# Anti-tumor Promoting Effects of Selected Ginsenosides and Their Underlying Molecular Mechanisms

Young-Joon Surh*, Ji-Yoon Lee, Kyung-Soo Chun and Hye-Kyung Na

*College of Pharmacy, Seoul National University, Seoul 151-742, South Korea*

The roots of rhizome of several varieties of *Panax* plants (e.g., *P. ginseng*, *P. notoginseng*, *P. japonicus* and *P. quinquefolium*) have been used in traditional oriental medicine for the treatment of many disorders, such as inflammation and cancer as well as for enhancing physical strength. Among them *P. ginseng* C.A. Meyer is most widely used in Asia. It has diverse biological and pharmacological effects which are mainly attributed to triterpenoid saponin constituents, collectively called ginsenosides (Attele et al., 1999). To date, more than 30 ginsenosides have been isolated from the roots and other parts of *P. ginseng*. Several individual ginsenosides as well as total or fractionated ginseng extracts have been tested for their anticarcinogenic or tumoricidal activities (Kubo et al., 1992; Matsunaga et al., 1994; Wakabayashi et al., 1997; Yun, 2001). For instance, Rg3, by activating the expression of p21 and p27 cyclin-kinase inhibitors, arrested human prostate carcinoma LNCaP cells at G1 phase, and subsequently inhibited cell growth through a caspase-3-mediated apoptosis mechanism (Liu et al., 2000). Besides ginsenoside Rg3, ginsenoside Rh2 with a similar dammarane skeleton has been shown to have a growth inhibitory effects towards various cancer cell lines (Kim et al., 1999). The Rh2-induced apoptosis of human hepatoma SK-HEP-1 cells was found to be mediated through activation of caspase-3, followed by proteolytic cleavage of its substrate, poly(ADP-ribose)polymerase (Park et al., 1997). In glioma cells, Bcl-xL-independent generation of reactive oxygen species (ROS) and activation of caspase cascades by ginsenoside Rh2 resulted in apoptosis (Kim et al., 1999; Park et al., 1997). In addition, ginsenoside Rh2 exerts anticarcinogenic effects by suppressing the initiation of malignant transformation in cultured BALB/c 3T3 cells (Tatsuka et al., 2001).

Correspondance to : Y.-J. Surh, e-mail: surh@plaza.snu.ac.kr
Phone: +82-2-880-7845, Fax: +82-2-874-9775

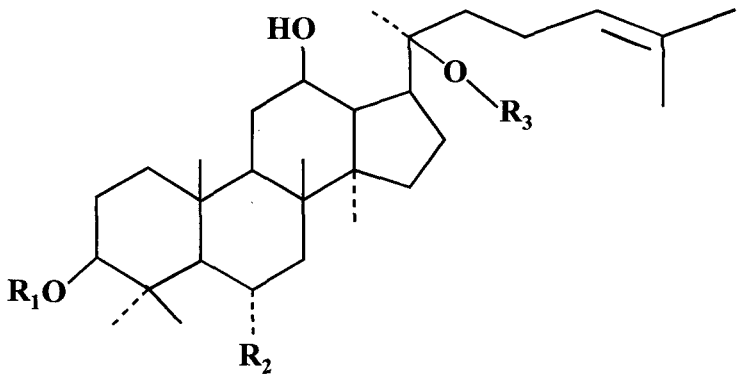
***Anti-tumor promotional activity of ginseng***

Recent studies have shown that ginseng extracts and its constituents could exert anti-tumor promoting activities (Kang et al., 2000, Konoshima et al., 1998; Surh et al., 2001; Tatsuka et al., 2001). Kang et al. (2000) reported the inhibitory effect of Rb2 on 12-*O*-tetradecanoylphorbol-13-acetate (TPA)- or hydrogen peroxide-induced gap junctional intercellular communication which is often altered during the tumor promotion. Topical application of the methanol extract of heat-processed ginseng onto shaven backs of mice before TPA treatment suppressed skin papillomagenesis initiated by 7,12-dimethylbenz[*a*]anthracene (Keum et al., 2000).

Ornithine decarboxylase (ODC) is a rate-limiting enzyme in the biosynthesis of polyamines that play pivotal roles in cell proliferation. Elevation of ODC activity is closely associated with tumor promotion (OBriene, 1976). Topical application of the heat-processed ginseng extract 30 min before TPA caused substantial reduction in epidermal ODC activity and also suppressed the expression of ODC mRNA (Keum et al., 2000). TPA-induced production of tumor necrosis factor-alpha (TNF-α) was similarly inhibited by heat-processed ginseng. One of the major ginsenosides present in heat-processed ginseng is Rg3. Topical application of Rg3 onto dorsal skins of mice prior to TPA also resulted in inhibition of ODC activity (Keum et al., 2002).

***COX-2 as a potential target for anti-tumor promotion by ginsenosides***

Because tumor promotion is closely linked to inflammation (Surh et al., 2001; Surh, 2002), compounds with potent anti-inflammatory activity are anticipated to possess anti-tumor-promoting potential. When various ginsenosides (e.g., Rc, Re, Rg1, and Rg3) were tested for their ability to inhibit TPA-induced mouse skin ear edema, Rg3 was found to be most potent in terms of exerting the anti-inflammatory activity (Surh et al., 2002). Considerable effort is being directed towards developing agents that can inhibit the activity of cyclooxygenase (COX). COX is a key enzyme in prostaglandin synthesis. There are two forms of COX enzymes, COX-1 and COX-2. While COX-1, which is a constitutive form present in most tissues, is involved in the physiological production of prostaglandins for maintaining normal homeostasis, COX-2 is barely detected in normal tissues, but is readily expressed in response to inflammatory cytokines, bacterial lipopolysaccharide, mitogens and ROS (Crofford, 1997). Substantial evidence has been accumulated to suggest that improper elevation of COX-2 is implicated in tumorigenesis. Thus, it was recognized that COX-2 was upregulated in transformed cells as well as in various forms of cancer, whereas levels of COX-1 remained unchanged (Subbaramaiah et al., 1996). Overexpression




| | | |
|---|---|---|
| $Rb_1$ ; $R_1$ = glu-2-1-glu | $R_2$ = H | $R_3$ = glu-6-1-glu |
| Rc ; $R_1$ = glu-2-1-glu | $R_2$ = H | $R_3$ = glu-6-1-ara |
| Re ; $R_1$ = H | $R_2$ = O-glu-2-1-rham | $R_3$ = glu |
| $Rg_1$ ; $R_1$ = H | $R_2$ = O-glu | $R_3$ = glu |
| $Rg_3$ ; $R_1$ = glu-2-1-glu | $R_2$ = H | $R_3$ = H |

**Fig. 1.** Chemical structures of various ginsenosides. Abbreviations : glu, glucose; rham, rhamnose; ara, arabinose

of COX-2 inhibited apoptosis and increased the invasiveness of the cells. Experimentally-induced null mutation of COX-2 reduced the number and size of colon tumors in a murine model of familiar adenomatous polyposis (Oshima et al., 1996).

To test whether Rg3 could inhibit TPA-induced COX-2 expression, this ginsenoside was applied topically 30 min prior to TPA treatment and sacrificed mice after 5 h later. Rg3 treatment caused marked suppression of TPA-induced COX-2 expression in mouse skin. TPA-induced expression of inducible nitric oxide (iNOS), another important enzyme responsible for mediating inflammation, was also inhibited by Rg3 pretreatment (Keum et al., unpublished observation). Besides Rg3, other structurally related ginsenosides including Rb1, Rc, Re and Rg1 (structures shown in Fig. 1) were also tested for their effects on TPA-induced COX-2 expression in mouse skin, but their inhibitory activities were found to be relatively weaker than that of Rg3 (Surh et al., 2002). Likewise, Rg3 strongly inhibited TPA-induced COX-2 expression in human breast epithelial cells (MCF-10A) in culture (Surh et al., 2001).

***Effects of ginsenosides on NF-κB activation***

It has been demonstrated that the intracellular signaling cascades regulating COX-2 induction and other proinflammatory events involves several distinct sets of protein kinases and transcription factors (Hwang et al., 1997; Tuyt et al., 1999). NF-κB is a ubiquitous eukaryotic transcrip-

tion factor that exists as a dimer composed of Rel family of proteins (Thanos and Maniatis, 1995). Several lines of evidence supports that NF-κB is implicated in cellular proliferation, inflammation, and malignant transformation. It has been observed that NF-κB activation is an essential early event, which occurs prior to malignant transformation and that NF-κB contributes to cell transformation by inhibiting cell death signal activated by oncogenic Ras (Mayo et al., 1997). Constitutive activation of NF-κB has been associated with proliferation of certain cancer cells and their resistance to apoptotic death (Bargou et al., 1997; Sovak et al., 1997). Thus, the role of NF-κB in oncogenesis is evident. NF-κB has been considered as a positive regulator of COX-2 expression in diverse cell types (reviewed by Surh et al., 2001 and references therein). The NF-κB binding consensus sequences have been found in the 5-flanking region of *cox-2* promoter.

In an attempt to elucidate the moleular mechanisms underlying suppression of TPA-induced expression of COX-2 by Rg3 and other ginsenosides, their effect on NF-κB activation were compared. TPA treatment caused dramatic DNA binding of NF-κB, and all of the ginsenosides tested exerted inhibitory effects to the different extent in mouse skin *in vivo* (Surh et al., 2002) and cultured MCF10A cells (Surh et al., 2001).

***Modulation of mitogen activated protein (MAP) kinases by ginsenosides***

The molecular signaling mechanisms that lead to the induction of COX-2 as well as activation of NF-κB in response to various external stimuli have not been fully clarified (Surh et al., 2001). One of the most extensively investigated intracellular signaling cascades involved in proinflammatory responses is the mitogen-activated protein (MAP) kinase pathway. Three distinct groups of well characterized major MAP kinases subfamily members include extracellular-regulated protein kinase (ERK), c-Jun $NH_2$-protein kinase (JNK)/stress-activated protein kinase (SAPK) and p38 MAPK that are serine/threonine protein kinases. The activated form of each of the above MAP kinases then phosphorylates and activates other kinases or transcription factors, thereby altering the expression of the target genes. The induction of COX-2 and resulting production of $PGE_2$ were abolished by the specific inhibitors of the corresponding MAP kinases, suggesting that the MAP kinase cascade is responsible, at least in part, for up-regulation of COX-2. Treatment of MCF-10A cells with 10 nM TPA led to rapid activation of ERK, whereas p38 MAP kinase was not much induced. When ginsenosides Rb1, Rc, Re, Rg1, and Rg3 were tested for their effects on ERK activation in TPA-stimulated MCF-10A cells, Rg3 exhibited the most

potent inhibitory activity (Surh et al., 2001). Similarly, topical application of 10 nmol TPA caused transient activation of ERK1/2, which was suppressed by aforementioned ginsenosides to a different extent (Surh et al., 2002).

### *Concluding remarks*

The heat-processed *Panax* ginseng has been reported to have potent antioxidative and anti-tumor promoting activities. Rg3, one of the major ginsenosides contained in heat-processed ginseng, also inhibits tumor promotion and ODC activity induced by TPA in mouse skin. Inflammatory tissue damage has been closely associated with tumor promotion. Rg3 inhibits the TPA-induced expression of the proinflammatory enzyme, COX-2 in both mouse skin and the human breast epithelial cell line (MCF-10A). Rg3 also inhibits TPA-induced activation of NF-κB and ERK, an upstream kinase known to play a role in regulating NF-κB activation. These findings,

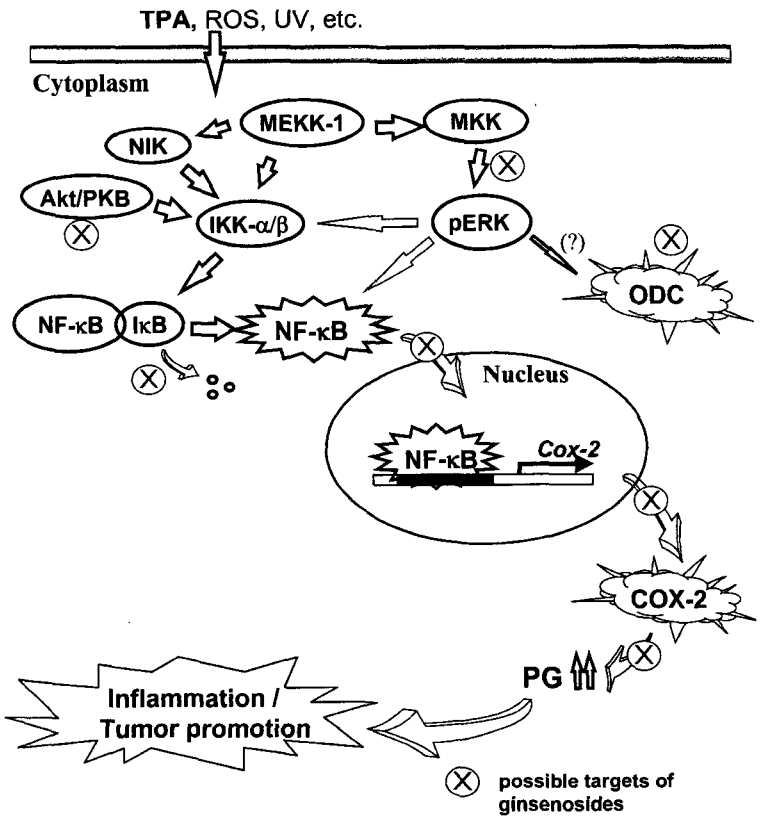



**Fig. 2.** Schematic representation of the possible targets of ginsenosides in the signal transduction pathway.

taken together, suggest that the previously observed anti-tumor promoting effects of heat-processed ginseng and its ingredient Rg3 are mediated through suppression of intracellular signaling cascades responsible for activation of NF-κB and and subsequent induction of COX-2. Other structurally related ginsenosides derived from P. ginseng also exhibit similar inhibitory effects on TPA-induced COX-2 expression and NF-κB activation. Figure 2 schematically represents the possible molecular targets of Rg3 and related ginsenosides in the intracellular signal transduction cascades related to tumor promotion.

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