Functional Mechanism of Ginsenoside Compound K on Tumor Growth and Metastasis

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Abstract

Ginsenosides, as the most important constituents of ginseng, have been extensively investigated in cancer chemoprevention and therapeutics. Among the ginsenosides, Compound K (CK), a rare protopanaxadiol type of ginsenoside, has been most broadly used for cancer treatment due to its high anticancer bioactivity. However, the functional mechanism of CK in cancer is not well known. This review describes the structure, transformation and pharmacological activity of CK and discusses the functional mechanisms of CK and its metabolites, which regulate signaling pathways related to tumor growth and metastasis. CK inhibits tumor growth by inducing tumor apoptosis and tumor cell differentiation, regulates the tumor microenvironment by suppressing tumor angiogenesis-related proteins, and downregulates the roles of immunosuppressive cells, such as myeloid-derived suppressor cells (MDSCs). There is currently much research on the potential development of CK as a new strategy when administered alone or in combination with other compounds.

Introduction

Ginseng, an herbaceous plant in the Araliaceae family, grows in the northern hemisphere of eastern Asia. Its ingredients are complex, including ginsenosides, polysaccharides, polyacetylenes, flavonoids and volatile oils. For thousands of years, ginseng has been one of the most widely used supplements and medical plants in Asian countries, especially in China, South Korea and Japan. In the past few decades, ginseng has become increasingly popular in the United States and Europe.

Ginseng exerts many pharmacological effects, such as anticancer, anti-inflammatory and treatment of diabetes, due to its important component, ginsenoside, which refers to a series of dammarane- or oleanane-type triterpenoid glycosides.[1] Based on the difference in the position and quantity of sugar moieties in glycosides, ginsenosides are classified into protopanaxadiol type (PPD), propylene glycol type, stearyl alcohol type and oleic acid type ginsenosides.[2] According to the amount found in cultivated ginseng, ginsenosides are grouped into major ginsenosides (Rb1, Rb2, Rc, Rd, Re, and Rg1) and minor ginsenosides (F2, Rg3, Rh1, Rh2, and Compound K). There is increasing evidence that the latter exhibit greater pharmacological activity than the former,[3] and minor ginsenosides also have more bioavailability and better permeability through cell membranes.[4] However, the natural rare ginsenoside content is very low and they must be produced through transformation.

Compound CK (CK), found in multiple ginseng species, is one of most important minor ginsenosides and has been studied for half a century, since it was found to exert increased pharmacological activity. CK was first discovered and

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identified in 1972 [5]; its molecular formula is $C_{36}H_{64}O_8$, and its structure is 20-O-β-(D-glucopyranosyl)-20(S)- protopanaxadiol. CK, also called M1, IH-901, and G-CK, is a protopanaxadiol-type saponin with the same core structural characteristics. Different protopanaxadiol-type saponins are composed of different sugar groups at the C-3 and C-20 positions. There is no CK in natural ginseng; however, after oral administration of ginsenosides Rb1, Rb2, and Rc, human intestinal bacterial enzymes gradually cleave the oligosaccharides linked to the aglycone from the terminal sugars and further decompose them into 20(S)-protopanaxadiol (PPD) by gastric acid and/or intestinal microorganisms. It has been reported that Rb1 and Rb2, the intermediate products of transformed CK, have anticancer effects, among which Rb1 targets chemotherapy-resistant ovarian cancer stem cells via simultaneous inhibition of Wnt/β-catenin signaling and the epithelial-to-mesenchymal transition,[6] while Rb2 inhibits tumor cells and their growth and metastasis in vivo.[7-10] In addition to the body, CK can also be converted from the main ginsenosides by heating, acid-base hydrolysis, enzyme conversion, and microbial conversion,[11] obtained by the cleavage of the sugar moiety at C-3 or C-20.[12]

CK has a variety of pharmacological activities, such as antitumor, anti-inflammatory and treatment of diabetes, and has the advantages of high safety and diverse biological functions, which are beneficial to the treatment of various clinical diseases.[13-15] This article reviews the structure, biotransformation, preparation, pharmacokinetics and pharmacological activities of CK. We focused on the functional mechanisms of CK and its metabolites, which regulate multiple signaling pathways related to tumor growth and metastasis.

The Chemical Structure of CK

CK is one of the main active metabolites of protopanaxadiol-type ginsenosides; its structure is 20-O-β-(D-glucopyranosyl)-20(S)-protopanaxadiol. Zhou et al[16] first determined the crystal structure of CK using both spectroscopy and X-ray diffraction. Its structure consists of a glucopyranosyl group and a tetracyclic aglycone.

Biotransformation of CK

Natural ginseng does not contain CK, which is usually produced by biotransformation of protopanaxadiol-type ginsenosides (such as ginsenosides Rb1, Rb2, Rc, etc.) in the presence of human intestinal bacteria, soil fungi or some commercial enzymes M (Figure 1).[11,12,17-19] The conversion pathway is Rb1/Rb2/Rc→Rd→F2/Rg3→CK.[20] Among these protopanaxadiol ginsenosides, Rb1 is the most abundant component in ginseng extracts. Therefore, the conversion pathway of ginsenoside Rb1→Rd→F2→CK is the

most important.[12,21] With in-depth research on CK, more transformation methods, such as biotransformation methods,[22] physical methods (such as heating)[23] and chemical methods (such as acid-base hydrolysis),[24] have been found. Compared with physical and chemical methods, biotransformation exhibits the advantages of high specificity, low cost and environmental protection.[25] The biotransformation of CK mainly includes both microbial and enzymatic assays.

Microbial Transformation of CK

Microbial transformation of CK includes both fungal transformation and enzymatic conversion.

Fungal transformation has become an important method, with the characteristics of fewer byproducts, mild reaction conditions, high transformation efficiency and no environmental pollution. Zhou et al[16] first used the fungus *Aspergillus niger* instead of intestinal bacteria to biotransform *Panax notoginseng* saponins (PNS) to produce CK with high quality. PNS can also be converted to CK through similar strains, such as *Fusarium racchari* fungus,[26] *Paecilomyces bainier* sp. 229,[19,27] *Fusarium racchari*,[28] and *Fusarium moniliforme*.[29] In addition, a rod-shaped bacterial strain isolated from a Korean ginseng field was designated strain DCY67T. Strain DCY67T contained β-glucosidase activity, which converts ginsenoside Rb1 to Compound K.[30] Strains similar to DCY67T include *Sphingomonas* GS-09,[12] *Platycodon grandiflorum* endophyte JG09,[21] *Bifidobacterium* K-103 and *Eubacterium* A-44.[31-40] Interestingly, the recombinant *Saccharomyces cerevisiae* strain BA21 expressing UGTPg1 is used to produce a large amount of CK from inexpensive monosaccharides. The whole method of CK synthesis is oxidosqualene→dammarenediol II→DMG→CK (CYP716A47) (Table 1).[41]

Enzymatic Transformation of CK

The enzymatic method is also widely used, with the characteristics of mild conditions without destroying the structure of saponins, strong specificity, high yield and no pollution. Ko et al[42] used β-galactosidase from *Aspergillus oryzae* to transform the main protopanaxadiol ginsenoside into CK. The enzymes similar to β-galactosidase include β-glycosidase from *Sulfolobus solfataricus* supplemented with α-L-arabinofuranosidase from *Caldicellulosiruptor saccharolyticus*,[43] recombinant β-glucosidase from Microbacterium *esteraromaticum*,[44] semirational design of *Sulfolobus solfataricus* β-glycosidase[45] and so on.[22,46-53] In addition, PPD-type gypenosides can be converted into CK through naringinase,[54] and rootlet ginseng can be converted into CK through pectinex containing pectinase and arabanase.[55] Choline chloride, as an enzymatic reaction medium, improves the ginsenoside conversion rate (Table 2).[56]
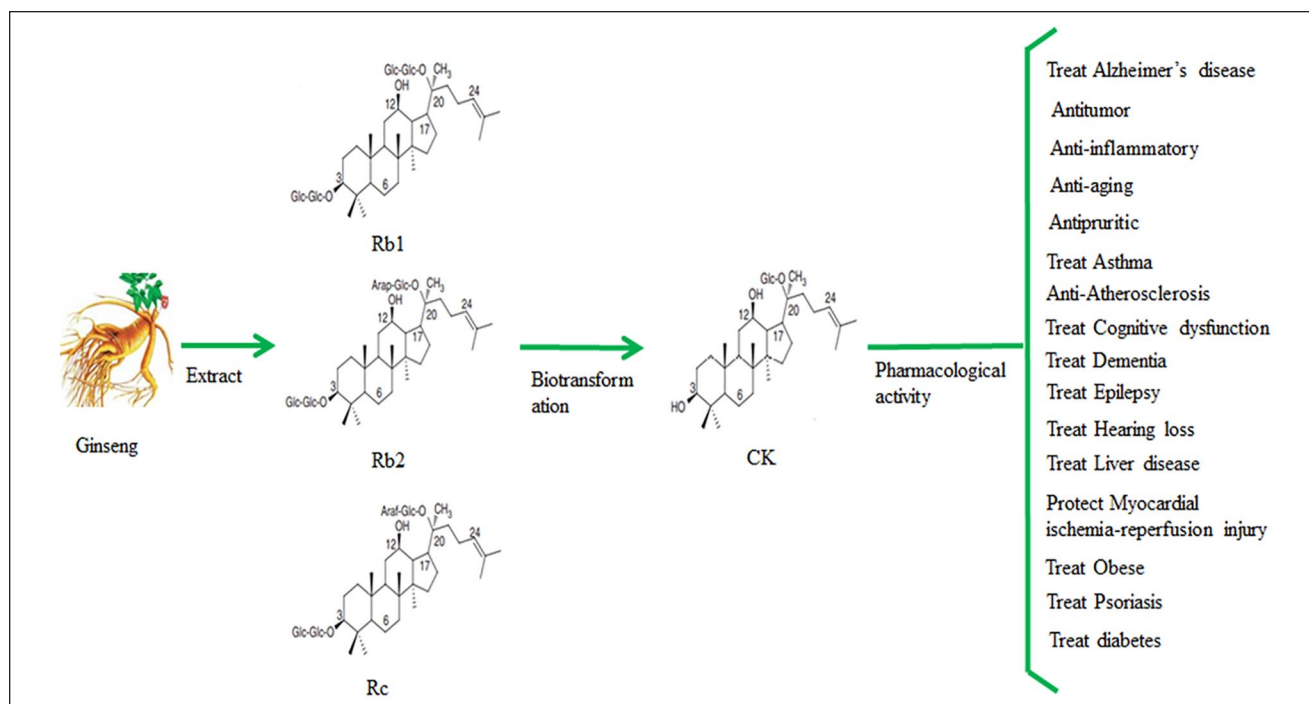
Figure 1. Transformation and pharmacological activity of Ginsenoside CK. Extract protopanaxadiol ginsenosides (such as ginsenosides Rb1, Rb2, Rc) from ginseng, and convert them into ginsenoside CK through biological transformation. The pharmacological potentials of ginsenoside CK include anti-tumor, anti-inflammatory, anti-aging, anti-seizure, anti-dementia, anti-pruritic, treating diabetes, treating asthma, treating atherosclerosis, treating Alzheimer's, treating obesity, treating psoriasis, treating myocardial ischemia-reperfusion injury, ameliorating cognitive impairment, recovery from hearing loss and hepatoprotective effects etc.
Abbreviations: Glc, glucose; Ara (P), Arabinose in pyranose form; Ara (f), Arabinose in furanose form; CK, Ginsenoside Compound K.

Other Methods of Transformation of CK

In addition to microbial and enzymatic conversion methods, glycerol is used as a carbon source to effectively improve the glycosylation efficiency of PPD to increase the output of CK.[57] In addition, the optimized *Cordyceps sinensis* was found by Dr. Qiu in our department for the first time to be an effective biocatalyst for the conversion of ginsenoside Rb1 to CK. Under optimized conditions, the molar conversion rate of Rb1 to CK is greater than 82%, which has a high efficiency and high selectivity and has raised new heights for CK production.[58]

CK Solubility

Appreciable antitumor activity of CK has been reported. However, the high polarity of CK leads to low solubility and poor oral bioavailability, which might also affect its biodistribution and efficacy. Thus, improving the slow dissolution rate of CK increases its pharmacological activity by covalently conjugating polyethylene glycol (PEG-CK) on the surface of CK with acid-labile ester bonds. PEG-CK was found to exhibit dose-dependent toxicity.[59]

The combination of Compound K and γ-cyclodextrin can improve the solubility of CK. These dissolution behaviors were reflected in the Cmax and Tmax values after oral administration in rats.[60] In addition, the new ester prodrug butyl octyl ester (CK-B and CK-O) improves the lipophilicity of CK through acylation to promote the transport of Caco-2 cells.[61] Prebiotic fiber can promote the metabolic transformation and gastrointestinal absorption of rat ginsenosides.[62] The structures of CK derivatives, as a novel class of LXRα activators, have been shown to have higher biological activity than CK.[63] The pharmacological effects of CK can be enhanced by the methods above.

CK Toxicity and Pharmacokinetics Study

A toxicity study of oral CK in beagle dogs was conducted, and all dogs received oral CK doses of 4, 12, or 36 mg/kg for 26 weeks. Animals in the 12 mg/kg group did not show any apparent toxicity for any of the measured parameters.[64] In the toxicity study of CK on mice and rats, some scholars found that in acute toxicity, oral CK in rats and mice did not cause death or toxicity at the maximum dose of 8 and

Table 1. Micro-Organism Methods Developed for CK Production.

Microorganisms	Transformation pathways	Optimum condition	Yield rate	Ref
Sphingomonas GS-098	Rb1→Rd→F2→CK Rb2→C-O→C-Y→CK Rc→C-Mc1→C-Mc→CK	No	No	Phi et al[8]
Aspergillus niger	*Panax notoginseng* saponins→CK	No	35.4%.	Chen et al[13]
Platycodon grandiflorum endophytes JG09	Rb1→Rd→F2→CK; Rb2→C-O→C-Y→CK; Rc→C-Mc1→C-Mc→CK	pH 4.0, 7 d	66.34%.	Chen et al[15]
Fusarium sacchari fungus	*Panax notoginseng* saponins→CK; C-Mx, and G-Mc	pH 5.5, 30°C 6 d	146.93 mg/g	Zhou et al[19]
Fusarium sacchari	Rb1→CK; G-Rc→CK	No	35.08%	Oh and Kim[20]
Fusarium moniliforme	*Panax notoginseng* saponin→CK	No	No	Cui et al[21]
Chryseobacterium yeoncheonense sp. nov DCY67T	Rb1→F2→CK	pH 6.0-6.5, 30°C	No	Noh et al[22]
Bifidobacterium K-103 and *Eubacterium* A-44 isolated from human fecal microflora	Rc→Rd→F2→CK; Rc→Mb→Mc→CK; Rc→Mb→F2→CK	pH 7.0, 37°C	No	Kim et al[23]
Bifidobacterium sp. Int57 and *Bifidobacterium* sp. SJ32	Rb2, Rc→Rd→F2→CK	pH 5.0, 37°C	No	Bae et al[24]
Esteya vermicola CNU120806	Rd→F2→CK	No	49.6%	He et al[25]
Acremonium strictum	G-Rb1→CK, other metabolites of bioactive ginsenosides	No	No	Han et al[26]
Aspergillus niger g.848	Rb1→Rd→F2→CK; Rb1→Gyp17→Gyp75→CK	No	No	Choi et al[27]
Leuconostoc citreum LH1	Rb1→gypenoside XVII, Rd→F2→CK	72 h, pH 6.0, 30°C	99%	Han et al[28]
Leuconostoc mesenteroides DC102	Rb1→gypenoside XVII, Rd→F2→CK	pH 6.0-8.0 and 30°C	99%	Yang et al[29]
Lactobacillus paralimentarius LH4	Rb1→gypenoside XVII, Rd→F2→CK	72 h, pH 6.0, 30°C	88%	Hoang et al[30]
Cladosporium cladosporioides GH21	Rb1→Rd→F2→CK; Rg1→F1→Rb1→Rd→F2→CK	No	74.2%	Bae et al[31]
β-glucosidase-producing microorganisms (K35)	*Panax ginseng* adventitious roots→CK	pH 7.0, 9 d	0.253	Chi et al[32]
Recombinant *Saccharomyces cerevisiae* strain BA21 expressing UGTPg1	Glucose→2,3-oxidosqualene→dammarenediol II→DMG→CK (CYP716A47)	No	No	Hou et al[33]

10 g/kg, respectively. In a 26-week toxicity study, rats were administered CK at doses of 13, 40, or 120 mg/kg. The NOA ELs of male and female rats were found to be 40 and 120 mg/kg, respectively.[65]

Pharmacokinetic studies have shown that oral ginsenosides pass through the stomach and small intestine and enter the large intestine without being decomposed by gastric juice or liver enzymes. In the large intestine, ginsenosides are decomposed by colonic bacteria.[66] CK, with the pharmacokinetic characteristics of minor ginsenosides, needs to be studied for its preclinical safety and effectiveness as a drug. The analysis and identification of CK metabolites are important aspects of CK research. The metabolism

of CK in rats has been reported. After oral administration of CK at a dose of 50 mg/kg, urine and feces were collected and subjected to ultra-performance liquid chromatography with electrospray ionization quadrupole time-of-flight tandem mass spectrometry. CK metabolites in urine and feces were detected and characterized, and various metabolites and metabolic pathways were analyzed in detail.[67] The absorption, dose linearity and pharmacokinetics of CK, the main intestinal bacterial metabolite of ginsenosides, have been studied.[68] After oral administration of CK in healthy people, CK was detected in both plasma and urine using LC–MS/MS and ESI-MS.[69-71] In addition, high-fat food was found to increase the absorption of CK in humans, and

Table 2. Enzymatic Methods Developed for CK Production.

Enzymes	Transformation pathways	Optimum condition	Yield rate	Ref
β-Glycosidase from *Sulfolobus solfataricus*	Rb1 or Rb2→Rd→F2→CK; Rc→C-Mc→CK	pH 4.5-6.5, 75°C	70%-80%	Zhou et al[16]
β-Galactosidase from *A. oryzae*	G-Rb1→G-Rd→G-F2→CK; G-Rb2→compound V→compound VII→CK; G-Rc→G-Rd→G-F2→CK; G-Rc→compound VI→compound VIII→CK	pH 4.5, 37°C	No	Chen et al[34]
β-Glycosidase from *Sulfolobus solfataricus*	G-Rb1→G-Rd→G-F2→CK; G-Rb2→G-Rd→G-F2→CK; G-Rc→G-Rd→G-F2→CK; G-Rc→C-Mc→CK	pH 6.0, 80°C	56%	Liu et al[35]
Recombinant β-glucosidase from *Microbacterium esteraromaticum*	Rb1→Rd→CK	pH 7.0, 40°C	77%	Quan et al[36]
Semi-rational design of *Sulfolobus solfataricus* β-glycosidase	G-Rb1→G-Rd→G-F2→CK	No	56%	Quan et al[37]
β-Glycosidase from *Pyrococcus furiosus*	Rb1, Rb2 or Rc→Rd→CK→APPD	pH 5.5, 95°C	79.5%	Quan et al[38]
Novel β-glucosidase MT619	Rd→F2→CK; Rb1→G17→F2→CK; Rb1→G17→G75→CK	pH 7.0, 37°C	79.2%	Wu et al[39]
β-Glucosidase from K-60	Rb1→F2→CK	pH 7.0, 40°C	No	Song et al[40]
β-Glucosidase from *Paecilomyces bainier*	Rb1→Rd→F2→CK	pH 3.5, 45°C	84.3%	Yan et al[41]
Recombinant β-glucosidase from *Terrabacter ginsenosidimutans*	Rb1→GypXVII→GypLXXV→CK	pH 7.0, 45°C	No	Ko et al[42]
Ginsenoside type I from *Aspergillus*	PPD type ginsenosides→F2, CK, Rh2	pH 5.0, 40°C	No	Shin et al[43]
β-Glycosidase from *Sulfolobus solfataricus*	Rb1→Rd→CK; Rb2→C-Y→CK	pH 5.5, 85°C	94%	Quan et al[44]
β-Glycosidase from *Microbacterium esteraromaticum*	Rb2→C-Y→CK	pH 7.0, 40°C	13.51%	Shin et al[45]
Naringinase	PPD type ginsenosides→CK	PH 4.1, 50°C, 71 h	65.44 ± 4.52%	Yoo et al[46]
Pectinex containing pectinase/arabanase	Rootlet ginseng→PG1, PG2, PG3 and CK	pH 5.0, 50°C	30%-65%	Cui et al[47]
Choline chloride	Rb1→Rd→F2→CK	pH 4.5, 60°C, 48 h	80.6%	Park et al[48]

the exposure of CK was higher in females than in males in Chinese subjects.[72,73] In a Japanese study, all subjects were equally divided into 2 groups and given tablets of *Lactobacillus paracasei* A221-fermented ginseng (FG) or nonfermented ginseng (NFG). This study intervention consisted of a single administration of 6 tablets. The CK contents of FG and NFG were 0.75 mg/tablet and 0.00 mg (not detected)/tablet, respectively. At 24 h after dosing, volunteers were measured using a validated LC–MS/MS assay, and plasma total testosterone concentrations in male volunteers were measured. The mean testosterone concentration in the fermented ginseng group significantly increased 24 h after administration.[74] In Korean subjects, the administration of fermented red ginseng extract promotes higher and faster absorption of CK in humans and rats compared with the treatment results of unfermented red ginseng.[75] The metabolic activity of ginsenosides in feces is positively correlated with the level of serum CK after conversion.[76]

Antitumor Activity of CK

Regulation of CK on Tumor Growth

CK exerts high antitumor roles with strong cytotoxic activity on tumor cells, such as mouse highly metastatic
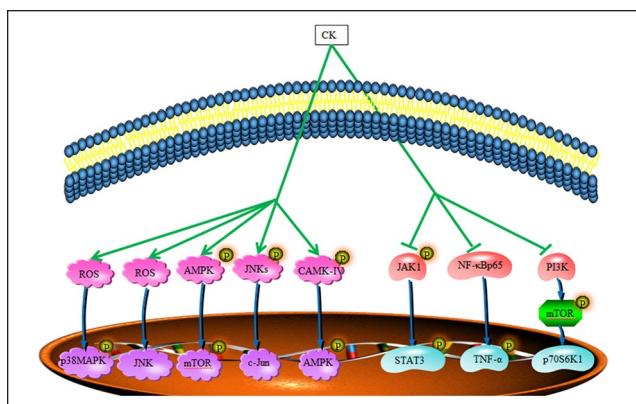
Figure 2. The functional mechanism of CK on different tumors. CK induces apoptosis and autophagy in non-small cell lung cancer cells through activating AMPK/mTOR and JNK pathways. CK induces bladder cancer cell apoptosis through activation of p38mapk pathway mediated by reactive oxygen species (ROS). CK induces apoptosis of colon cancer cells through the activation of CAMK-IV/AMPK pathway. CK induces autophagy and apoptosis of human colon cancer cells through increasing the level of ROS and activating JNK signal. CK induces osteosarcoma cell apoptosis and inhibits its proliferation and invasion through inhibition of PI3K/mTOR/p70S6K1 pathway. CK induces apoptosis of human multiple myeloma cells through inhibiting JAK1/STAT3 signal. CK inhibits TNF-α-promoted colon cancer metastasis in mice through inhibiting NF-κB signaling. Blue lines demonstrate the promotion (→) or inhibition (⊣) roles of signal pathways. Green lines indicate the promotion (→) or inhibition (⊣) roles of CK.

melanoma (B16-BL6), human liver cancer (HepG2), human myeloid leukemia (K562) and human highly metastatic lung cancer (95-D) cell lines, compared with those of other ginsenosides.[16]

CK inhibits tumor growth through different signaling pathways. Under hypoxia, CK can reduce the expression levels of HK-II, PDK1, and LDHA to inhibit the expression of HIF-1α and its downstream gene GLUT1, further blocking the growth of lung cancer.[15] CK induces apoptosis and autophagy in lung cancer cells A549 and H1975 through the AMPK/mTOR and JNK pathways.[77] CK also induces the production of ROS and the activation of p38MAPK to promote the apoptosis of bladder cancer T24 cells.[78] CK significantly reduces human multiple myeloma U266 cells through the JAK1/STAT3 pathway (Figure 2).[79]

For some rare tumor types, CK has been found to exert strong antitumor activity. CK induces ROS-mediated apoptosis and autophagy flux to suppress neuroblastoma.[80] For nasopharyngeal carcinoma (NPC), CK-induced HK-1-cell apoptosis is mediated through the mitochondrial pathway.[81]

Increasing evidence has revealed that CK blocks one type of cancer, colon cancer, and also that its regulatory mechanism differs.[82] CK induces the apoptosis of colon cancer HT-29 cells. CK inhibits the expression and activity

of deoxyribonucleic acid methyltransferase 1 to achieve demethylation of the RUNX3 gene and induce the expression of Smad4 and Bim mediated by RUNX3, indicating that CK significantly inhibits the growth of colon cells by inhibiting DNMT1 and reactivating epigenetically silenced genes.[83] In addition, CK downregulates cell survival proteins, including Mcl-1 and Bcl-2, upregulates cell proapoptotic proteins, including Bax and tBid, and induces the expression of the TRAIL death receptor DR5 on the cell surface, which promotes the apoptosis of colon cancer cells.[84] CK can increase the mRNA and protein expression of RUNX3, as well as p21, a downstream target of RUNX3. Blocking histone deacetylase activity induces the apoptosis of colon cancer cells.[85] CK induces the apoptosis of HT-29 cells and the destruction of mitochondrial membrane potential by regulating the CAMK-IV/AMPK pathway to achieve inhibition of colon cancer cells (Figure 2).[86] Furthermore, CK mediates ROS production to hinder the growth of human colon cancer HT-29 and HCT-116 cells by regulating the mitochondrial-dependent apoptosis pathway and MAPK pathway (Figure 2).[87,88] Studies have also shown that CK increases Ca^{2+} influx through TRPC channels and by targeting AMPK, thereby producing effective anticancer effects on colon cancer CT-26 cells.[89]

The protective role of CK was also investigated against liver cancer. CK significantly suppressed the proliferation of MHCC97-H human liver cancer cells and induced their apoptosis through the caspase-dependent pathway mediated by Fas and mitochondria.[90] Another study showed for the first time that CK prevented the interaction between Annexin A2 and the NF-κB p50 subunit and NF-κB nuclear colocalization to reduce the activation of NF-κB and activate caspase9 and caspase3, further blocking the growth of liver cancer.[91]

Studies have reported the effect of CK against leukemia. CK induces apoptosis of HL-60 human leukemia cells through a caspase-8-dependent pathway.[92] The G1 cell cycles of Kasumi-1 and MV4-11 are significantly arrested with CK treatment.[93] CK treatment contributes to G1 blockade of U937 cells through upregulation of p21 and the activation of JNK.[94] Similar results regarding the regulation of the cell cycle were reported in gastric cancer. CK induces apoptosis of BGC823 and SGC7901 cells and arrests the G2 cell cycle by upregulating the expression of p21 and downregulating the expression of cdc2 and cyclin B1. CK also effectively prevents tumor formation of SGC7901 gastric cancer cells in nude mice.[95] In addition, CK downregulates cyclin D1 levels to result in cell cycle arrest I then G1 phase, further retarding the proliferation of MCF10CA1a breast cancer cells.[96] CK also induces the apoptosis of MCF-7 breast cancer cells by suppressing the phosphorylation of GSK3β to reduce the expression of β-catenin and cyclin D1.[97] CK inhibits the growth of different colon cell lines through multiple mechanisms,

Table 3. Ameliorative Effects of CK on Tumor Growth.

Cancer type	Cell Line	Description	Ref.
lung cancer	NCI-H46, A549, NCI-H1299	To down-regulate the expression of HIF-1α and its downstream gene GLUT1 to suppress the growth of lung cancer cells	Xie et al[67]
Lung cancer	A549, H1975	To induce cancer cell apoptosis and autophagy through AMPK/mTOR and JNK pathway	Paek et al[68]
Bladder Cancer	T24	To induce the production of ROS and activation of p38MAPK, promoting cancer cell apoptosis	Chen et al[69]
Myeloma	U266	To downregulate the phosphorylation of STAT3/JAK1 to prevent tumors	Yang et al[70]
Neuroblastoma	SK-N-BE, SH-SY5Y, SK-N-SH	To induce ROS-mediated apoptosis and autophagy flux to inhibit neuroblastoma	Tawab et al[71]
NPC	HK-1	To induce cancer cell apoptosis through mitochondrial pathway	Chen et al[72]
Colon cancer	HCT-116, SW480	To suppress the proliferation and promote apoptosis	Chen et al[73]
Colon cancer	HT-29	To block DNMT1 and reactivates epigenetic silenced genes	Fukami et al[74]
Colon cancer	HT-29	To upregulate DR5 through autophagy-dependent and independent (p53-CHOP pathway) to enhance TRAIL-induced apoptosis	Choi et al[75]
Colon cancer	HT-29	To inhibit histone deacetylase activity to inhibit growth/promote apoptosis of cancer cells	Kim et al[76]
Colon cancer	HT-29	To induce cancer cell apoptosis through CAMK-IV/AMPK pathway	Li et al[77]
Colon cancer	HT-29	To regulate the mitochondrial-dependent apoptotic and MAPK pathway	Wang et al[78]
Colon cancer	HCT-116	To increase ROS production and JNK activation for inducing autophagy and apoptosis of cancer cells	Park et al[79]
Colon cancer	CT-26	To increase Ca2 + influx through TRPC channel/target AMPK to repress the growth of cancer cells	Oh et al[80]
Liver cancer	MHCC97-H	To retard the proliferation of liver cancer cells and induce their apoptosis	Law et al[81]
Liver cancer	HepG2	To attenuate the activation of NF-κB and the expression of their downstream genes, and activate caspase 3, 9 to induce anti-cancer effects	Wang et al[82]
Leukemia	HL-60	To induce leukemia cell apoptosis through caspase-8 dependent pathway	Kang et al[83]
Leukemia	Kasumi-1, MV4-11	To arrest cell cycle in G1 and promote apoptosis	Chen et al[84]
leukemia	U937	To upregulate p21 and activate JNK to block G1 phase of cancer cells	Kang et al[85]
Gastric carcinoma	BGC823 SGC7901	To upregulate the expression of p21, down-regulate the expression of CDC2/Cyclin B1, inducing cancer cell apoptosis and arresting cancer cell cycle	Kim et al[86]
Breast cancer	MCF10CA1	To down-regulate cyclin D1 level, lead to cell cycle arrest in G1 phase, inhibiting tumor cell proliferation	Lee et al[87]
Breast cancer	MCF-7	To reduce GSK3β phosphorylation and the expression of both β-catenin and cyclin D1 to induce cancer cell programed necrosis	Kim et al[88]

Abbreviations: DR5, TRAIL-R2; DNMT1, DNA methyltransferase 1; MMP9, Matrix Metalloproteinase 9; NPC, nasopharyngeal carcinoma; TNF-α, tumor necrosis factor-α; TRPC, transient receptor potential canonical.

indicating that CK may bind to different proteins or have multiple targets.

The bioactivity of CK has been described by inhibiting viability and proliferation and inducing the apoptosis of tumor cells (Table 3).

Impact of CK on Tumor Invasion and Metastasis

Tumor metastasis is regarded as a major obstacle to successful cancer therapy. Blocking metastasis provides more survival opportunities for cancer patients.[98] Recent investigations of the regulation of tumor metastasis have involved one family of enzymes, the matrix metalloproteinase (MMP) family, which exacerbates tumor metastasis in the TME.[99] These data were consistent with our previous findings.[100,101] Thus, the downregulatory roles of CK on the activity of MMPs may attenuate tumor migration/metastasis. CK effectively suppresses TNF-α-induced NF-κB activation and matrix metalloproteinase 9 (MMP-9) expression, further limiting the migration and invasion of

Table 4. Anti-Tumor Metastasis of CK.

Cancer type	Cell Line	Description	Ref.
Colon cancer	CT-26	To reduce TNF-α-induced NF-κB activation and MMP-9 expression to prevent the migration and invasion of cancer cells	Kang et al[94]
Liver cancer	MHCC97-H	To diminish the expression of NF-κBp65 nuclear export and MMP2/9 to inhibit tumor metastasis.	Hu et al[95]
Astroglioma	U87MG U373MG CRT-MG	To inhibit the expression of AP-1 and PMA-mediated activation of p38 MAPK/ERK/JNK, inhibiting the MMP-9 expression on cancer cells.	Lee et al[96]
Osteosarcoma	MG-63 U2-OS	To suppress tumor proliferation, promote apoptosis and migration through PI3K/mTOR/p70S6K1 signal pathway	Kwak et al[97]
Breast cancer	MCF-7	To induce apoptosis through PI3K/AKT pathway	Ma et al[98]
GBM	U87MG U373MG	To inhibit proliferation and promote apoptosis through PI3K/Akt/mTOR signal pathway	Kessenbrock et al[99]

Abbreviations: GBM, glioblastoma; AP-1, c-jun and c-fos.

colon cancer.[102] In addition, CK was found to diminish the expression of NF-κBp65 nuclear export and MMP2/9, retarding the metastasis of MHCC97-H liver cancer cells (Figure 2).[103] Similar studies have been conducted in astroglioma. CK reduces the expression of MMP-9 in human astroglioma cells by blocking the expression of AP-1 and PMA-mediated activation of p38 MAPK, ERK, and JNK.[104] Recent studies have shown that CK inhibits the proliferation of MG-63 and U2-OS osteosarcoma cells and reduces the expression of MMP-2/9 to prevent the migration and invasion of tumor cells through the PI3K/mTOR/p70S6K1 pathways (Figure 2).[105] The epithelial–mesenchymal transition (EMT) has been shown to promote tumor metastasis. CK and DDP alone or in combination inhibit MCF-7-cell proliferation and the EMT through the PI3K/AKT pathway.[106] Similarly, CK suppresses the self-renewal ability and invasiveness of glioblastoma U87MG and U373MG GBM stem cells through the PI3K/AKT/mTOR signaling pathway (Table 4).[107]

Impact of CK on MDSCs in the Tumor Microenvironment

The tumor microenvironment (TME) is a complicated system in which tumor cells are supported and allowed to flourish by many cells, such as endothelial cells, fibroblasts and myeloid suppressor cells.[108] In the TME, myeloid suppressor cells, including tumor-associated macrophages (TAMs), myeloid-derived suppressor cells (MDSCs) and tumor-associated DCs (TADCs), play important roles in promoting the invasion and metastasis of tumors.[109]

Here, we focus on discussing the roles of CK in tumor MDSCs. MDSCs originate from the bone marrow and are composed of bone marrow progenitor cells and immature bone marrow cells (IMCs). MDSCs, which are important

myeloid suppressor cells, accumulate in the TME and exhibit strong immunosuppressive activity against T-cell antitumor responses. In mice, MDSCs are divided into 2 subgroups according to their epitope-specific antibodies: monocyte $CD11b^+LY6G^-LY6Chi$ phenotype and granulocyte $CD11b^+LY6G^+LY6$ slow phenotype. Mononuclear MDSCs and granulocyte MDSCs use different inhibitory mechanisms.[110] Mononuclear MDSCs produce few reactive oxygen species (ROS) but produce high levels of nitric oxide (NO) and consist of IMCs that have the ability to differentiate into macrophages and DCs. In contrast, granulocyte MDSCs express high levels of ROS and very little NO and are the main population of MDSCs in tumor-bearing mice. In humans, MDSCs in cancer patients are defined by a combination of functional markers (eg, CD14, CD33, CD11b, and CD66b).[110-112] A study in a CT26 colorectal cancer xenograft-bearing mouse model demonstrated that CK had a significant effect against tumor MDSCs. CK promotes the apoptosis of MDSCs by downregulating the expression of Cox-2 and Arg-1 in MDSCs and reduces the secretion of the inflammatory cytokines IL-1β, IL-6, and IL-17, inhibiting the growth and proliferation of tumor cells.[113] These results are consistent with ours (unpublished data).[114]

Synergistic Antitumor Effects of CK and Other Methods/Compounds

The antitumor activity of CK has been widely investigated. However, CK has certain drawbacks that restrict its clinical use since it has low solubility and poor absorption. Therefore, some scientists have combined CK with other clinical antitumor compounds or assays (such as irradiation) to enhance antitumor activity in clinical practice.[115] For example, CK enhances gamma-ray-induced apoptosis of lung cancer cells and inhibits tumor growth in vivo at a dose of 30 mg/kg/day.[116]

Wang et al[117] synthesized *Dendropanax* AgNPs (D-AgNPs) and *Dendropanax* AuNPs (D-AuNPs) and found that 2 CK-added nanoparticles had a strong synergistic anticancer effect on A549 lung cancer. According to reports, A54 peptide was utilized to fabricate CK-loaded micelles (APD-CK) and chitosan nanoparticles loaded with CK (CK-NPs) to more efficiently suppress liver cancer cell proliferation and promote apoptosis.[118-120] In addition, the combination of CK with other compounds has also been studied in the treatment of gastric adenocarcinoma, colon cancer and hippocampal nerve cells. DPPH-scavenging gold nanoparticles (DCY51T-AuNps), CK-bearing glycol chitosan conjugates and novel ester prodrugs, such as butyl and octyl ester (CK-B and CK-O), exert better effects on improving the absorption of CK and contributing to its antitumor effect.[61,121,122] These studies provide an idea for antitumor therapy of CK in clinics.

Conclusions and Prospect

CK has attracted an increasing number of scientific workers and is widely used due to its outstanding pharmacological activity. CK enhances human immunity, has antitumor, anti-inflammatory, and antiaging properties, protects the nervous system and treats cardiovascular diseases, especially in the treatment of cancer. This article introduces the chemical structure, biotransformation, preparation, pharmacokinetics and antitumor activity of CK. This article also summarizes the antitumor mechanisms of CK, which inhibits tumor growth by inducing tumor apoptosis and tumor cell differentiation and blocks tumor invasion and metastasis via multiple signaling pathways and the functional inhibition of MDSCs. In the future, the effects of CK against the roles of other immunosuppressive cells need to be investigated, further displaying the immune roles of CK on the TME. Interestingly, CK exhibits antitumor activity through multiple signaling pathways, indicating that CK may target distinct proteins that need to be studied for clinical treatment. We have sorted and integrated different aspects of CK to clarify relevant treatment ideas and help people better understand CK and its broad application prospects.

Author Contributions

PQ conceived and designed the work. ZL coordinated technical support and funding. JL and PQ wrote the manuscript. YW, ZY, GL, XH, HL and CM acquired, analyzed, and interpreted the data. All authors read and approved the final manuscript.

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