



Original article

Anti-tumor and anti-metastatic roles of cordycepin, one bioactive compound of *Cordyceps militaris*Ye Jin^a, Xue Meng^a, Zhidong Qiu^a, Yanping Su^b, Peng Yu^{a,*}, Peng Qu^{c,*}^a Department of Pharmacy, Changchun University of Chinese Medicine, Changchun, Jilin, China^b Department of Histology and Embryology, Taishan Medical College, Taian, Shandong, China^c National Cancer Institute, National Institutes of Health, Frederick, MD, USA

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ABSTRACT

Public interest in complementary and alternative medicine has been increased worldwide, due to its wide applications in cancer prevention and treatment. Cordycepin is one of the most common and crucial types of complementary and alternative medicine. Cordycepin (3'-deoxyadenosine), a derivative of adenosine, was first isolated from medicine drug *Cordyceps militaris*. Cordycepin has been widely used as one compound for antitumor, which has been found to exert antiangiogenic, anti-metastatic, and antiproliferative effects, as well as inducing apoptosis. However, the mechanism of its anti-tumor activity is not well known. This review will clarify anti-tumor mechanisms of Cordycepin, which regulate signaling pathways related with tumor growth and metastasis. Cordycepin inhibit tumor growth via upregulating tumor apoptosis, inducing cell cycle arrest and targeting cancer stem cells (CSCs). Cordycepin regulates tumor microenvironment via suppressing tumor metastasis-related pathways. Thus, Cordycepin may be one of important supplement or substitute medicine drug for cancer treatment.

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1. Introduction

The number of people suffering from cancer in the world will continue to grow steadily, according to the latest report about the trend of global cancer released by the World Health Organization (WHO). It is estimated that the number of newly-increased cancer patients will reach 19 million or even more by 2025. Cancer is the second killer of human death in the world (Anderson and Flanigan, 2015). Cancer is caused by an imbalance between the progression of cell cycle and programmed cell death (Apoptosis) (Lowe et al., 2004). Therefore, the majority of anticancer medical drugs exert their anti-proliferative activity through cell cycle arrest and induction of apoptosis (Bai et al., 2017; Evan and Vousden, 2001). The cytotoxic nucleoside analogues were the first

chemotherapeutic agents used for the therapy of cancer. The some researched cytotoxic nucleoside analogues are isolated from *Cordyceps militaris* (Tian et al., 2015).

Cordycepin was first found from the fermented broth of the medicinal mushroom *Cordyceps militaris*, which is the fungus that grows parasitically on lepidopteron larvae and insect pupae (Cunningham et al., 1950). The genus *Cordyceps* is well-known in traditional Chinese medicine and exhibits a variety of clinical health effects including immunomodulatory, anticancer, antioxidant, anti-inflammatory and anti-microbial activities (Tuli et al., 2014; Yue et al., 2013). Recently, more and more studies have demonstrated Cordycepin, as one bioactive compound of *Cordyceps militaris*, have abroad roles of anti-tumor (Hsu et al., 2017; Hwang et al., 2017a; Wang et al., 2017a, 2017b; Zeng et al., 2017). However, little is known about the active ingredients as well as the mechanism underlying these roles. The review summarizes the anti-tumor mechanism of Cordycepin.

2. Main active components of *Cordyceps militaris*

2.1. Chemical features of cordycepin

The structure of Cordycepin is very much similar with cellular nucleoside, adenosine and acts like a nucleoside analogue (Li et al., 2015). The structure of cordycepin comprises a purine

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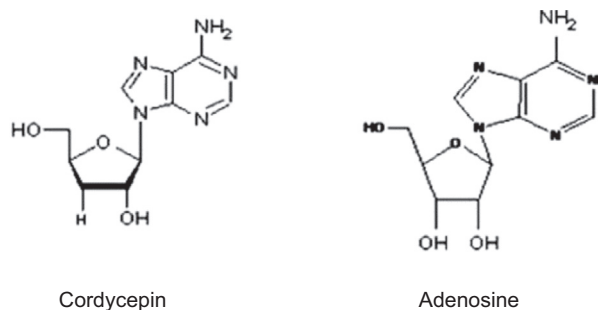


Fig. 1. The difference of chemical structures between Cordycepin and adenosine.

(adenine) nucleoside molecule attached to one ribose sugar moiety. The chemical synthesis of cordycepin is completed mainly through the replacement of the OH group at the 3'-position in the ribofuranosyl moiety with H, generating a deoxy analogue of adenosine (Fig. 1) (Tuli et al., 2013).

2.2. Function of cordycepin

Cordycepin has many biological and pharmacological actions in immunological, hepatic, renal, cardiovascular systems as well as an anti-cancer agent. Those functions are related to its structure (Tuli et al., 2014). During the process of RNA synthesis (transcription), some enzymes are not able to distinguish between an adenosine and Cordycepin which leads to incorporation of Cordycepin to induce premature termination of transcription (Chen et al., 2008; Holbein et al., 2009). In addition, The IC₅₀ (the concentration at which 50% inhibition of cell growth was achieved) of cordycepin in human gallbladder cancer cell lines NOZ and GBC-SD cells at 48 h was approximately 19.2 µg/mL and 398.1 µg/mL, respectively (Wang et al., 2014). For human lung cancer cell lines, IC₅₀ of cordycepin was 60 µg/ml (Hwang et al., 2017b). The function of Cordycepin treatment in tumor is dependent on tumor types and concentration (Cho and Kang, 2018; Fong et al., 2018).

The high dosage of Cordycepin can block mTOR (mammalian target of rapamycin) signaling pathway (Wong et al., 2010). The name mTOR has been derived from the drug rapamycin, because the drug inhibits mTOR activity. Some of mTOR inhibitors have been tested as anti-cancer drugs, since they suppress cancer through mTOR signaling pathway (Bjornsti and Houghton, 2004; Sabatini, 2006). The study found that Cordycepin can activate AMPK which blocks the activity of mTORC1/mTORC2 complex. The inactivated complex cannot activate AKT 1 kinase fully, which suppress mTOR signal transduction inhibiting translation, and further cell proliferation and growth (Wong et al., 2010). Those functions of Cordycepin elucidate their structure–function relationship, and further explain the anti-tumor roles of the compound. Cordycepin had been shown to regulate AMPK/mTORC1 signaling pathway to down-regulate multiple drug resistant to HIF-1α in GBC-SD gallbladder cancer cells (Wu et al., 2014). The anti-tumor roles and mechanisms of Cordycepin are described detail below.

3. Cordycepin inhibits tumor growth

Many complementary and alternative medicine are developed in applications of cancer prevention and therapy due to chemotherapy resistance and metastasis (Wong et al., 2017). Traditional Chinese medicine is one treatment for complementary and alternative therapy (Wong et al., 2015; Ye et al., 2018). Cordycepin is an active compound and has been used in cancer treatment in past studies.

3.1. Induction of tumor apoptosis

Cordycepin can induce cancer cell apoptosis in caspase-dependent pathways. Apoptosis of Human liver cancer (HepG2) cells were induced by the activation of caspase, interaction between Fas and FADD, and modulation of the protein levels of Bid and tBid (Shao et al., 2016). Cordycepin also decreased human bladder carcinoma cells (T24 cells) survival, which was regulated by the activation of A₃ adenosine receptor and the subsequent inactivation of Akt pathways, leading to the increases in cleaved Caspase-3 and apoptosis (Cao et al., 2017). In addition, Cordycepin reduced cell viability, inhibited cell proliferation, and enhanced lactate dehydrogenase release and reactive oxygen species (ROS) accumulation of human breast cancer cell (MCF-7 and MDA-MB-231 cells) through up-regulating the activation of pro-apoptotic proteins, such as caspase-3, 8, 9 and suppressing the expression of the anti-apoptotic protein, B-cell lymphoma 2 (Bcl-2) (Wang et al., 2016).

Cordycepin induced the apoptosis of human renal cancer cells by triggering the MKK7-JNK signaling pathway through inhibition of anti-apoptotic protein cellular caspase 8 (FLICE)-like inhibitory protein (c-FLIP) expression and the consequent activation of the Bax/caspase-3/PARP-mediated pathway (Hwang et al., 2017a). In human Non-Small Cell Lung Cancer (NSCLC), Cordycepin-induced apoptosis was also associated with down-regulation of protein c-FLIP, which inhibited the activity of caspase-8. Cordycepin inhibited cell growth by inducing apoptosis and autophagy. The cordycepin-stimulated autophagy were mediated by suppressing mTOR signaling pathway in lung cancer cells. In addition, suppression of autophagy could also elevate the protein level of c-FLIP which indicated cordycepin-triggered autophagy promoted the degradation of c-FLIP. Therefore, Cordycepin induced apoptosis through autophagy-mediated downregulation of c-FLIP in human NSCLC cells. In addition, cordycepin also inhibits the ERK/Slug signaling pathway through the activation of GSK3β which, upregulates Bax to result in apoptosis of lung cancer cells (Hwang et al., 2017b). Taken together, cordycepin may serve as one promising therapeutic compound, which acts on multiple molecular targets in lung cancer treatment (Yu et al., 2017).

Cordycepin also induces cancer cell apoptosis in caspase-independent pathways. Cordycepin decreased cell mitosis and EGFR signaling in one murine oral tumor mouse model. In accordance, the treatment distinctly reduced the levels of ki-67 and EGFR signaling molecules to induce cancer cell apoptosis (Hsu et al., 2017). For human lung adenocarcinoma, Cordycepin induced cancer cell apoptosis by caveolin-1-upregulated JNK/Foxo3a signaling pathway, and significantly decreased tumor volume in nude mice (Joo et al., 2017). Cordycepin also increase ROS levels and induce apoptosis in MA-10 mouse Leydig tumor cells but not cause cell death of primary mouse Leydig cells on moderate concentration through down-regulating the p38 MAPK and PI3K/AKT signaling pathways (Pan et al., 2015) (Table 1).

3.2. Cell cycle arrest

Cordycepin incorporates mitochondrial-mediated apoptosis in gastric cancer cell (SGC 7901 cells) with regulating mitochondrial extrinsic pathways by inhibition of A₃ adenosine receptor (A₃AR) and drive activation of death receptor DR3, which promote the activation of PI3K/Akt protein expression as well as collapse of mitochondrial membrane potential (MMP). Phosphorylation of PI3K/Akt and DNA damage by cordycepin induced the production of ROS and regulated SGC 7901 cell cycle cessation at S phase (Nasser et al., 2017). Cordycepin also increased radio-sensitivity in human uterine cervical cancer cells, such as ME180 and HeLa cells, and induced the increased number of those tumor cells in

Table 1
Inhibition roles of Cordycepin on tumor growth.

Tumor types (cell lines)	Mechanism of anti-tumor	Molecular targets	References
Human gastric cancer (SGC-7901)	Induction of apoptosis	PI3K/AKT↓	(Nasser et al., 2017)
Human non-small cell lung cancer cells	Induction of apoptosis/autophagy	c-FLIPL↓	(Yu et al., 2017)
Human liver cancer (HepG2)	Induction of apoptosis	Caspase-8, Fas, FADD↓	(Shao et al., 2016)
Human renal cancer cells (TK-10)	Induction of apoptosis	MKK7, JNK↓	(Hwang et al., 2017a)
Human uterine cervical cancer cell (ME180 and HeLa cell)	Induction of G2/M arrest	Cyclin A2↓	(Seong da et al., 2016)
Human Leukemia cells (NB-4 and U937 cells)	Induction of apoptosis/cell cycle arrest	Cyclin A2, cyclin E, and CDK2↓ p53↑	(Liao et al., 2015)
Human Bladder cancer (T-24)	Induction of apoptosis	A3 adenosine receptors↑	(Cao et al., 2017)
Human bladder cancer cell (5637 and T-24 cells)	G2/M cell cycle arrest	Phosphorylation of c-Jun	(Lee et al., 2009)
Human breast cancer cells (MCF-7 and MDA-MB-231)	Induction of apoptosis	Caspase-3,8,9↓, BCL-2↓	(Wang et al., 2016)
Murine oral cancer (4NAOC-1)	Induction of apoptosis, decrease cell mitosis and EGFR signaling	Caspase-3↑ EGFR, IL-17RA↓	(Hsu et al., 2017)
Murine Leydig tumor cell (MA-10)	Induction of apoptosis	p38 MAPK↑	(Hsu et al., 2017)

the G2/M phase, which is related to the induction of p53-mediated apoptosis and modulation of the expression of cell cycle checkpoint molecules (Seong da et al., 2016). The increased expression of p53 by Cordycepin treatment promoted the release of cytochrome c from mitochondria to the cytosol, to further activate caspase-9 and promote the apoptosis of leukemia cells (NB-4 and U937 cells) (Liao et al., 2015). In addition, cordycepin inhibits the expression of cyclin A2, cyclin E, and CDK2, which leads to the accumulation of those leukemia cells in S-phase through the activation of Chk2-Cdc25A pathway (Liao et al., 2015).

Recently, Lee et al. found that Cordycepin causes p21WAF1-mediated G2/M cell cycle arrest by upregulating c-Jun N-terminal kinase activation in human bladder cancer cells. They blocked JNK function using JNK-specific inhibitor and small interfering RNA of JNK to rescue cordycepin-dependent p21WAF1 expression and decrease of cell cycle proteins (Lee et al., 2009). These results suggest that cordycepin could be an effective treatment for bladder cancer.

3.3. Resistance of cancer stem cell

Cancer stem cells (CSCs) are a limitless cell source for the initiation and maintenance of cancer cells. CSCs can generate cancer cells through the stem cell processes of self-renewal and differentiation into multiple tumor cell types (Battle and Clevers, 2017; Visvader, 2011). Thus, The intrinsic resistance of CSCs to conventional therapy is regarded as a potential therapeutic target of cancer (Reya et al., 2001). Activation of the Wnt/ β -catenin pathway is required for the survival and development of CSCs, such as leukemia stem cells (LSCs) (Nusse and Clevers, 2017). Therefore, targeting β -catenin is considered a therapeutic strategy for the treatment of leukemia. cordycepin can block the effect of β -catenin in leukemia cells by regulating GSK-3 β to inhibit the growth of LSCs (Ko et al., 2013). CSCs escape chemotherapy and lead to chemoresistance due to the induction of TGF- β . Cordycepin efficiently inhibited cell viability, the percentage of ovarian cancer stem cells, and the levels of matrix metalloproteinases (MMPs) in TGF-beta-induced SKOV-3 ovarian cancer cells. Thus, cordycepin acted as a complementary agent for ovarian cancer therapy that against chemoresistance (Wang et al., 2017c).

4. Regulation of cordycepin on tumor microenvironment

4.1. Inhibition of migration and invasion of tumor cell

Cordycepin inhibited the migration and invasion of human oral squamous cell carcinoma (OSCC) cell through upregulating E-cadherin and downregulating N-cadherin protein expression, implying the inhibition of Cordycepin on epithelial-mesenchymal transition (EMT) (Yu et al., 2017). In addition, Cordycepin have

been shown to suppress the migration of the human glioblastoma cell lines U87MG and LN229 in transwell and wound healing assays *in vitro*, since Cordycepin decreased protein expression of integrin α 1, focal adhesion kinase (FAK), p-FAK, paxillin and p-paxillin. The lysosomal inhibitor NH4Cl can block the ability of cordycepin to inhibit focal adhesion protein expression and glioma cell migration. The protein phosphatase inhibitors Calyculin A and okadaic acid also blocked the cordycepin-mediated reduction in p-Akt, p-FAK and further suppress tumor cell line migration. Hematoxylin and eosin staining of mouse xenografts demonstrated that brain tumor sizes were reduced after Cordycepin treatment *in vivo*. Thus, cordycepin inhibited the migration and invasion of human glioblastoma cells by affecting lysosomal degradation and protein phosphatase activation (Hueng et al., 2017). These data are in consistent with the finding that cordycepin inhibits the migration and invasion of LNCaP cells (human prostate carcinoma cells). Cordycepin significantly downregulated the activity of tight junctions and suppressed the expression and activity of MMP-2 and MMP-9, which regulated tumor metastasis. These anti-metastatic roles were mediated by inactivation of the phosphoinositide 3-kinase (PI3K)/Akt pathway in LNCaP cells (Jeong et al., 2012).

4.2. Blockage of tumor metastasis

Anti-metastatic activities of cordycepin were demonstrated in mouse models where cordycepin inhibited B16 mouse melanoma liver metastasis *in vivo* (Kubo et al., 2010). The potential roles of cordycepin in melanoma cell metastasis and the underlying molecular mechanisms were addressed further. Zhang et al. found that cordycepin could suppress melanoma invasion via MMPs and metastasis via actomyosin machinery through LXR/RXR activation-dependent upregulation of miR-33b. Cordycepin also suppressed the expressions of HMGA2, Twist1 and ZEB1 through miR-33b. The up-regulation of miR-33b by cordycepin inhibited melanoma metastasis *in vivo* (Zhang et al., 2015). In another *in vivo* mouse melanoma studies, Yoshikawa et al. demonstrated that adenosine-5'-diphosphate (ADP)-induced platelet aggregations accelerated lung metastasis on mouse melanoma. Cordycepin treatment reduced the number of metastatic lung nodules through blocking ADP-induced platelet aggregations (Yoshikawa et al., 2009). Those data indicated that cordycepin inhibit melanoma metastasis through different anti-metastatic mechanisms.

Cordycepin can suppress mitochondrial fusion-induced EMT in ovarian carcinoma cells through inhibiting estrogen-related receptor (ERR)- α , which is a co-transcription factor for gene expressions associated with mitochondrial fusion. Thus, cordycepin suppresses metastasis and migration of ovarian carcinoma cells via inhibiting mitochondrial activity (Wang et al., 2017b). In addition, Cordycepin also blocked EMT through regulating TGF- β (Wang et al., 2017c).

Table 2
Inhibition roles of Cordycepin on tumor migration and metastasis.

Tumor types (cell lines)	Anti-metastatic mechanism	Molecular targets	References
Human oral squamous cell carcinoma	Inhibition of epithelial-mesenchymal transition (EMT)	E-cadherin, N-cadherin↓	(Su et al., 2017)
Human hepatocellular carcinoma (HepG2)	Anti-metastatic and anti-angiogenic		(Lu et al., 2014)
Human glioblastoma cell (U87MG and LN229)	Inhibition of tumor cell motility	Lysosomal degradation, protein phosphatase activation↑	(Hueng et al., 2017)
Human prostate carcinoma (LNCaP)	Inhibition of migration and invasion of tumor	AKT↓	(Jeong et al., 2012)
Human ovarian carcinoma (OVCAR-3)	EMT	Mitochondrial activity↓, Estrogen-related receptor α↓	(Wang et al., 2017b)
Human melanoma	Inhibition of invasion and metastasis	miR-33b↓, HMG2, Twist1, ZEB1↑	(Zhang et al., 2015)

Solid tumors grow fast if they induce the development of new blood vessels, a process known as tumor angiogenesis, which is the main process of tumor growth and metastasis (Carmeliet and Jain, 2000, 2011). Angiogenesis was assessed using a tube formation assay (Yang et al., 2008). Anti-angiogenic drugs have been broadly used for clinical studies to suppress the growth and metastasis of tumors (Ferrara and Adamis, 2016). Cordycepin inhibited tube formation (total length of tubular structure) of human umbilical vein endothelial cell line (HUVEC) and the migration of those cells. Cordycepin also efficiently suppressed the invasion and migration of hepatocellular carcinoma cell line (HepG2) (Lu et al., 2014) (Table 2).

4.3. Disruption between cancer cells and mesenchymal stromal cells (MSCs)

Mesenchymal stromal cells (MSCs), as the main cell type of tumor microenvironment, promote tumor growth and metastasis, and stromal cells support tumor progression and resistance to chemotherapy (Ridge et al., 2017; Wan et al., 2013). Thus, targeting the niche-based microenvironment may be one new approach for cancer therapy (Singh et al., 2018). Cordycepin reduces the numbers of CD34+CD38-cells in leukemia such as U937 and K562, and induces Dkk1 expression to disrupt the association of both leukemia and MSCs. Cordycepin also suppressed cell attachment of leukemia with MSCs and downregulates N-cadherin in leukemia and VCAM-1 in MSCs (Liang et al., 2017). Therefore, the results indicated the potential of cordycepin as a multitarget drug in anti-metastatic therapy.

5. Conclusion and future prospect

Numerous studies have shown that Cordycepin as one valuable compound, can inhibit many malignant tumors through different pathways. Since Cordycepin-induced death of cancer cells are performed via multi-target pathways, it is difficult to some extent for cancer cells to develop drug resistance. Moreover, another advantage of Cordycepin is that the small side effect is shown when inhibiting the growth and progression of cancer cells. Therefore, Cordycepin may be considered as one wonderful drug candidate for cancer treatment.

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