Anticancer effects of genistein, green tea catechins, and cordycepin on oral squamous cell carcinoma

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Abstract

Oral squamous cell carcinoma (OSCC) is the most frequent form of oral cancer and holds the eighth position in the cancer incidence ranking. OSCC patients are treated by classical therapeutic modalities consisting of surgery, radiotherapy, and/or chemotherapy. But OSCC still shows significant mortality rates. Thus, new therapeutic approaches have been investigated and the most promising one is naturally acquired agents with known anti-cancer effects. Genistein is a compound extracted from soy bean. Its anti-cancer effect on breast cancer is well established now and it is investigated whether it has similar effect on OSCC. It inhibited the growth and invasiveness of OSCC cells in vitro, but these effects did not work in living animals in vivo. Catechin is a compound from green tea and its anti-cancer effect on OSCC is known better than other agents. Catechin showed its anti-cancer effect in vitro via induction of apoptosis, cell cycle arrest, inhibition of growth, and down-regulation of invasion/metastasis. These effects were confirmed in vivo with mouse model. Cordycepin is one of major pharmacologically important components in Cordyceps Militaris and may exert its anti-cancer effect as an adenosine receptor agonist. In recent study, it inhibited the proliferation of OSCC cells via A3 adenosine receptor. But because there is very scarce evidence on this effect, more researches are needed on this theme.

Key words
Mouth Neoplasms, Squamous Cell Carcinoma, Genistein, Cordycepin, Catechin

INTRODUCTION

Oral cancer holds the eighth position in the worldwide cancer incidence ranking[1] and oral squamous cell carcinomas (OSCC) encompass about 90% of all oral cancers. In general, OSCC patients are treated by one or a combination of the three classical principal therapeutic modalities consisted of surgery, radiotherapy, and/or chemotherapy. But OSCC still shows quite significant mortality and morbidity rates[2]. Thus, the main concern has been focused to identify new chemotherapeutic agents. Recently, many researchers paid their attention to naturally acquired compounds for new candidates of chemotherapeutic agents. In fact, natural dietary agents have been used in traditional medicines for thousands of years and have been drawing a great deal of attention from both the general public owing to their possible ability to suppress cancers. The first idea for the use of natural compounds in cancer treatment in scientific community was aroused from epidemiologic evidences. Epidemiological studies have indicated that populations that consume food rich in fruits and vegetables have a lower incidence of almost all the kinds of cancers[3,4].

We have experienced and treated a lot of patients with OSCC and directly observed their relatively poorer prognoses than those of others in our hospital (Department of Oral and Maxillofacial Surgery, Seoul National University Dental Hospital) despite our ceaseless efforts. Thus we have endeavored to find out new treatment

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modalities and chemotherapeutic agents. For these aims, we have tried some naturally acquired agents, that is, genistein, catechin, and cordycepin. So the purpose of this study is to report the anticancer effects of these agents from our studies with the review of the literatures.

NATURAL AGENTS AND THEIR POSSIBLE TARGETS

The active components of dietary phytochemicals that most often appear to be protective against cancer are curcumin from turmeric, genistein from soybean, resveratrol from red grapes, diallyl sulfide, S-allyl cysteine, and allicin from garlic, lycopene from tomato, capsaicin from red chilli, diosgenin, 6-gingerol from ginger, ellagic acid from pomegranate, ursolic acid from basil, silymarin from artichoke, anethol from fennel, catechins from tea, eugenol and isoeugenol from cloves, and so on[5].

Despite the accurate targets for these agents and modes of actions have not been understood completely yet, these exert the antitumor activities through regulation of different cell signaling pathways. The possible targets surmised are transcription factors (e.g., NF-kB, AP-1, STAT3), anti-apoptotic proteins (e.g., Akt, Bcl-2, Bcl-XL), proapoptotic proteins (e.g., caspases, PARP), protein kinases (e.g., IKK, EGFR, HER2, JNK, MAPK), cell cycle proteins (e.g., cyclins, cyclin-dependent kinases), cell adhesion molecules, COX-2, and growth factor signaling pathways[5]. The most important factors and mechanisms in carcinogenesis and cancer treatment are listed and illustrated in Fig. 1.

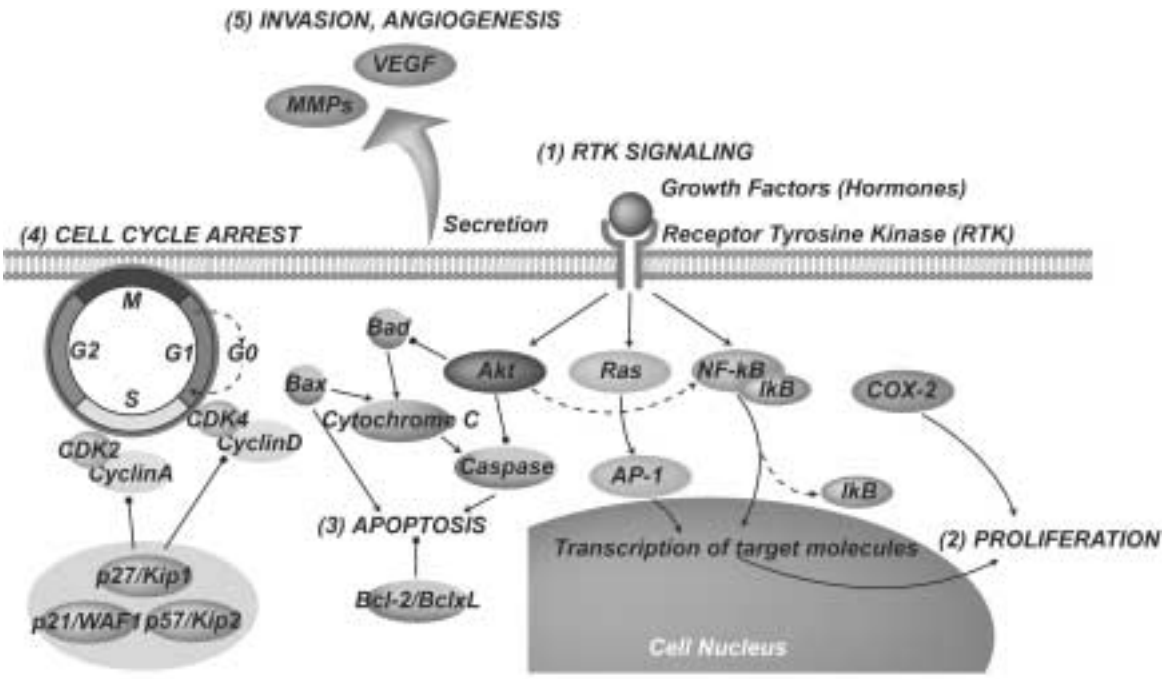

Fig. 1. Molecular mechanisms of OSCC carcinogenesis. (1) Many growth factors are overexpressed in OSCC and can activated receptor typrosine kinases (RTK). RTK can stimulate intracellularly many signal pathways containing proliferation via NF-κB, Ras, and Akt. (2) NF-κB and AP (Activation Protein)-1 are most famous transcription factors, important in cancer cell survival. COX-2 and Akt are similarly important in carcinogenesis via cell proliferation pathway. (3) Apoptosis pathway is initiated by Bad and Bax. It can be inhibited by Akt and Bcl-2/BclxL. They are overexpressed in some cancer cells with inhibiting apoptosis. (4) Arresting cell cycle is another important mechanism of anticancer agents. There are two check points in cell cycle, ie G1/S and S/G2 check points. They are regulated by the adhesion of CDKs and cyclins. p27/Kip1, p21/WAF1, and p57/Kip2 can inhibit the progression of cell cycle and are overexpressed in cancer cells. (5) Cancer cells excrete molecules related to tumor invasion and angiogenesis, ie, MMPs or VEGFs. They can determine tumor invasiveness and potential for metastasis.

GENISTEIN

(1) General Overview

Epidemiologic evidences showed that the incidence of breast cancer in Western countries is higher than that in Eastern countries[6]. The low incidence of breast cancer in Asians has been attributed, in part, to the high intake of soy products. Since then, many studies on soybean and its extracts have shown them to be potent anticancer agents. Soy contains several potential anticancer agents, including protease inhibitors, phytosteroids, saponins, phytates, and isoflavones in a high level[7]. Genistein, one of the most studied isoflavones, is considered as the principle compound responsible for soy's beneficial effects (Fig. 2)[8]. The proposed biologic activities of genistein are; (1) antioxidant and anti-inflammatory effect; (2) phenolic phytoestrogic activity; (3) inhibition of ornithine decarboxylase; (4) inhibition of prostaglandin synthetase; (5) inhibition of tyrosine kinase activity; (6) anti-angiogenic effect[9].

(2) Mechanisms of Anticancer Effects

The first mechanism proposed for the anticancer effect of genistein was inhibition of receptor tyrosine kinase[10,11]. Since then, other possible mechanisms for the cancer inhibiting effects were found. Reports showed that activated Akt by other agents was inhibited by genistein in cancer cells, suggesting that anticancer effects of genistein may be partially mediated by the Akt pathway[12]. NF-κB inducing activity of other agents was completely abrogated by genistein pretreatment in prostate, breast, lung, and pancreatic cancer cells, suggesting that genistein pretreatment inactivates NF-κB[13]. Genistein combined with docetaxel or gemcitabine significantly inhibited Bcl-2, Bcl-XL, and survivin and induced p21WAF1, suggesting that combination treatment regulates the important molecules in the apoptotic pathway[14]. The inhibition of COX-2 pathway was proposed for another possible effect of genistein. Finally, some reports showed that genistein has anti-angiogenic effect and antimetastatic effect[15].

(3) Anticancer Effects in OSCC

Historically, the main concern of anticancer effect of genistein was concentrated on steroid hormone related cancer (breast and prostate cancer) because it is an antiestrogen agent. So, its effect on OSCC is a recent issue. Some studies have tested the anticancer of genistein on OSCC (Table 1). In vitro studies using OSCC cell lines

Table 1. Studies on anticancer effects of genistein for OSCC

Author	Study Model	Results	Comment
Yang, 2006[19]	Hamster cheek	Carcinogenesis (−), vascular density (−)	Genistein has no inhibitory effect on tumerogenesis and vascular density in this model.
Ye, 2004[18]	SCC cell line	Proliferation (↓), Apoptosis (−), COX-2 (↓)	Anticancer activity of genistein is mainly due to inhibition of proliferation via COX-2 down-regulation.
Liu, 2004[60]	Xenografted mouse (ACC)	Metastasis (↓), Apoptosis (↑), VEGF & MMP expression (↓)	Genistein has antimetastatic effect.
Myoung, 2003[15]	SCC cell line Xenografted mouse	VEGF (↓), bFGF (−), MMP-2 (−), in vitro invasion (↓), in vivo tumor growth & meastasis (−)	Genistein has anticancer effect on OSCC. Antiangiogenic effect is insufficient.
Shirataki, 2001[17]	SCC cell line	Cytotoxic activity (↑)	Genistein produced higher cytotoxic activity against OSCC cell lines than normal cells.
Elattar, 2000[16]	SCC cell line	Cell growth & proliferation (↓ ↑)	Effects on cell growth and proliferation were biphasic depending on concentration. The anticancer effect was inferior to cisplatin and curcumin.

showed that genistein had cytotoxic activity, inhibitory effect on cancer cell proliferation partly due to COX-2 inhibition, inhibition of VEGF and MMP expression (proteins related to invasion and metastasis)[15-18]. Two in vivo studies tested the antiangiogenic effect using xenografted nude mouse or hamster[15,19]. These studies showed different results on in vivo angiogenesis. That is, one concluded that genistein reduced neovascularization around tumor mass significantly (Fig. 3)[15], while the other showed opposite result. But both concluded that genistein had no significant effect on OSCC carcinogenesis or tumor growth[19]. So it can be concluded that genistein may have cytotoxic or anti-angiogenic effect on OSCC in vitro, but its effect is too weak to show a significant anticancer effect in vivo. But because the published reports are rare and it is found that genistein has synergistic effects with chemotherapeutic agents[14], further research is recommended to confirm that genistein may be employed as an adjunct treatment modality for OSCC.

GREEN TEA CATECHINS

(1) General Overview

Although there are some debates, epidemiologic studies suggest that the consumption of tea, especially green tea, is linked to a decreased incidence of various cancers[20]. Green tea and black tea are derived from the same plant, *Camellia sinensis*. But it is generally thought that the anticancer effect is stronger in green tea. In the production of green tea, freshly harvested leaves are rapidly heat-treated to inactivate enzymes, producing a product rich in catechins. Among the polyphenols, (-)-epigallocatechin gallate (EGCG) is the most abundant (40-60%), followed by (-)-epicatechin gallate (ECG) (10-20%), (-)-epigallocatechin (EGC) (10-20%), (-)-epicatechin (EC) (4-6%), and (-)-catechin (C) (2-4%)[21]. Thus, EGCG appears to be the most potent compound in tea with respect to inhibiting cell proliferation and inducing apoptosis in cancer cells (Fig. 2).

(2) Mechanisms of Anticancer Effects

EGCG has various anti-cancer effects, including inhibition of oxidative stress, inhibition of carcinogen-induced mutagenesis, induction of apoptosis, and inhibition of angiogenesis. EGCG mainly inhibits various RTKs, thus suppressing many intracellular pathways important in carcinogenesis[22]. Recently, Masuda et al. extended this finding into the more sophisticated mechanisms[23,24]. They found that EGCG inhibits activation of the EGFR, and also HER2, and multiple downstream signaling pathways in human HNSCC and breast cancer cell lines. Thus, they concluded that EGCG inhibits activation of ERK, inhibits basal and TGFα-stimulated c-fos and cyclin D1 promoter activity, and causes a decrease in cellular levels of the cyclin D1 and Bcl-xL proteins. As a result, they could get the assumption that the effect on cyclin D1 may explain why the EGCG-treated cells were arrested in G1 and the effect on Bcl-xL may contribute to the apoptotic effect of EGCG. In vivo[25] and in in vitro[26] studies indicated that EGCG inhibit both the potent transcrip-

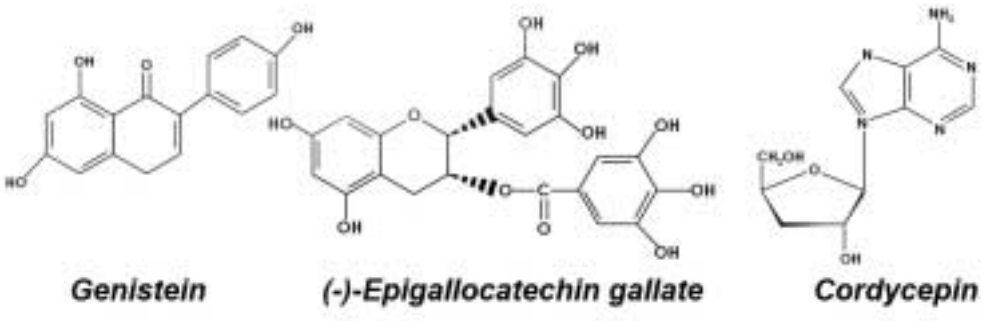

Fig. 2. Molecular structures of genistein, green tea catechins (EGCG), and cordycepin.

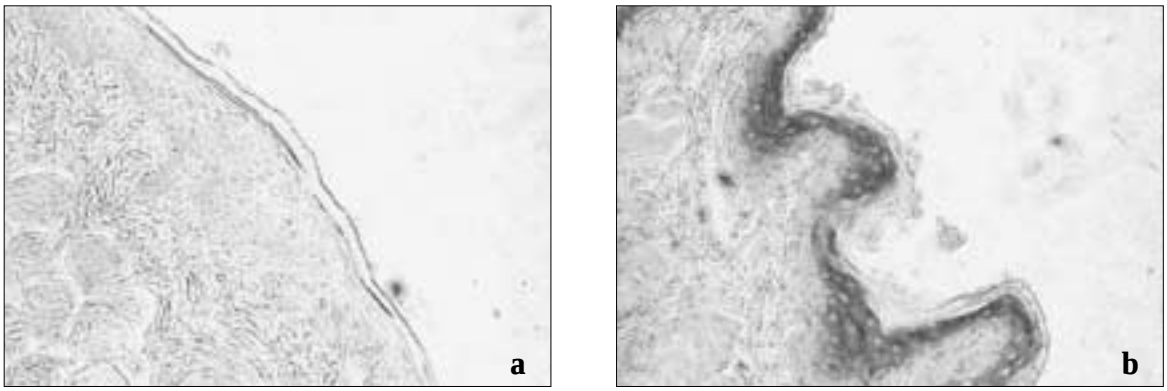

Fig. 3. Immunohistochemical expression of VEGF in hamster buccal pouch oral carcinogenesis model. a. Brown stained cytoplasms of cells were shown markedly in the 8week control group (× 200). b. VEGF expression in the 8 week genistein-treated group (× 200) note the brown stained cytoplasms in were shown rarely or sparsely[29].

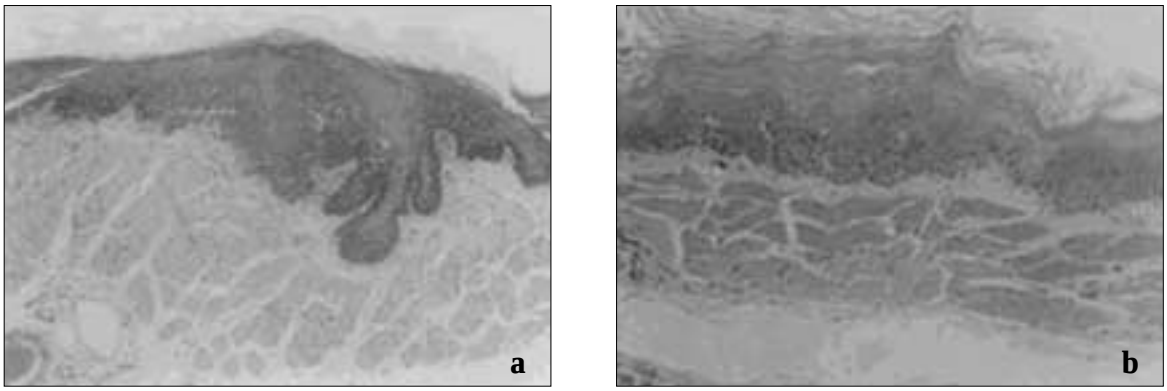

Fig. 4. Carcinoma in situ in hamster buccal pouch oral carcinogenesis model. a. Appearance of carcinoma cells with keratin apposition is observed markedly in the control group (× 100) in 12 weeks. b. Appearance of carcinoma cells is also observed in the experimental (catechins applied) group (× 100) in 12 weeks although it was weaker than control group.

tion factors, AP1 and NF-κB, thus provide evidence that EGCG has significant inhibitory effect on cancer cell proliferation and malignant cell transformation. Also, it was found that EGCG inhibits VEGF production in human HNSCC and breast cancer cells, apparently by inhibiting both the activation of Stat3 and NF-κB in these cells. This effect could contribute to the anti-angiogenic effects of EGCG[27].

(3) Anticancer Effects in OSCC

The anticancer effects of green tea catechins, tea extracts, or EGCG alone on OSCC have been tested in many in vitro and in vivo studies. The results were relatively consistent and promising (Table 2). EGCG or green tea extract showed selective inhibition for cancer cell proliferation, not disturbing normal cells[28-30]. This cancer cell-selective effect may be a superior characteristic to conventional chemotherapeutic agents because the side effects by normal cell damage can be diminished. In vitro studies indicated that the anticancer effect of EGCG was mainly attributable to induction of apoptosis. Elattar et al. found that EGCG showed dose-dependent inhibitory effect on OSCC cell growth and dose-dependent cell morphology changes were observed. The cell morphologic change were representatives of apoptosis[31]. Since then, additional in vitro studies the similar results and they concluded that the apoptotic pathway related to EGCG was intrinsic, that is, mitochondria-related. Caspase 3 is an important mediator in intrinsic apoptosis

Table 2. Studies on anticancer effects of green tea or catechins for OSCC

Author	Study Model	Results	Comment
Ko, 2007[34]	Hamster cheek SCC cell line	Carcinogenesis(↓), APP(↓)	Green tea ingredients (EGCG) might diminish carcinogenesis by down-regulating APP
Chiang, 2006[61]	SCC cell line	MMP-13(↓)	The effects of EGCG in tumor inhibition may act partially through the modulation of MMP-13.
Schwartz, 2005[35]	Human cytology	DNA damage(↓), cell growth(↓), cells in S phase(↓), markers of apoptosis(↑)	Drinking green tea reduced the number of damaged cells in smokers by inducing cell growth arrest and apoptosis.
Hsu, 2005[32]	SCC cell line	p21WAF1(↑)	p21WAF1 is involved in EGCG-induced growth arrest of SCC cells, which may facilitate caspase 3-mediated apoptosis.
Yamamoto, 2004[62]	SCC cell line Salivary gland cell line	Protection of both cell type from irradiation or chemical induced damage	Combination of green tea consumption with cancer therapy requires further evaluation.
Weisburg, 2004[28]	SCC cell line Normal fibroblast cells	More cytotoxicity in SCC cell line, hydrogen peroxide(↑)	EGCG acts as a prooxidant, with the cancerous cells more sensitive to oxidative stress than the normal cells.
Srinivasan, 2004[36]	Hamster cheek	cellular thiols(↑)	Green tea supplementation enhances the cellular thiol status thereby mitigate oral cancer.
Hsu, 2003[30]	SCC cell line	caspase 3 null cells did not undergo apoptosis	Green tea polyphenol-induced apoptosis is a mitochondria-targeted, caspase 3-executed mechanism.
Li, 2002[37]	Hamster cheek	Carcinogenesis(↓), proliferatiom(↓), apoptosis(↑)	Green tea had inhibitory effects against oral carcinogenesis and such inhibition may be related to the suppression of cell proliferation, induction of apoptosis.
Hsu, 2002[63]	SCC cell line Normal keratinocyte	Selective apoptosis of SCC cell(↑), SCC cell growth(↓), invasion(↓)	Chemopreventive effects of green tea may involve a p57 mediated survival pathway in normal epithelial cells, while SCC cells undergo an apoptotic pathway.
Elattar, 2000[31]	SCC cell line	Cell growth(↓), apoptosis(↑)	Dose-dependent inhibitory effect on cell growth and. dose-dependent cell morphology changes were observed.
Li, 1999[39,40]	Hamster cheek	Tumor size(↓), carcinogenesis(↓), AgNOR(↓), PCNA(↓), EGFR(↓)	Tea preparations could effectively inhibit oral carcinogenesis. Protection from DNA damage and suppression of cell proliferation could be important mechanisms of the anticarcinogenic effects.
Khafif, 1998[29, 64]	SCC, premalignant, normal cell line	Cell cycle arrest in G1 phase, effect intensity in normal>premalignant> SCC cell, synergism with curcumin	EGCG and curcumin, were noted to inhibit growth by different mechanisms, a factor which may account for their demonstrable interactive synergistic effect.
Azuine, 1994[41]	Hamster cheek	Carcinogenesis(↓)	Catechin and turmeric are effective as chemopreventive agents and show synergistic effect.
Fan, 1992[33]	SCC cell line	Proliferative survival(↓)	One of the mechanisms of cell growth inhibition by catechin may probably due to inhibition of DNA synthesis.

pathway. Caspase 3 null OSCC cells did not undergo apoptosis with control OSCC cells showed significant apoptosis when exposed to EGCG[30]. Also, Hsu et al. found that p21WAF1 is involved in EGCG-induced growth arrest of SCC cells, which may facilitate caspase 3-mediated apoptosis[32]. Other anticancer effects attributable to EGCG proposed were, (1) cytotoxicity agent as prooxident, (2) inhibition of invasion / metastasis through the modulation of MMP-13, (3) cell cycle arrest, and (4) inhibition of cell growth / proliferation[29,33].

The most in vivo model for testing anticancer effects of tea catechins were hamster cheek pouch carcinogenesis model (Fig. 4)[34-41]. Azuine et al. used this model first and they showed that EGCG had antitumor effect for oral carcinogenesis[41]. Li et al. also found the anticancer effect of EGCG in hamster model and concluded that this effect is via TKR and subsequent proliferation mechanisms[39,40]. They studied the topic further and finally concluded that green tea and its extract had inhibitory effects against oral carcinogenesis and such inhibition may be related to the suppression of cell proliferation, induction of apoptosis[37,38]. Recently, Schwartz et al. examined the effect of green tea on the protection of oral mucosa from smoking using cytology from 3 smokers and 3 non-smokers[35]. They found that drinking green tea reduced the number of damaged cells in smokers by inducing cell growth arrest and apoptosis.

With the results from the published data, it can be concluded that the green tea and EGCG, its major extract has anticancer effect on OSCC. The proposed mechanisms were inhibition of proliferation, induction of apoptosis, selective cytotoxicity, and inhibition of cancer cell invasion / metastasis. So, more epidemiologic studies and animal studies using other than hamster model are needed in future.

CORDYCEPIN

(1) General Overview

Cordyceps is a genus of ascomycete fungi. All *Cordyceps* species are parasitic, mainly on insects and other arthropods. The genus has a worldwide distribution and more than 300 species are currently known. Of them, *Cordyceps militaris* and *Cordyceps sinensis* are most famous for their pharmacologic effects in traditional medicine of Korea, China, and Japan. They are consisted of cordycepin, neucleodide, and various polysaccharides, but most of the pharmacologic effects of Cordyceps are attribuied to cordycepin[42]. Cordycepin is a nucleoside analogue (3' -deoxyadenosine) and was first isolated from *Cordyceps militaris* by Cunningham et al (Fig. 2)[43]. It is a natural antibiotics but other important biologic effects have been known; (1) selective antibacterial effect on harmful species; (2) Maturations of antigen-presenting dendritic cell (improvement of immunoregulatory function); (3) Antifungal *(Candida albicans)* activity; (4) Antiviral effect (Newcastle disease virus); and most importantly (5) Anticancer effect[44-48].

(2) Mechanisms of Anticancer Effect

Compared with the above mentioned agents (genistein and green tea catechins), the mechanism of anticancer effect of cordycepin is known much less. But Yoshikawa et al. found that cordycepin has antitumor effect on melanoma cells in vitro and they speculated that it is mediated by inhibition of nucleic acid methylation and polyadenylation by preventing the addition of the poly (A) tail to the 3' -cleaved mRNA[49]. Placing methyl groups onto specific locations in DNA is achieved through a process called DNA methylation. DNA methylation tells cells which genes need to be expressed or "turned on." Thus, it may be an important step in transcription of cancer-related molecules. Kredich et al. found that cordycepin could inhibit nucleic acid methylation via in vivo study[50]. Most cytoplasmic mRNAs have a poly A tail (3' end) of 50-250 adenosine and it promotes mRNA stability so enhances translation. There are reports that cordycepin can reduce the production of nucleotides and proteins via specific inhibition of RNAs with poly (A) tail51. Recently, Nakamura et al. showed anticancer effect of cordycepin in mouse and cancer cell line. They used melanoma and lung carcinoma cells and cordycepin consistently inhibited carcinogenesis and cancer growth[47-49]. They took notice of the structural similarity between adenosine and cordycepin and tested cordycepin as adenosine A3 receptor agonist. And they found that cordycepin exerted inhibitory effects on the growth of mouse melanoma and lung carcinoma cells, at least in part, by stimulating adenosine A3 receptors on tumor cells[48].

(3) Anticancer Effects in OSCC

There is no published report on the anticancer effect of

cordycepin upon OSCC. So, we are now testing cordycepin as an anticancer agent on OSCC. Hereafter, some obtained data with review of literatures will be presented.

As stated earlier, Nakamura et al. found that the anticancer effect of cordycepin is mediated by adenosine A3 receptor. They showed that cordycepin and selective adenosine A3 receptor agonist notably inhibited the growth of both mouse tumor cell lines. In addition, the tumor growth inhibitory effect of cordycepin was antagonized by selective adenosine A3 receptor antagonist. So they concluded that cordycepin exerts inhibitory effects on the growth of cancer cells by stimulating adenosine A3 receptors on tumor cells. Adenosine is released into the extracellular environment from metabolically active or stressed cells and it has various cellular activities (growth, differentiation, death)[52]. Its various biologic effects may be attributable to the receptor type of recipient cells. There are 4 types of adenosine receptors; A1, A2a, A2b, and A353. It has been shown that adenosine A3 receptor agonist has anticancer effect on various solid and hematogenic cell types. In general, It is thought that adenosine A3 receptor agonist has two antitumor mechanisms; (1) via cell cycle arresting in low concentration; and (2) via induction of apoptosis in high concentration[54-59]. When A3 adenosine receptor agonist was applied to cancer cells in low concentration, cell count decrease and cell cycle arrest (in G0/G1 phase) were consistently observed. It was consistently shown that cell count was decreased and cancer cell s cell cycle arrest At low concentration, it was showed that A3 adenosine receptor agonist consistently inhibited cell cycle. Lee et al. found that cell cycle arrest was due to decrease of cyclin D1 and c-myc[58]. At higher concentration, DNA fragmentation with cancer cell apoptosis was also consistently observed. It was postulated that Bcl-2 might not be involved but decreasing Akt (p-Akt) and activated GSK (p-GSK) were the main mechanism for apoptosis[58]. Furthermore, Madi et al. found that primary and metastatic tumor tissues from colon and breast carcinoma highly expressed A3 adenosine receptor indicating that high receptor expression is a characteristic of solid tumors.

Thus, we decided to start a new experiment that can inspect the anticancer effect of cordycepin to OSCC as an A3 adenosine receptor agonist. Cordycepin decreased OSCC cell lines (HSC-3, KB) significantly at higher concentrations. Similar to adenosine A3 receptor agonists, cordycepin induced apoptosis to OSCC cells. When adenosine receptor antagonists were applied with cordycepin, the anticancer effect was significantly reversed only by A3 adenosine receptor antagonist. Now we are ongoing in vivo study with xenografted mouse.

From these results with literatures, it can be concluded that cordycepin may be an anticancer agent. Although the exact mechanisms are not found yet, it may suppress cancer cells via activating A3 adenosine receptor. As a result, it can induce cell cycle arrest and apoptosis. Anticancer effect of cordycepin to OSCC is not verified yet, so further study is needed. But data from ongoing study showed positive results.

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