, silencing of the PTP??gene reversed the GGC-promoted abrogation of STAT3 activation and apoptosis. Moreover, GGC exposure significantly reduced NSCLC tumor growth without demonstrating significant adverse effects via decreasing levels of p-STAT3 in mice tissues. Conclusions and Implications: Overall, the findings support that GGC may exhibit anti-neoplastic actions by mitigation of STAT3 signaling cascade in NSCLC.

## Abrogation of STAT3 activation cascade by Ginkgolide C mitigates tumorigenesis in lung cancer preclinical model

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**Running Title:** GGC negates carcinogenesis in NSCLC.

**Summary**

**Background and purpose:** Ginkgolide C (GGC) isolated form *Ginko biloba* (Ginkgoaceae) leaf can demonstrate pleiotropic pharmacological actions although. its anti-oncogenic impact in non-small cell lung cancer (NSCLC) model has not been reconnoitered. As signal transducer and activator of transcription 3 (STAT3) cascade can promote tumor growth and survival, we contemplated that GGC may interrupt this signaling cascade to expend its anti-cancer actions in NSCLC.

**Experimental approach:** The effect of GGC on STAT3 activation, associated protein kinases, STAT3-regulated gene products, cellular proliferation, and apoptosis was examined. The *in vivo* effect of GGC on the growth of human NSCLC xenograft tumors in athymic *nu/nu* female mice was also investigated.

**Key results:** GGC attenuated the phosphorylation of STAT3 and varying upstream kinases effectively. Exposure to pervanadate modulated GGC-induced down-regulation of STAT3 activation and promoted an elevation in the level of PTPε protein. Indeed, silencing of the *ΠΤΠϵ* gene reversed the GGC-promoted abrogation of STAT3 activation and apoptosis. Moreover, GGC exposure significantly reduced NSCLC tumor growth without demonstrating significant adverse effects via decreasing levels of p-STAT3 in mice tissues.

**Conclusions and Implications:** Overall, the findings support that GGC may exhibit anti-neoplastic actions by mitigation of STAT3 signaling cascade in NSCLC.

**Keywords:** Ginkgolide C, NSCLC, STAT3, xenograft, PTPε.

## Introduction

Lung cancer contributes largely to cancer-related mortality worldwide (Huang et al., 2016; Lee, Kim, Sethi & Ahn, 2015; Mason, 1949; Siegel, Miller & Jemal, 2015). Human lung cancer consists of non-small lung cancer (NSCLC) and small cell lung cancer (SCLC) (Zhang et al., 2016; Zhu et al., 2018). NSCLC is more common form and five-year survival rate is generally less than 5% in advanced cases (Lee et al., 2019a; Reck, Heigener, Mok, Soria & Rabe, 2013; Wang et al., 2018; Yang, Lee, Ko, Jung, Sethi & Ahn, 2019). Surgery operation, radiation therapy, chemotherapy, targeted therapy etc are are routinely used for management of NSCLC patients (Lee, Chinnathambi, Alharbi, Shair, Sethi & Ahn, 2019; Lee, Kim, Lee, Sethi & Ahn, 2018b; Yang et al., 2005). However, these traditional treatment modalities may be associated with low specificity and serious side effects on patients (Artal Cortes, Calera Urquizu & Hernando Cubero, 2015; Ko, Nam, Um, Jung, Sethi & Ahn, 2018; Lee et al., 2018; Liu, Kuang, Wu, Jin & Sun, 2016). Hence, the evolution of novel agents for the NSCLC therapy are still required.

Signal transducer and activators of transcription (STAT) family is made up of STAT1, STAT2, STAT3, STAT4, STAT5α, STAT5β, and STAT6 (Ashrafizadeh et al., 2019; Loh, Arya, Naema, Wong, Sethi & Looi, 2019; Mohan et al., 2020; Schindler, Levy & Decker, 2007; Wong, Hirpara, Pervaiz, Eu, Sethi & Goh, 2017; Yu, Pardoll & Jove, 2009). Among these proteins, STAT3 activated has been found to be deregulated in varied human cancer cells, such as head and neck, lung, breast, prostate, kidney, pancreas, liver cancer lymphomas, multiple myeloma (Arora, Kumar, Arfuso, Chng & Sethi, 2018; Hwang et al., 2019; Kim et al., 2014; Lee et al., 2014; Lee, Kim, Lee, Um, Sethi & Ahn, 2019; Lee et al., 2019b; Loh, Arya, Naema, Wong, Sethi & Looi, 2019; Yang et al., 2019). STAT3 can regulate varying hallmarks of cancer and promote expression of various tumorigenic genes (Baek et al., 2017; Kim et al., 2018; Lee et al., 2017; Lee, Kim, Kim, Sethi & Ahn, 2015; Shanmugam et al., 2015). The activation of STAT3 can be mediated by upstream kinases (JAK1/2 and Src) and undergo dimerization, translocation into nucleus, DNA binding, thus promoting transcription (Baek et al., 2016a; Fathi, Rashidi, Khodadadi, Shahi & Sharifi, 2018; Lee, Kim, Lee, Sethi & Ahn, 2018a; Wang & Sun, 2014). In addition, diverse protein tyrosine phosphatases (PTPs) such as SHP-1, SHP-2, PTEN, and PTPε can also control STAT3 pathway. PTPε occurs in both transmembrane (PTPε M) and cytosolic (PTPε C) forms (Nakamura, Mizuno & Kikuchi, 1996; Yang et al., 2019). PTPε M can be expressed in brain, testis, and lung, whereas PTPε C is expressed in spleen thymus, and peritoneal macrophages (Elson & Leder, 1995;

Tanuma, Nakamura & Kikuchi, 1999; Tanuma, Shima, Nakamura & Kikuchi, 2001). Interestingly, PTPε C was found to abrogate IL-6-stimulated JAK/STAT casacde in murine leukemia cells (Tanuma, Nakamura, Shima & Kikuchi, 2000).

Natural products remain the mainstay for discovery of novel anti-cancer drugs (Dai et al., 2015; Shanmugam, Warrier, Kumar, Sethi & Arfuso, 2017; Siveen et al., 2014; Tewari et al., 2018; Zhou et al., 2020). *Ginkgo biloba*(Ginkgoaceae) is one of the common ancient trees, and has been used as a medical herb for a long time in clinical therapy in oriental and western medicine (Yeh, Shou, Lin, Chen, Chiang & Yeh, 2015). Ginkgolide C (GGC) isolated from *Ginkgo biloba*leaves, is a flavone with multiple biological functions (Huang et al., 2014; Huang, Chen, Liu, Wu & Liou, 2018; Liou, Lai, Chen, Wang, Wei & Huang, 2015; Zhang et al., 2018). GGC has been implicated to increase lipolysis and suppress adipogenesis in 3T3-L1 adipocyte through modulation of AMPK signaling cascades (Liou, Lai, Chen, Wang, Wei & Huang, 2015). In addition, GGC can alleviate myocardial ischemia/reperfusion injury through suppression of CD40-NF-kB signaling pathway (Zhang et al., 2018). GGC also has been reported to reduce oleic acid-induced lipid accumulation via the Sirt1/AMPK pathway (Huang, Chen, Liu, Wu & Liou, 2018). However, up to now, there is little research on the impact of GGC on the progression of human cancers. STAT3 has been implicated to regulate tumorigeensis (Dai et al., 2016; Li et al., 2013), it may be possible that GGC may exert its anti-cancer actions through affecting this pathway.

In this study, we investigated whether GGC induce apoptosis and affect NSCLC growth by modifying the activation of STAT3 pathway. We noted that GGC can mitigate STAT3 activation by affecting PTPε tyrosine phosphatase in NSCLC cells that mediates its anti-oncogenic impact. Thus, GGC can act as a potent blocker of STAT3 signaling pathway and abrogate tumor growth effectively in NSCLC model.

**Materials and Methods**

Reagents and cell lines

Ginkgolide C (GGC) was purchased from Weikeqi Biological Technology (Chengdu, Sichuan. China). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and bovine serum albumin (BSA) were purchased from Sigma-Aldrich (St. Louis, MO). Human lung carcinoma cells (A549 and H1299 cells) were obtained from the American Type Culture Collection (Manassas, VA, USA). A549 cells were grown in DMEM low glucose. H1299 cells was cultured in RPMI 1640 medium and maintained at 37 under 5% $CO_2$atmosphere.

MTT assay

Cell viability was measured using an MTT assay as described earlier (Lee et al., 2019b).

Western blot analysis

After treating with GGC for indicated concentrations and time points, western blotting was executed as elaborated previously (Lee et al., 2019b).

EMSA for STAT3-DNA binding

STAT3-DNA binding was analyzed by electrophoretic mobility shift assay (EMSA) as elaborated previously (Lee et al., 2019b).

Immunocytochemistry

A549 and H1299 cells were treated with GGC 30 μM for 12 h and thereafter immunocytochemistry was done (Lee et al., 2019b).

Transfection with PTPε siRNA

To inhibit PTPε expression by RNA interference, A549 cells were transfected with 50 nM STAT3 siRNA or 100 nM scrambled siRNA using NEON Transfection system (Invitrogen). Then cells were incubated in 10% FBS-supplemented DMEM low glucose medium for 24 h.

Transfection of plasmids

To establish the link between anti-cancer effects of GC and involvement of STAT3 signaling pathway, A549 cells were transfected with STAT3-C Flag pRc/CMV containing plasmid DNA. Cells ($5 \times 10^4$cells/well) were transfected with STAT3C DNA (300 ng/well) in serum-free media for 48 h by iN-fect transfection Reagent. After 48 h, cells were washed with 1x PBS and treated with GGC (30 μM) in complete media for 24 h.

Cell cycle analysis

Cell cycle analysis was performed to examine the effects of GGC on cell cycle progression as described before (Lee et al., 2019c).

Annexin V and TUNEL assays

Annexin V assay was performed to determined apoptosis in A549 and H1299 cells as elaborated upon previously (Lee et al., 2019b).

Real-time cell proliferation analysis

Cell growth behavior was performed using the Roche xCELLigence Real-Time Cell Analyzer (RTCA) as reported previously (Lee et al., 2019c).

Reverse transcription polymerase chain reaction (RT-PCR)

A549 cells were treated with GGC 30 μM for 24 h and H1299 cells were pre-treated with GGC 30 μM for 12 h and treated with IL-6 (20 ng/ml) for 24 h. Total RNA was extracted and RT-PCR was done.

Experimental Protocol

All procedures involving animals were reviewed and approved by Kyung Hee University Institutional Animal Care and Use committee [KHUASP(SE)-18-124]. A549 xenograft model was established and athymic nu/nu female mice were randomized into following four different treatment groups (n=6/group). Tumor volumes were measured by Digimatic caliper every 5 days and mice body weight was measured about 2 or 3 day intervals. Mice were killed 5 days later after last therapy and tumor tissues were further processed as described before (Jung et al., 2019)

Western blot analysis of tumor tissues

Lung tumor tissues were subjected to western blotting as described earlier (Jung et al., 2019).

Immunohistochemical study

Immunohistochemistry was done as elaborated previously (Jung et al., 2019).

Statistical analysis

All experiments are presented as the mean ± standard deviation (SD). Statistical significance of the data compared with the non-treated control was determined using the Mann-Whitney U test. Significance was set at $P < 0.05$.

## Results

### GGC exhibited cytotoxic activity against lung cancer cells.

First, we found that GGC mitigated the viability of A549 and H1299 cells significantly in dose response studies (Fig. 1B). Interestingly, it demonstrated lower cytotoxicity against HEL 299 normal lung cells as compared to tumor cells.

### GGC alters STAT3 phosphorylation substantially.

We examined the impact of GGC on STAT3 activation using western blotting (Fig. 1C). We noted that GGC suppressed the phosphorylation of STAT3 (Tyr 705), but had minimal impact on STAT5 activation

(Fig. 1G). We next determined whether GGC can modulate DNA binding property of STAT3. As shown in Fig. 1D, GGC can effectively reduce DNA binding ability as well as attenuate STAT3 translocation from the cytoplasm to nucleus in A549 cells (Fig.1E).

**GGC represses activation of kinases controlling STAT3 phosphorylation.**

As STAT3 can be activated by upstream kinases, we deciphered the actions of GGC on the phosphorylation of JAK1, JAK2, and Src. GGC inhibited p-JAK1, p-JAK2, and p-Src levels (Fig. 1F) without altering total JAK1, JAK2, and Src proteins. We also found that IL-6 promoted phosphorylation of STAT3 was maximally induced at 15 min after IL-6 treatment (Fig. 2A). As shown Fig. 2B and 2C, GGC attenuated IL-6-driven STAT3 phosphorylation and upstream signaling proteins in time dependent manner. In addition, H1299 cells were transfected with STAT3-luciferase construct and when the cells exposed to GGC prior to stimulation, IL-6-induced STAT3 activity was repressed significantly.

**Overexpression of STAT3 relieves GGC-promoted apoptosis and reduction in viability.**

In addition, to confirm whether the mitigation of STAT3 activation was crucial in GGC-driven apoptosis, A549 cells were transfected with a STAT3C-expressing construct followed by GGC exposure. As shown Fig. 2H, STAT3C-overexpression remarkably increased p-STAT3(Tyr705) and STAT3 levels. Moreover, this increase led to a substantial decrease in GGC-regulated apoptosis and its effect on viability as compared with control cells (Fig. 1F and Fig.4F).

**ΓΓ‟ ρομοτεδ τηε εξπρεσσιον οφ ΠΤΠε προτειν.**

To study the mechanism of GGC-mediated inhibition of STAT3 phosphorylation, we elucidated the impact of GGC by modulating of protein tyrosine phosphatase. Pretraetment with sodium pervanadate abrogated reduction in p-STAT3 levels caused by GGC (Fig. 2E). Moreover, we noted that GGC led to a dramatic increase in PTPε protein levels (Fig. F) however at mRNA level an augmentation was noted only in PTPε M, but not PTPε C. Moreover, in A549 cells with PTPε siRNA, PTPε expression was substantially reduced ((Fig. 2H) and GGC was unable to affect STAT3 phosphorylation in PTPε knockdown cells (Fig. 2I). In addition, PTPε knockdown attenuated GGC-induced PARP cleavage suggesting that this phosphatase may have a vital role in regulating anti-cancer properties of GCC.

**GGC induces apoptotic cell death in NSCLC.**

To analyze the apoptotic effect of GGC, we performed various assays to measure programmed cell death. As shown Fig. 3A, GGC caused subG1 phase arrest in A549 and H1299 cells. Early apoptosis was promoted and peak was shift to right side with apoptosis. In addition, as shown in Fig. 3B, GGC promoted apoptosis through modulating caspase-3 and PARP cleavage. Next, we noted that cell proliferation was significantly reduced upon GGC exposure in both A549 and H1299 cells (Fig. 3C). Moreover, GGC also attenuated the expression of various oncogenic molecules at protein and mRNA levels (Fig. 4A and C). Moreover, IL-6 promoted articulation of oncogenic markers was also affected upon GGC exposure (Fig. 4B and D).

**GGC exerts antitumor effects in preclinical model.**

We examined the impact of GGC to modulate tumor growth in xenograft model as per the protocol specified in Fig. 5A. The results suggested that in control group I, tumor volume sharply increased but markedly decreased in groups II and III (Fig. 5C). Moreover, tumor size and weight were attenuated in groups II, and III as compared to control (Fig. 5B and D), without altering the body weight (Fig. 5E).

**GGC alters levels of various oncogenic markers.**

First as measured by immunohistochemical analysis it found that GGC treatment caused a marked down-regulation in the expression of p-STAT3, Ki-67, and VEGF proteins (Fig. 6A). Thereafter, as shown Fig. 6B, GGC increased PTPε protein level but decreased the levels of phopho-STAT3 and different phosphorylated kinases as measured by western blotting. Next, cleaved caspase-3 and PARP levels were significantly elevated

in tissues harvested from GCC exposed mice (Fig. 6D). Finally, GGC reduced the expression of diverse oncogenic proteins that can regulate tumorigenesis in NSCLC model (Fig. 5E).

## Discussion

In traditional Chinese medicine, *Ginkgo leaf* extract is widely used as medical herb to treat cardiovascular disorders (Xu, Hu, Shen & McQuillan, 2015). It has been reported that GGC isolated from *Ginkgo leaf* , can exhibit diverse pharmacological properties (Huang et al., 2014; Huang, Chen, Liu, Wu & Liou, 2018; Liou, Lai, Chen, Wang, Wei & Huang, 2015; Zhang et al., 2018). This is the first investigation that has analyzed the potential impact of GGC and its mechanism in NSCLC model. We found that GGC diminished activation of STAT3 as well as that of JAK1, JAK2, and c-Src phosphorylation. This inhibition led to an induction in the expression of phosphatase PTPε. GGC also mitigated proliferation and promoted apoptosis through down-regulating STAT3 signaling cascade. Moreover, it attenuated tumor growth and survival in a xenograft mouse model without displaying any toxicity.

Aberrant STAT3 activation has been associated with various tumors, including head and neck, lung, breast, prostate, kidney, pancreas, liver cancer, lymphomas, and multiple myeloma (Banerjee & Resat, 2016; Bharti, Donato & Aggarwal, 2003; Chai et al., 2016; Hwang et al., 2019; Jung et al., 2018). We first investigated if GGC could alter constitutive and inducible STAT3 phosphorylation in NSCLC cells. We noted that GGC abrogated STAT3 phosphorylation and localization of this protein into the nucleus. Phosphorylation of JAKs has been linked to STAT3 activation (Ahn, Sethi, Sung, Goel, Ralhan & Aggarwal, 2008; Baek et al., 2017; Bowman, Garcia, Turkson & Jove, 2000). Interestingly, GGC mitigated JAK1, JAK2, as well as c-Src phosphorylation substantially and overexpression of STAT3 not only alleviated the inhibitory actions of GGC on p-STAT3 levels but also relieved apoptosis caused by this agent.

Recently, many studies have reported that protein tyrosine phosphatases (PTPs) may negatively control STAT3 signaling pathway (Ahn, Sethi, Sung, Goel, Ralhan & Aggarwal, 2008; Baek et al., 2016a; Baek et al., 2016b; Kim, Morales, Jang, Cho & Kim, 2018). We noted that GGC may have altered STAT3 activation by causing the modulation of PTPs. Interestingly, GGC promoted PTPε expression at protein and mRNA levels. Moreover, GGC could increase PTPε M expression also at mRNA levels. Moreover, the silencing of PTPε expression repudiated GGC-driven attenuation of STAT3 activation and induction of apoptosis. However, how GCC can stimulate PTPε levels and if GCC can also alter, its activity needs additional investigations.

It has been reported that reduction of STAT3 activity may decrease the survival ability of tumor cells (Aoki, Feldman & Tosato, 2003). Hence, we observed if GGC could alter proliferation and promote apoptosis in NSCLC cells. GGC induced apoptotic death was detected by cell cycle, annexin V and TUNEL assays. We demonstrated that GGC caused sub-G1 arrest, early apoptosis, and mediated the activation of caspase-3 and PARP. In addition, we noted that GGC dramatically reduced the expression of oncogenic molecules such as anti-apoptotic and metastasis promoting proteins that may contribute to its varying anti-neoplastic activities.

In a NSCLC preclinical model, GGC significantly suppressed lung tumor growth, altered the levels of p-STAT3, PTPε, and caspase-3 in treated groups. These modifications are consonant with its observed *in vitro* actions and were noted in the absence of any adverse effects. Thus, our preclinical studies imply that GGC may have a potential as a novel therapeutic agent for the management of NSCLC.

In summary, it was noticed that GGC can exert pleiotropic anti-neoplastic effects through modulating STAT3 signaling pathway by affecting the PTPε expression. Taken together, GGC may act as an effective inhibitor of STAT3 phosphorylation and its function can be investigated further in alleviation of different malignancies.

## Abbreviations

GGC: Ginkgolide C;

STAT3: signal transducer and activator of transcription 3;

c/w : Cell per well;

DAPI: 4',6-Diamidino-2-Phenylindole, Dihydrochloride;

FBS: Fetal bovine serum;

GAPDH: Glyceraldehyde 3-phosphate dehydrogenase;

HRP: Horseradish peroxidase;

IHC: Immunohistochemistry;

ip: Intraperitoneal injection;

MMP: Matrix metalloproteinase;

DMEM: Dulbecco's Modified Eagle Medium;

NSCLC: Non-small cell lung carcinoma;

NT : Non treat;

P/S: Penicillin-streptomycin;

RTCA: Real-time cell analysis;

RT-PCR: Reverse transcription polymerase chain reaction;

SFM: Serum free media;

PTP: Protein tyrosine phosphatase;

JAK: Janus kinase;

PARP: Poly (ADP-ribose) polymerase;

VEGF: vascular endothelial growth factor

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**Author's Contributions:**

MHY conducted all the experiments. JYU analyzed the data. G.S. and K.S.A. supervised and wrote the manuscript.

**Declaration of competing interest**

The authors have no conflicts of interest to declare.

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**Figure legends**

**Fig. 1. GGC alters STAT3 activation. (A)** The structure of Ginkgolide C (GGC). **(B)** A549, H1299, and HEL 299 cells were treated with the indicated concentration of GGC for 24 h and viability was measured. **(C)** A549 cells were treated for indicated time intervals with GGC 30 μM and western blotting was executed. **(D)**A549 cells were treated as described above in panel **(C)** and STAT3 levels were determined by EMSA. **(E)** A549 cells were treated with GGC 30 μM for 12 h and STAT3 distribution was measured.**(F)** A549 cells were incubated with various concentrations of GGC and western blotting was executed. **(G)** Western blotting to analyze STAT5 (Tyr694/Tyr699) expression. **(H)** A549 cells were transfected with empty vector (pcDNA) or STAT3-C Flag pRc/CMV (300 ng) for 24 h. Whole-cell extracts were prepared and Western

blot was executed. **(I)** A549 cells were transfected with Empty vector (pcDNA) or STAT3-C Flag pRc/CMV were treated with or without of GGC for 24 h and cell viability was analyzed.

**Φιγ. 2. ΓΓ˜ βλοχκς ΙΛ-6 προμοτεδ ΣΤΑΤ3 αχτιατιον ανδ ινχρεασες ΠΤΠε λεελς.** **(A)** H1299 cells were treated with the indicated concentrations of GGC for 12 h and IL-6 (20 ng/ml) for 15 min. Thereafter western blot was executed. **(B)** H1299 cells were treated with GGC 30 μM and stimulated with IL-6 as described above. Western blot was executed on the cell lysates. **(C)** A549 cells were treated as described above in panel (B) and western blotting was executed. **(D)** H1299 cells were transfected with STAT3 and treated with GGC for 12 h. After stimulation with IL-6 (20 ng/ml) for 15 min, luciferase assay was conducted. **(E)** A549 cells were exposed to pervanadate and 30 μM of GGC for 12 h followed by western blotting. **(F)** A549 cells were treated with GGC for 12 h and western blot analysis was executed. **(G)** A549 cells were processed as elaborated above and RT-PCR was done. **(H)** A549 cells were transfected with PTPε-specific siRNA and scrambled RNA. After 24 h, the cells were exposed to GGC 30 μM for 12 h. Lysates from the cells were determined by Western blot. **(I)** A549 cells were transfected and treated with as described above in panel (H) and western blotting was executed.

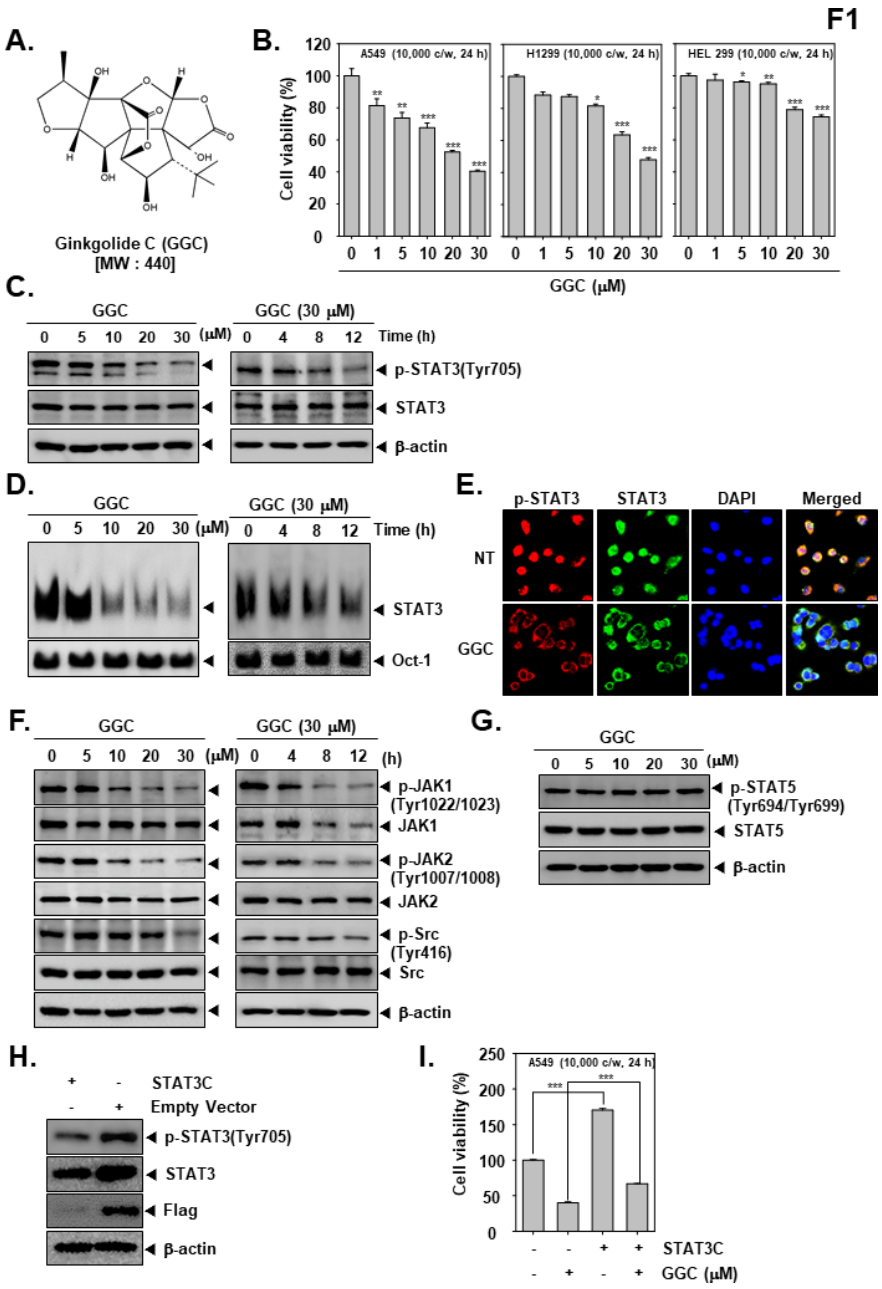
**Fig. 3. GGC promotes apoptosis and suppresses proliferation.** **(A)** A549 cells were treated with GGC 30 μM for 24 h. and apoptosis was examined by Cell cycle Analysis, Annexin V, and TUNEL assays. H1299 cells were treated with GGC 30 μM. After 12 h, cells were exposed to IL-6 (20 ng/ml) for 12 h and apoptosis was determined **(B)** A549 cell were treated with GGC for 24 h. H1299 cells were exposed to GGC. After 12 h, IL-6 (20 ng/ml) was added in these cells for 12 h. Thereafter western blotting was executed. **(C)** The cells were seeded in 16-well E-plates and treated with various concentration of GGC and IL-6 as elaborated above and proliferation assay was performed.

**Fig. 4. GGC attenuates levels of tumorigenic proteins.** **(A)** A549 cells were treated with GGC for 24 h and western blotting was executed.**(B)** H1299 cells were exposed to GGC 30 μM. After 12 h, IL-6 (20 ng/ml) was added for 24 h and western notting was conducted.**(C)** A549 cells were treated with various concentration of GGC and RT-PCR was done for different genes. **(D)** H1299 cells were treated with GGC and IL-6 as elaborated above. (B) and RT-PCR was conducted. **(E)** A549 cell were transfected with PTPε-specific siRNA and scrambled RNA. After 48 h, the cells were treated with GGC 30 μM for 24 h and western blotting was executed. **(F)** A549 cells transfected with STAT3-C Flag pRc/CMV (300 ng) were treated with or without of GGC for 24 h and western blot analysis was conducted.

**Fig. 5. Anti-tumor actions of GGC.** **(A)** In vivo experimental design. **(B)** Necropsy photographs of mice carrying implanted tumors. **(C)** Tumor volume data. ** $p < 0.01$ compared the control. **(D)** Tumor weight was measured on Day 25. * $p < 0.05$ compared the control. **(E)** Body weight was calculated on the indicated days.

**Fig. 6. GGC alters the levels of oncogenic markers in tissues.** **(A)** GGC treated mice tissues were studied by immunohistochemical staining. Quantification data was represented as mean ± SD on right panel. **(B-E)** Western blot data for different markers in whole cell extracts from mouse tissues.

F1



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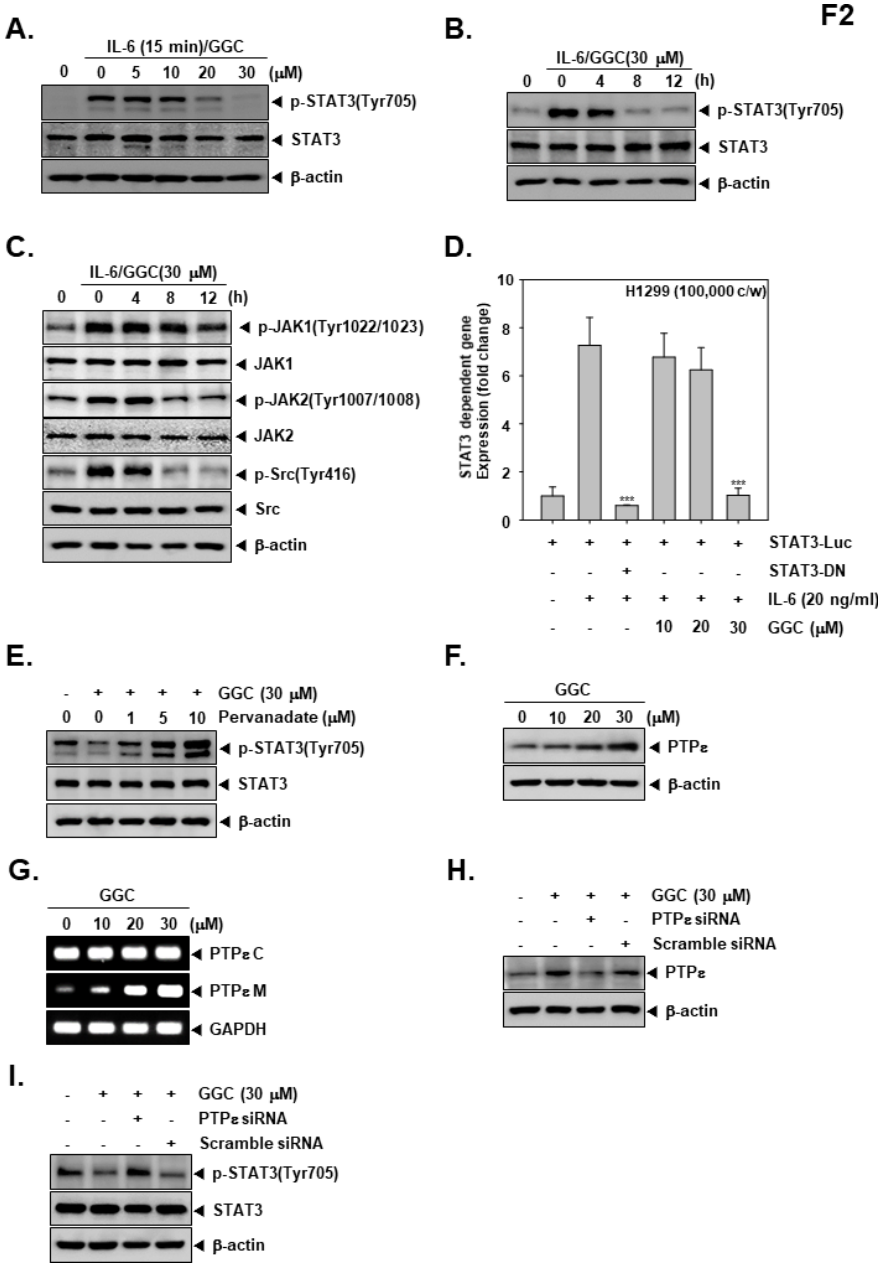

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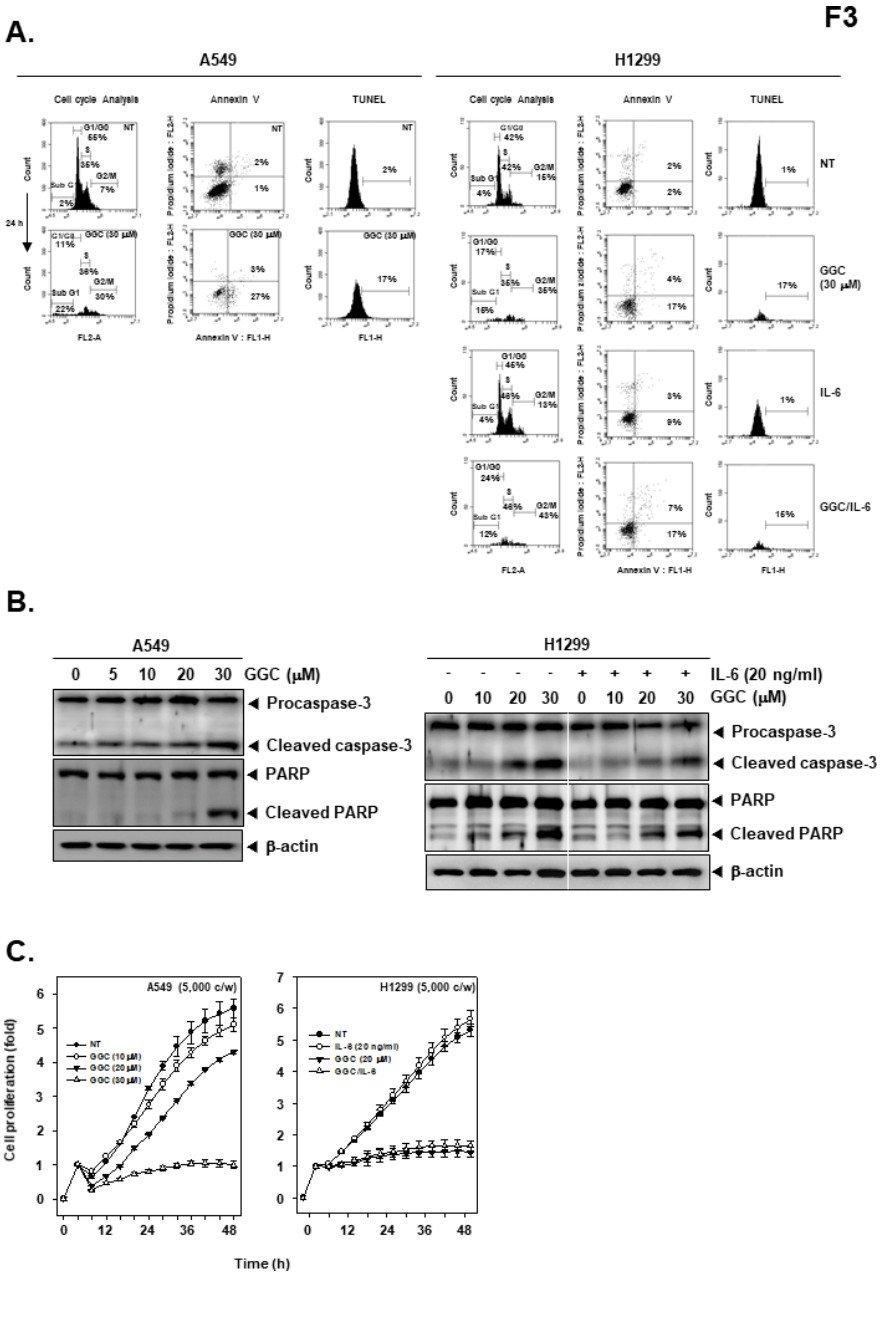

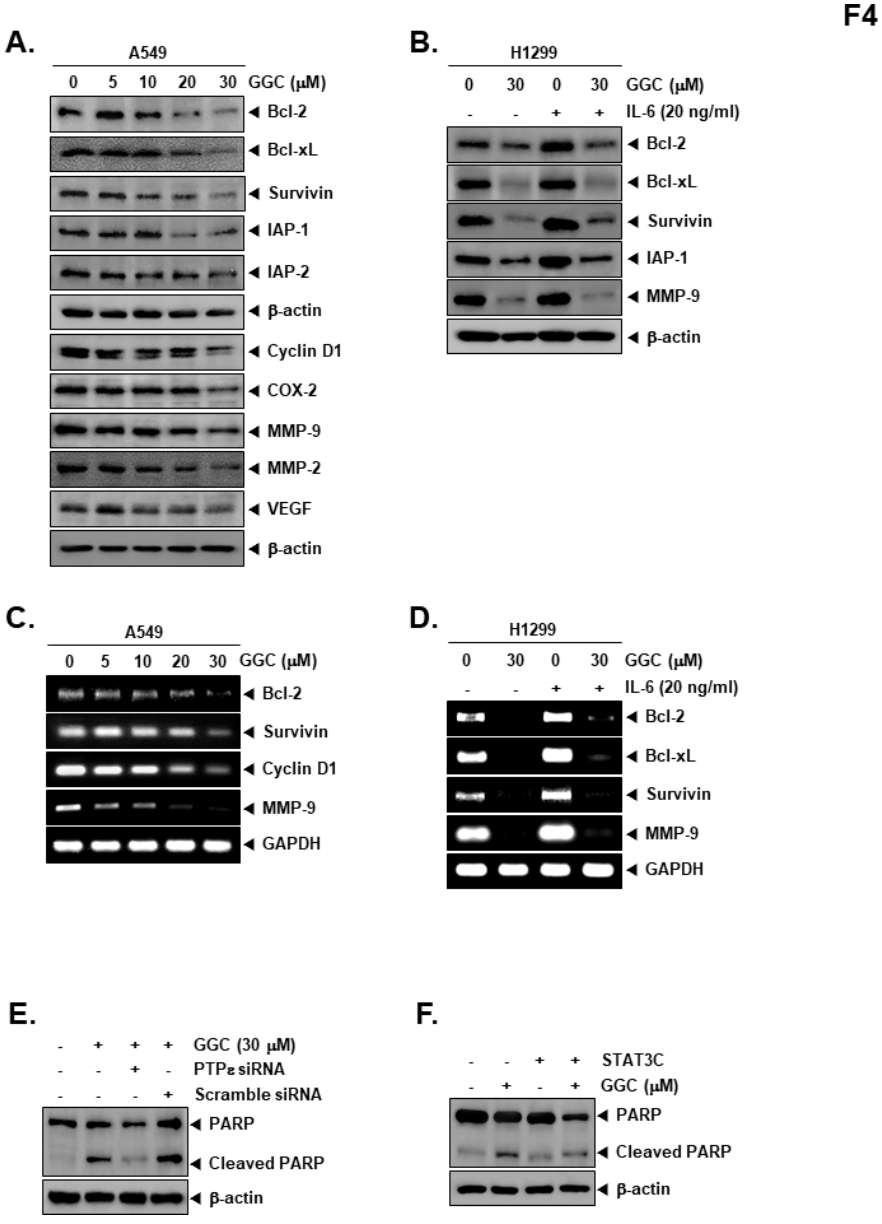

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