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ORIGINAL ARTICLE

The apoptotic effect of cordycepin on human OEC-M1 oral cancer cell line

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Abstract Cordycepin (3′-deoxyadenosine), a pure compound of *Cordyceps sinensis*, has been illustrated with anti-tumor effects. In the present study, the apoptotic effect of cordycepin on OEC-M1, a human oral squamous cancer cell line, was investigated by morphological observations, cell viability assay, annexin V-FITC analysis and flow cytometry methods. Results demonstrated that the number of rounded-up cell increased as treatment duration of cordycepin (100 μM) increased from 3 to 48 h, and the plasma membrane blebbing could be observed after 12 h treatment. In cell viability assay, cell surviving rate significantly decreased as the dosage and duration of cordycepin treatment increased ($P < 0.05$). Moreover, phosphatidylserine flipping on cell membrane could be detected with 3, 6 and 12 h cordycepin treatment, which indicated an early apoptotic phenomenon. Furthermore, cell cycle studies illustrated that the percentage of G1 phase cell declined as the dosages of cordycepin increased (10 μM to 5 mM), while the percentages of G2M and subG1 phase cell increased ($P < 0.05$) in 12, 24 and 48 h cordycepin treatment. These results further confirmed the apoptotic event. In conclusion, cordycepin significantly induced cell apoptotsis in OEC-M1 human oral squamous cancer cells.

Keywords Cordycepin · Apoptosis · OEC-M1 · Oral cancer · Cell cycle · Cell viability

Introduction

Cordycepin (3′-deoxyadenosine) is a pure substance extracted from *Cordyceps sinensis*, and was considered as an active component (Cunningham et al. 1950). It has been illustrated that cordycepin has effects altering cytokines secretion (Zhou et al. 2002), improving lung function, increasing energy levels and sex drive (Cui 1999), and having antitumor effect on mouse melanoma and lung carcinoma cells (Nakamura et al. 2006). It has also been demonstrated that cordycepin could inhibit polyadenylate polymerase (PAP; Thomadaki et al. 2005) or inactivate mRNA polyadenylation (Lallas et al. 2004) to induce tumor cell apoptosis, which is characterized by the cellular rounding-up, cytoplasmic contraction, plasma membrane blebbing, chromatin condensation, DNA fragmentation and many biochemical characteristics, including the activation of death receptor pathway, mitochondrial pathway and/or caspases cascades, etc. (Gupta 2001; Daniel et al. 2006; Feldmann 2006). Apoptosis in early stage is also accompanied by a loss of membrane phospholipid asymmetry, resulting in the exposure of phosphatidylserine at cell surface (Fadok et al. 2000).

Betel quid-related oral cavity cancer is a unique type of squamous cell carcinoma of the head and neck

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(HNSCC), which is endemic in certain areas, including Taiwan, with areca nut chewing habit (Gupta and Warnakulasuriya 2002). It has been suggested that the cause of HNSCC might be induced by mechanical irritation and/or carcinogenic components from betel quid (Jin et al. 1996, 2001). Noteworthy, the incidence rate of intra-oral cancers, mostly betel quid-related, is still increasing in Taiwan (Ho et al. 2002).

Oral cavity cancer not only takes out lives but also injures the appearance of patient face. The main treatment of early-staged HNSCC is surgery and/or radiotherapy. For late-staged HNSCC patients, chemotherapy is often used in various combinations with surgery and radiotherapy to improve the poorer survival rate (Forastiere et al. 2003; Psyrri et al. 2004). Thus, the exploration of cordycepin-induced cell death will be valuable to design more effective chemotherapy agents against HNSCC. In this study, human HNSCC cell line, OEC-M1, was used to investigate whether cordycepin has apoptotic effect.

Materials and methods

Chemicals

Cordycepin, penicillin-streptomycin, 3-3-[4,5-dimethylthiazol]-2,5-dyphenylatetrazolium bromide (methylthiazoletetrazolium or MTT), dimethyltetrazolium bromide (DMSO), RNase A, propidium iodine (PI), Annexin V-FITC (apoptosis detection kit) were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). Fetal bovine serum and RPMI 1640 medium were purchased from Gibco Co. (Grand Island, NY, USA). Sodium hydroxide was purchased from Merck Co. (Dermstadt, Germany). HEPES was purchased from Mallinckrodt Baker, Inc. (Philipsburg, NJ, USA). Sodium bicarbonate, sodium carbonate and sodium chloride were purchased from Riedel deHaen (Seelze, Germany).

Cell culcuture

OEC-M1 is a cell line derived from gingival epidermal carcinoma, an indigenous oral cancer cell line in Taiwan, which is a generous gift from Prof. Kuo-Wei Chang (National Yang-Ming University, Taipei, Taiwan). OEC-M1 cells were maintained in RPMI 1640 medium supplemented with 24 mM $NaHCO_3$, 25 mM HEPES, 10,000 U penicillin, 10,000 μg streptomycin and 10% heat-inactivated fetal bovine serum, pH 7.4, incubated in a humidified atmosphere containing 95% air, 5% CO_2 at 37°C (Shneyvays et al. 2000).

Morphology study

OEC-M1 cell line was seeded in 6 cm dish with 2 ml serum medium, which contained 6×10^5 cells. After cells reached 70–80% confluence, cells were treated with serum free medium contained 100 μM cordycepin for various time points (3, 6, 12, 24, 48 h). Cell morphology was then observed and recorded under light microscopy (Olympus, CK 40). Apoptosis is characterized by the loss of cellular contact with the matrix and the appearance of plasma membrane blebbing (Gupta 2001).

MTT assay

Methylthiazoletetrazolium assay was used to determine cell viability (Denizot and Lang 1986). OEC-M1 cells were seeded in 96-well plate containing 1×10^4 cells with 100 μl serum medium. After cells reached 70–80% confluence, cells were treated with serum free medium containing 10 μM, 100 μM, 1 mM, 2 mM and 5 mM cordycepin for 3, 6, 12, 24 and 48 h. MTT were added at different time points with the final concentration of 0.5 mg/ml and then incubated for 4 h at 37°C. The medium was removed and DMSO (50 μl) was added into each well to dissolve the crystals by shaking the plate weakly for 20 min in dark. The O.D. values in each treatment were then determined at 590 nm by an ELISA reader (Opsy MR, Dynex, USA).

Annexin V-FITC assay

To detect whether cordycepin could induce the flipping of phosphatidylserine at the cell surface, treated cells were double stained with annexin V-FITC and PI and under the examination of FL1 (530 nm for annexin V-FITC) and FL2 (585 nm for PI; Nicoletti et al. 1991; Koopman et al. 1994). OEC-M1 cell line was seeded in 6 cm dish with 2 ml serum medium, which contained 6×10^5 cells. After cells reached 70–80% confluence, cells were treated with free medium containing 100 μM cordycepin for 3, 6 and 12 h. The distribution of cells was analyzed with CellQuest™ software (Becton-Dickinson, Mountain View, CA, USA).

Flow cytometric analysis

In order to investigate whether cordycepin could induce cell apoptosis, DNA fragmentation and the redistribution of cell cycle, flow cytometric analysis was used with propidium iodine stain (Nicoletti et al. 1991; Wang et al. 2005). OEC-M1 cell line was seeded in 6 cm dish with 2 ml serum medium, which contained

6×10^5 cells. After cells reached 70–80% confluence, cells were treated with free medium containing 10 μM, 100 μM, 1 mM, 2 mM or 5 mM cordycepin for 12 h; and 500 nM, 1 μM, 10 μM, 100 μM, 1 mM, 2 mM or 5 mM cordycepin for 24 and 48 h, respectively. Cordycepin-treated cells were harvested with trypsin, washed with PBS, and fixed in 75% ethanol for at least 2 h at −20°C. After fixation, cells were washed in cold PBS and then collected by centrifugation and stained with PI solution containing 40 μg/ml in PBS, 100 μg/ml RNase. The stained cells were analyzed using a fluorescence-activated cell sorter (FACScan, Becton-Dickinson, Moutain View, CA, USA) at $\lambda = 488$ nm using CellQuest™ software (Becton-Dickinson, Moutain View, CA, USA). The DNA content distribution of normal growing cells is characterized by two peaks—G1/G0 and G2/M phase. G1/G0 phase possesses normal functioning and resting state of cell cycle with most diploid DNA content, while the DNA content in G2/M phase are more than diploid. Cells in sub G1 phase have least DNA content in cell cycle distribution, called hypodiploid. The hypoploid DNA contents represent the DNA fragmentation (Wang et al. 2005).

Statistics

All data are expressed as mean ± SEM of at least three independent experiments. Statistically significant differences between control and treatments were determined by one-way analysis of variance (ANOVA) and then the least significance difference (LSD). Statistical significance was set at $P < 0.05$.

Results

Effects of cordycepin on morphological changes in OEC-M1 cell line

OEC-M1 cells were treated without or with cordycepin (100 μM) for 3, 6, 12, 24 and 48 h, respectively, and morphological changes were examined under light microscopy.

Cells without cordycepin treatment showed polygonal shape with healthy appearances, blurred outline and firm attachment, which is normal cell growth phenomenon (Fig. 1a, c, e, g, i). After 3 h cordycepin treatment, cells appeared rounded-up phenomenon but still adherent to the ground matrix (Fig. 1b). Cordycepin treatment for 6 h caused more cells rounded-up plus some floated cells (Fig. 1d). After 12 h treatment, the adherent cells appeared black-dotted outline, and some cells expressed plasma membrane blebbings with more floated cells (Fig. 1f). After 24 and 48 h, more adherent cells appeared membrane blebbings, and there were more floating cells (Fig. 1h, j). These phenomena suggest that cordycepin might induce apoptotic cell death in OEC-M1 human head and neck cancer cell line.

Effects of cordycepin on OEC-M1 cell viability

The morphological changes suggested the involvement of cell death induced by cordycepin in OEC-M1. Thus, MTT assay was used to investigate the effect of cordycepin on cell viability in OEC-M1 cells. OEC-M1 cells were treated with 10 μM, 100 μM, 1 mM, 2 mM or 5 mM cordycepin for 6 and 12 h, respectively; and 500 nM, 1 μM, 10 μM, 100 μM, 1 mM, 2 mM or 5 mM cordycepin for 3, 24 and 48 h, respectively. Results showed that cordycepin had significantly negative effect on cell viability in OEC-M1 cells with time- and dose-dependent manners (Fig. 2).

Figure 2a illustrates that cell viability significantly decreased 18–40% by 3 h cordycepin treatment at 100 μM or higher dosages ($P < 0.05$) in OEC-M1 cells. Interestingly, cordycepin at 1 mM or higher dosages after 6 and 12 h treatments had significantly negative effects on cell viability from 40 to 60% on OEC-M1 cells (Fig. 2b, c; $P < 0.05$). After 24 h cordycepin treatment, 1 μM or higher dosages treatments had significantly negative effects from 18 to 62% on OEC-M1 cell viability (Fig. 2d; $P < 0.05$). Cordycepin at 500 nM with 48 h treatment would have significantly negative effects from 10 to 65% on OEC-M1 cell viability (Fig. 2e; $P < 0.05$).

Effect of cordycepin on phosphatidyserine membrane translocation in OEC-M1 cell line

To determine whether the inhibition of cell growth by cordycepin resulted from the induction of apoptosis, the early translocation of phosphatidyserine (PS) from the internal to external leaflet, a hallmark of early apoptosis, was demonstrated by incubating OEC-M1 cells with 100 μM cordycepin for 3, 6 and 12 h, respectively (Fig. 3).

The annexin V-FITC staining cell distribution regarding the normal cell (82.33%) and apoptotic trend cell (17.67%) without cordycepin treatment, or the normal cell (79.52%) and apoptotic trend cell (20.48%) with cordycepin treatment for 3 h are demonstrated in Fig. 3a and b, respectively. Figure 3c illustrates the distribution of normal cell (78.02%) and apoptotic trend cell (21.98%) without cordycepin treatment for 6 h, and Fig. 3d demonstrates the distri-

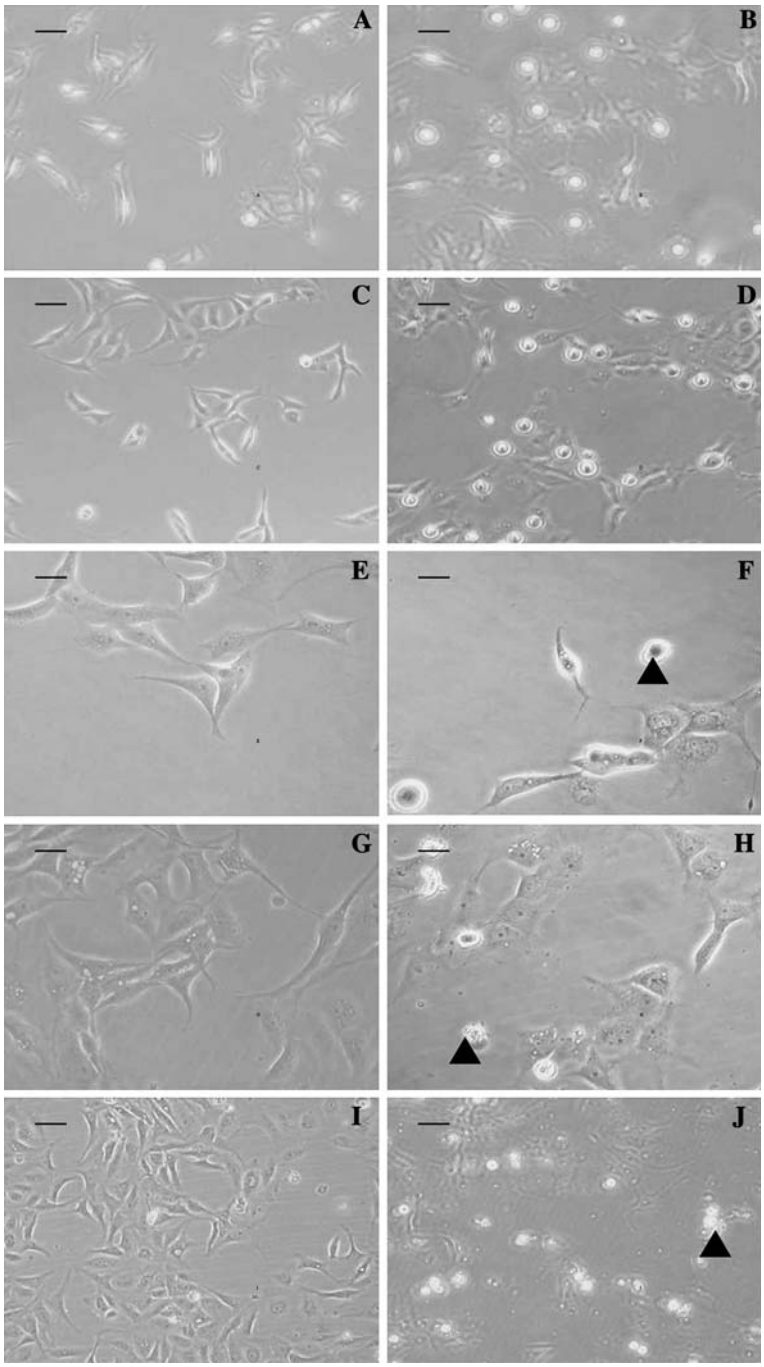

Fig. 1 Effects of cordycepin on morphological changes in OEC-M1 cells. Cells were cultured in 24-well plates until 70–80% confluence and then treated without or with 100 μM cordycepin for 3 h (**a**, **b**), 6 h (**c**, **d**), 12 h (**e**, **f**), 24 h (**g**, **h**) and 48 h (**i**, **j**). Morphological changes of cells were examined under light microscopy (*bar* 0.1 mm, *arrow heads* membrane-blebbed cells)

bution of normal cell (56.93%) and apoptotic trend cell (43.07%) with cordycepin treatment for 6 h. Figure 3e illustrates the distribution of normal cell (64.83%) and apoptotic trend cell (35.18%) without cordycepin treatment for 12 h, and Fig. 3f demonstrates the distribution of normal cell (31.97%) and apoptotic trend cell (68.03%) with cordycepin treatment for 12 h.

Figure 3g demonstrates the statistical analysis regarding the change in normal cell or apoptotic trend cell between control and cordycepin-treated OEC-M1 cells after 3, 6 or 12 h treatment, respectively. There was no significant change of cell number in percentage between control and cordycepin-treated groups in normal cell or in apoptotic trend cell (Fig. 3g). However, in 6 h treatment, the percentage of apoptotic trend cell significantly increased between control and cordycepin-treated groups ($18.65 \pm 3.32\%$ at control vs. $36.31 \pm 1.79\%$ at treatment, $P < 0.05$), while the percentage in normal cells significantly decreased between control and cordycepin-treated groups ($77.70 \pm 2.21\%$ at control vs. $56.36 \pm 2.52\%$ at treatment, $P < 0.05$; Fig. 3g). In 12 h treatment, the percentage of apoptotic

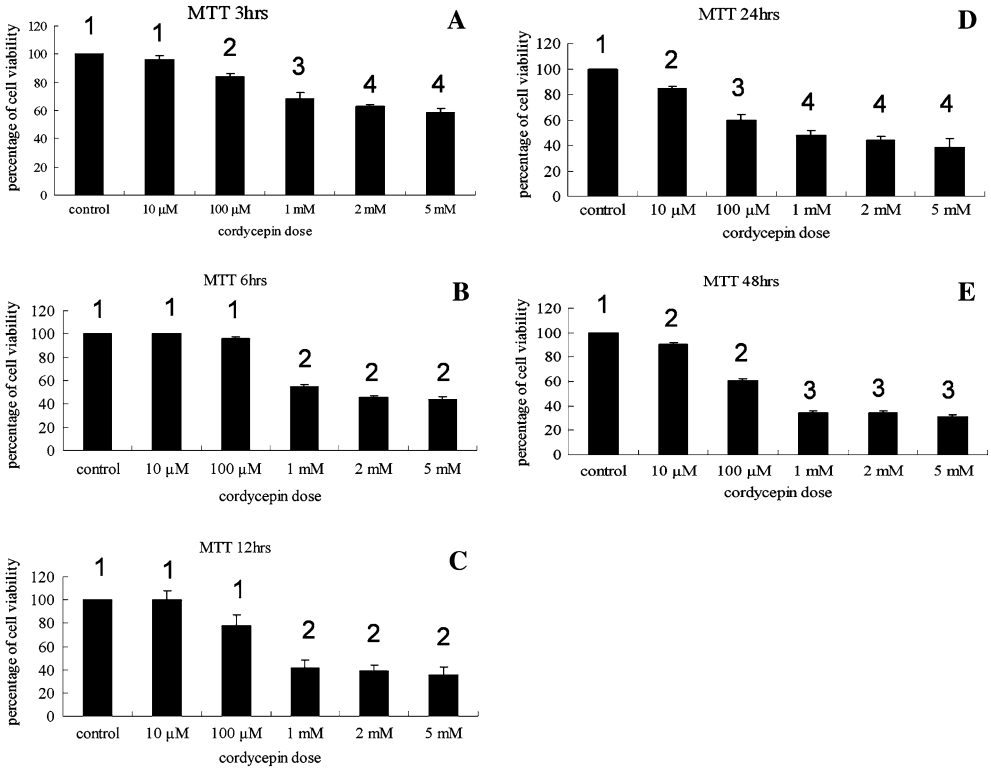

Fig. 2 Effects of cordycepin on cell viability of OEC-M1 cells. Cells were cultured in 96-well plates until 70–80% confluence and various concentrations of cordycepin (10 μM to 5 mM) were then added to cells for 3 h (**a**), 6 h (**b**), 12 h (**c**), 24 h (**d**) and 48 h (**e**). Methylthiazoletetrazolium (MTT) assay was used to detect the viability of the cells. The O.D. value of control was regarded as 100%. Each data point in the figure represents the mean ± SEM in percentage of cell viability from more than three experiments. The *different number* above each *bar* indicates the significant difference among each treatment ($P < 0.05$)

trend cell significantly increased (28.37 ± 5.06% at control vs. 60.54 ± 10.84% at treatment, $P < 0.05$), while the percentage of normal cell significantly decreased between control and cordycepin-treated groups (64.68 ± 1.79% at control vs. 31.58 ± 8.59% at treatment, $P < 0.05$; Fig. 3g).

The apoptotic effects of cordycepin in OEC-M1 cell

Previous results illustrated that cordycepin would cause cell death with early apoptosis phenomenon in OEC-M1 cell. Flow cytometry analysis was used to further confirm whether DNA fragmentation occurred and if there was any change in cell cycle progression. The subG1 phase in cell cycle distribution represents the occurrence of DNA fragmentation. The distribution of PI-stained OEC-M1 cells after 12 h cordycepin (control: 10 μM, 100 μM, 1 mM, 2 mM or 5 mM) treatment (Fig. 4a), 24 h cordycepin (control: 500 nM, 1 μM, 10 μM, 100 μM, 1 mM or 5 mM) treatment (Fig. 4b) and 48 h cordycepin (500 nM, 1 μM, 10 μM, 100 μM, 1 mM, 2 mM or 5 mM) treatment (Fig. 4c) were illustrated, respectively.

In 12 h treatment, G1 phase slightly decreased as the cordycepin dosages increased, while subG1 phase slightly increased in 100 μM and 1 mM cordycepin treatments as G2/M phase remained constantly (Fig. 4a). After 24 h treatment, G1 phase decreased as the cordycepin dosages increased, while subG1 phase slightly increased in 100 μM cordycepin treatment as G2/M phase gradually increased (Fig. 4b). After 48 h treatment, G1 phase decreased as the cordycepin dosages increased with a dramatically drop at 100 μM cordycepin, while the subG1 phase increased with significant elevation by 100 μM cordycepin as G2/M phase increased from 100 μM to 5 mM cordycepin treatments (Fig. 4c).

The tendency and analysis of cell cycle under cordycepin influence in OEC-M1 cell

Statistical analysis from three independent experiments of Fig. 4 with different concentrations of cordycepin after 12, 24 or 36 h treatments regarding the change of subG1, G1 and G2/M phases of cell cycle in percentages was analyzed and illustrated in Fig. 5.

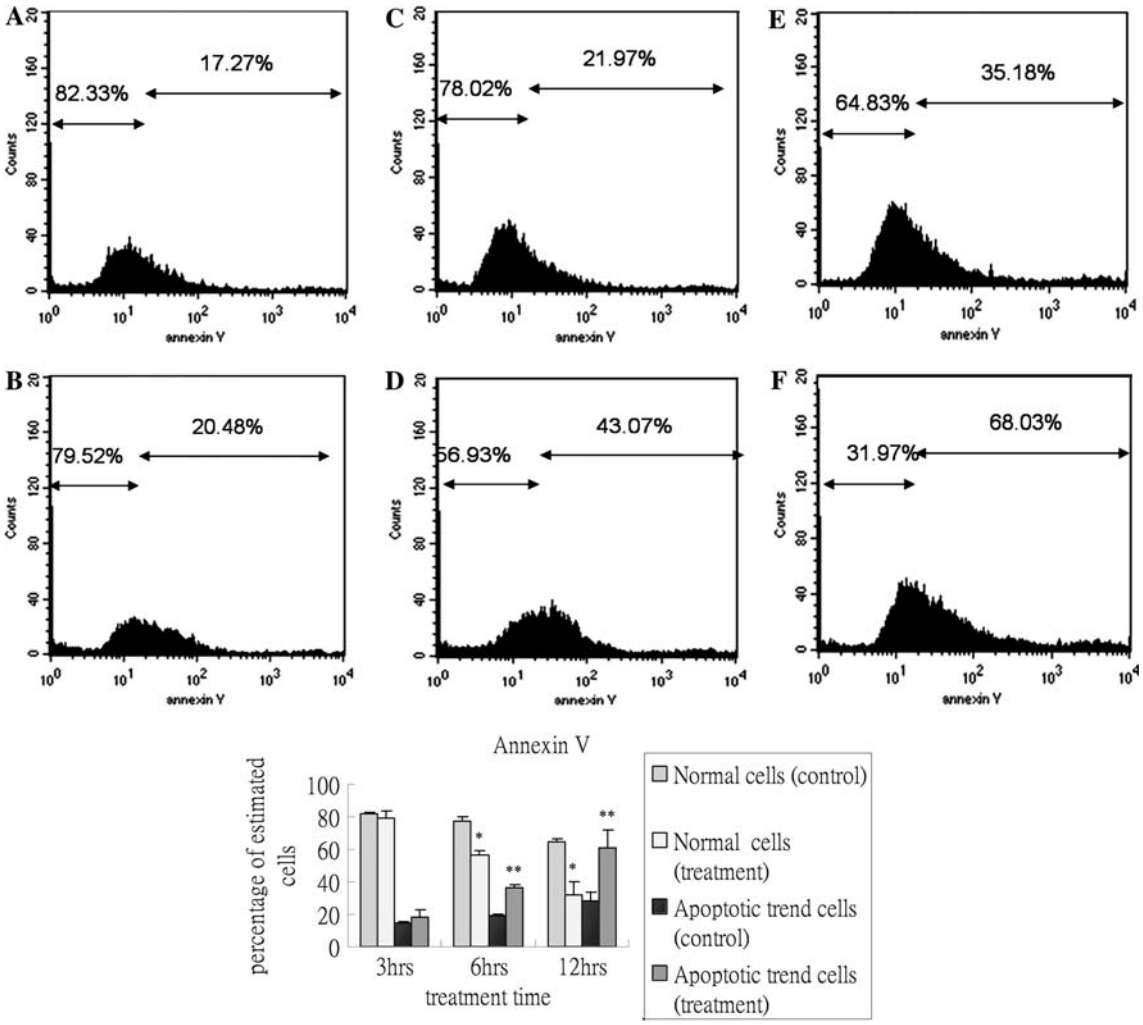

Fig. 3 Effects of cordycepin on membrane phosphatidyserine translocation in OEC-M1 cells. Cells were treated without (control) or with 100 μM cordycepin (treatment) for 3 h (**a** control, **b** treatment), 6 h (**c** control, **d** treatment) and 12 h (**e** control, **f** treatment) and apoptotic cell distribution by annexin V-FITC staining was quantitated by flow cytometry. Cell distribution graph (**a**–**f**) represents the observations from one single experiment, which was repeated at least three times. Each data point in (**g**) represents the mean ± SEM of cell distribution in percentage between normal cell and apoptotic trend cell of three experiments. * above the *bars* in **g** indicates the significant difference of normal cell in percentage between control and cordycepin-treated groups ($P < 0.05$) in each time point, and ** above the *bars* in **g** indicates the significant difference of apoptotic trend cell in percentage between control and cordycepin-treated groups ($P < 0.05$) in each time point

In 12 h treatment, subG1 phase significantly increased at 100 μM cordycepin (2.13 ± 0.62% at control vs. 4.43 ± 0.45% at 100 μM cordycepin treatment, $P < 0.05$; Fig. 5a), while G1 phase significantly decreased at 100 μM cordycepin (49.86 ± 1.30% at control vs. 35.77 ± 4.56% at 100 μM cordycepin treatment, $P < 0.05$; Fig. 5b). There was no difference of G2/M phase among control and treatments ($P > 0.05$; Fig. 5c).

Although there was no significant increase of subG1 phase in 24 h treatment, a gradual tendency did occur with the maximal increase at 100 μM cordycepin (Fig. 5d). G1 phase significantly decreased by cordycepin at 10 μM, 100 μM, 1 mM, 2 mM and 5 mM ($P < 0.05$; Fig. 5e), while the G2/M phase significantly increased at 10 μM, 100 μM, 1 mM, 2 mM and 5 mM ($P < 0.05$; Fig. 5f). The increasing G2/M phase with the decrease of cell viability in 24 h treatment suggests the possibility of cordycepin-induced G2/M phase arrest effect on OEC-M1 cells.

In 48 h treatment, subG1 phase significantly increased at 100 μM cordycepin (35.77 ± 4.56% at 100 μM cordycepin treatment vs. 49.86 ± 1.30% at control, $P < 0.05$; Fig. 5g), while G1 phase significantly decreased at 100 μM, 1 mM, 2 mM and 5 mM cordycepin ($P < 0.05$; Fig. 5h) and G2/M phase significantly increased at 100 μM, 2 mM and 5 mM cordycepin ($P < 0.05$; Fig. 5i).

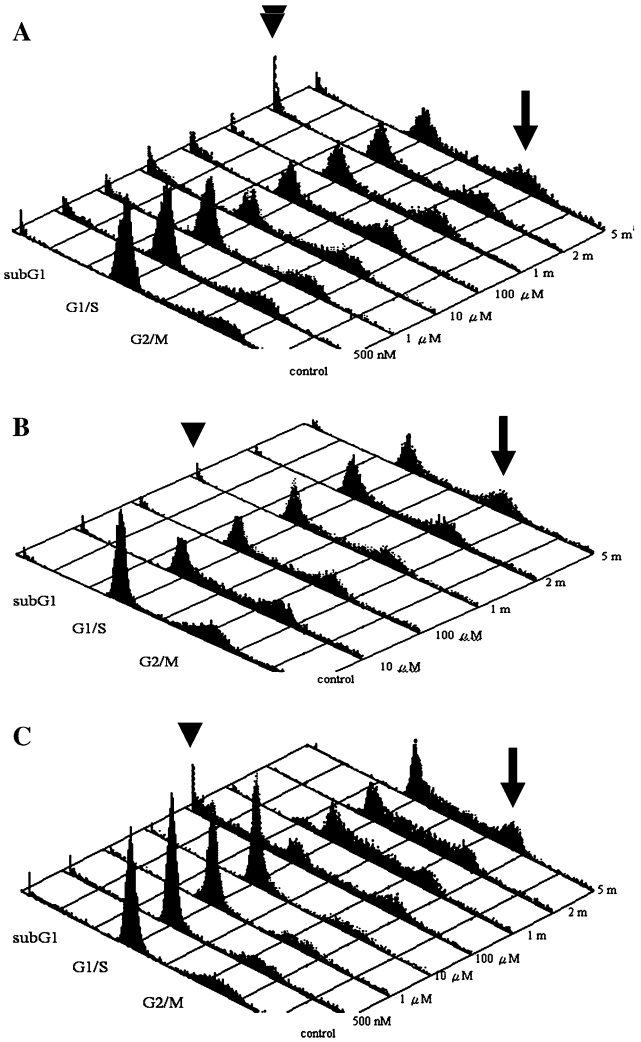

Fig. 4 The apoptotic effects of cordycepin in OEC-M1 cells. Cells were cultured in 6 cm dish until 70–80% confluence and treated with various concentrations of cordycepin (500 nM to 5 mM) for 12 h (**a**), 24 h (**b**) and 48 h (**c**). Cells were then harvested and fixed by 70% alcohol and stained with propidium iodide for analyzing the cell cycle progression by flow cytometry, CellQuest™. The 3D graph of quantification in percentage of cell number in subG1, G1 and G2/M phases under different dosages of cordycepin treatments was illustrated. The graph of flow cytometry analysis represents the observations from a single experiment, which was repeated at least three times. *Arrow heads* indicate the increase of subG1 phase and arrows represent the increase of G2/M phase

Comparison among subG1, G1 and G2/M cell cycle phases in percentages at 100 μM cordycepin among 12, 24 and 48 h treatments (Fig. 5j), significant positive trend of subG1 phase correlated with a significant negative trend of G1 phase plus a significant increase of G2/M phase could be observed ($P < 0.05$; Fig. 5j). This phenomenon might be postulated a cell cycle shift from G1 to subG1 phase as the duration of cordycepin treatment increased.

Discussion

In the present study, cordycepin significantly induced cell apoptotsis in OEC-M1 human oral squamous cancer cells. Cordycepin would induce OEC-M1 cells rounded-up with blebbed membrane. Cordycepin also induced OEC-M1 cells death in a time- and dose-dependent manner. In cytometry analysis, the flipping of phosphatidylserine on cell surface, an early apoptotic phenomenon, significantly increased in 6 and 12 h cordycepin treatments, which correlated with the increase of subG1 phase, occurrence of DNA fragmentation, in 12, 24 and 48 h with 100 μM cordycepin treatment. Meanwhile, there was a significant decrease of G1 phase cells with significant increase of G2/M phase cells. It has been demonstrated that cordycepin could arrest the dividing cells at the onset of mitosis (Zieve et al. 1987; Zieve and Roemer 1988). Thus, our observation is not unprecedented. Indeed, quantitative analysis of flow cytometric results (Fig. 5) showed that cordycepin-induced G2/M arrest seemed to precede the onset of apoptosis (G2/M significant increased at 12 h and subG1 significant increased at 24 h).

It has been demonstrated that hyperphosphorylation of PAP from p34 cyclin B kinase during mitosis leads to the inactivation of PAP, which will contribute to the reduction of mRNA and protein synthesis during the M phase of the cell cycle (Colgan et al. 1998). Besides, PAP activity levels are significantly elevated at the G1/S phase of the cell cycle, along with the increased rate of mRNA polyadenylation and accumulation in the cytoplasm (Martincic et al. 1998). In the present study, treatment of OEC-M1 cells with cordycepin lead to G2/M arrest. It is possible that cordycepin inhibited polyadenylation, which was necessary at the G1/S phase of the cell cycle in order for all the required mRNAs to be polyadenylated, transferred to the cytoplasm and translated, in order for mitosis to take place. As a result, mitosis will not finish and cells will arrest at the onset of apoptosis, since not all necessary proteins are translated.

Eventually, a portion of OEC-M1 cells did survive after cordycepin treatment at different doses or times. These phenomena highly imply that head and neck tumor cells have the resistance to cordycepin. It has been proposed that several mechanisms might be able to induce drug resistance (Orr et al. 2003). It is also possible that our cell population was not synchronized, and no drug treatment kills every single cell at the same time in a mixed cell population at different phases of the cell cycle. Thus, synchronized cell population will be used to further examine the apoptotic effect of cordycepin on OEC-M1 oral cancer cell line, which will

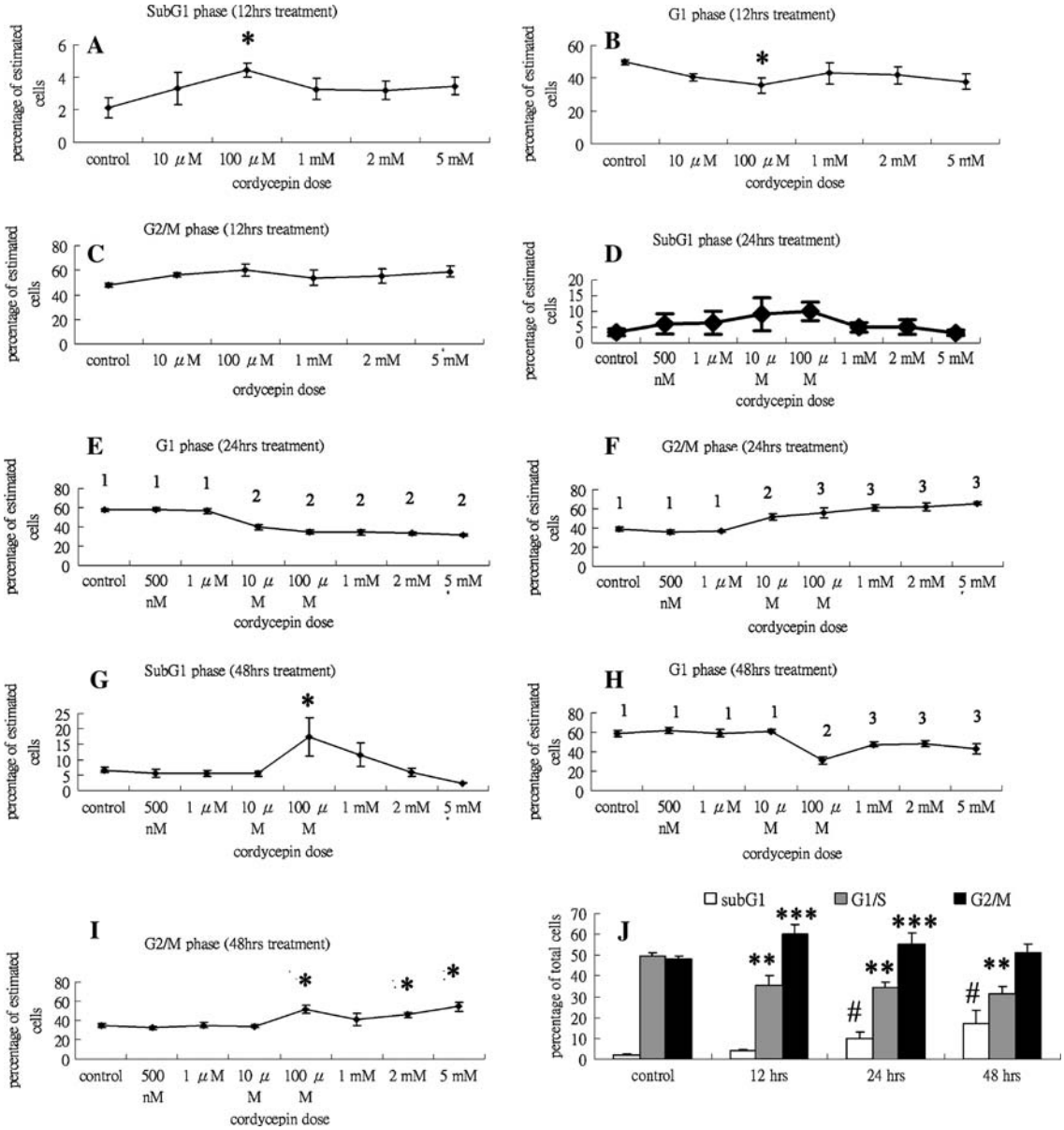

Fig. 5 The analysis of cell cycle progression under cordycepin influence in OEC-M1 cells. Statistical analysis from three independent experiments of Fig. 4 with different concentrations of cordycepin after 12, 24 or 48 h treatments regarding the change of subG1, G1 and G2/M phases of cell cycle in percentages was analyzed and illustrated (**a** 12 h of subG1, **b** 12 h of G1, **c** 12 h of G2/M, **d** 24 h of subG1, **e** 24 h of G1, **f** 24 h of G2/M, **g** 48 h of subG1, **h** 48 h of G1, **i** 48 h of G2/M). Comparison of subG1, G1 and G2/M cell cycle phases in percentage at 100 μM cordycepin among 12, 24 and 48 h treatments is illustrated in **j**. * above the *bars* in **a**, **b**, **g** and **i** indicates the significant difference between control and treatment ($P < 0.05$). *Different number* above each bar in **e**, **f**, **g** and **h** indicates the significant difference among each other ($P < 0.05$). #, ** and *** above the *bar*s in **j** indicate the significant difference between control, 12, 24 and 48 h treatments of subG1, G1 and G2/M, respectively ($P < 0.05$)

be very helpful to reveal the detail apoptotic pathway regarding cordycepin-induced cell death and resistance on head and neck cancers.

It has been shown that cordycepin is an analogue of adenosine. The possibility of adenosine receptor involved in apoptotic effect on some types of cancer cell is also proposed recently (Yoshikawa et al. 2004). Thus, it is possible that cordycepin might associate with adenosine receptor to activate apoptosis in OEC-M1 cells. Future works regarding the investigation on the mechanism of cordycepin-induced apoptosis and the relations to adenosine receptor in OEC-M1 cells will be valuable.

In conclusion, cordycepin could induce G2/M cell arrest and the increase of subG1 cell number, followed by significant apoptotic cell death in OEC-M1 oral can-

cer cell line. This promising observation about the apoptotic effect of cordycepin on oral cancer cell may be considered as a good approach in search and development of new anti-oral cancer drug.

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References

Cui XM (1999) Asia-Pacific Biotech news 3:333–337

Cunningham KG, Hutchinson SA, Manson W, Spring FS (1950) Cordycepin, a metabolic product isolated from cultures of Cordyceps militaris. Nature 166:949

Colgan DF, Murthy KGK, Zhao W, Prives C, Manley JL (1998) Inhibition of poly (A) polymerase requires p34cdc2/cyclin B phosphorylation of multiple consensus and non-consensus sites. EMBO 17:1053–1062

Daniel PT, Koert U, Schuppan J (2006) Apoptolidin: induction of apoptosis by a natural product. Angew Chem Int Ed Engl 45:872–893

Denizot F, Lang R (1986) Rapid colorimetric assay for cell growth and survival. Modifications to the tetrazolium dye procedure giving improved sensitivity and reliability. J Immunol Methods 89:271–277

Fadok VA, Bratton DL, Rose DM, Pearson A, Ezekewitz RA, Henson PM (2000) A receptor for phosphatidylserine-specific clearance of apoptotic cells. Nature 405:85–90

Feldmann G (2006) Liver apoptosis. Gastroenterol Clin Biol 30:533–45

Forastiere AA, Goepfert H, Maor M, Pajak TF, Weber R, Morrison W, Glisson B, Trotti A, Ridge JA, Chao C, Peters G, Lee DJ, Leaf A, Ensley J, Cooper J (2003) Concurrent chemotherapy and radiotherapy for organ preservation in advanced laryngeal cancer. N Eng J Med 349:2091–2098

Gupta S (2001) Molecular steps of death receptor and mitochondrial pathways of apoptosis. Life Sci 69:2957–2964

Gupta PC, Warnakulasuriya S (2002) Global epidemiology of areca nut usage. Addict Biol 7:77–83

Ho PS, Ko YC, Yang YH, Shieh TY, Tsai CC (2002) The incidence of oropharyngeal cancer in Taiwan: an endemic betel quid chewing area. J Oral Pathol Med 31:213–219

Kopman G, Reutelingsperger CP, Kuijten GAM, Keehnen RMJ, Pals ST, van Oers MHJ (1994) Annexin V for flow cytometric detection of phosphatidylserine expression on B cells undergoing apoptosis. Blood 84:1415–1420

Jeng JH, Chang MC, Hahn LJ (2001) Role of areca nut in betel quid-associated chemical carcinogenesis: current awareness and future perspectives. Oral Oncol 37:477–492

Jin YT, Tsai ST, Wong TY, Chen FF, Chen RM (1996) Studies on promoting activity of Taiwan betel quid ingredients in hamster buccal pouch carcinogenesis. Eur J Cancer B Oral Oncol 32B:343–346

Lallas GC, Courtis N, Havredaki M (2004) K562 cell sensitization to 5-fluorouracil- or interferon-alpha-induced apoptosis via cordycepin (3′-deoxyadenosine): fine control of cell apoptosis via poly(A) polymerase upregulation. Int J Biol Markers 19:58–66

Martincic K, Campbell R, Edwalds-Gilbert G, Souan L, Lotze MT, Milcarek C (1998) Increase in the 64-kDa subunit of the polyadenylation/cleavage stimulatory factor during the G0 to S phase transition. Proc Natl Acad Sci USA 95:11095–11100

Nakamura K, Yoshikawa N, Yamaguchi Y, Kagota S, Shinozuka K, Kunitomo M (2006) Antitumor effect of cordycepin (3′-deoxyadenosine) on mouse melanoma and lung carcinoma cells involves adenosine A3 receptor stimulation. Anticancer Res 26(1A):43–47

Nicoletti I, Migliorati G, Pagliacci MC, Grignani F, Riccardi C (1991) A rapid and simple method for measuring thymocyte apoptosis by propidium iodide staining and flowcytometry. J Immunol Methods 139:271–279

Orr GA, Verdier-Pinard P, McDaid H, Horwitz SB (2003) Mechanisms of taxol resistance related to microtubules. Oncogene 22:7280–7295

Psyrri A, Kwong M, DiStasio S, Lekakis L, Kassar M, Sasaki C, Wilson LD, Haffty BG, Son YH, Ross DA, Weinberger PM, Chung GG, Zelterman D, Burtness BA, Cooper DL (2004) Cisplatin, fluorouracil, and leucovorin induction chemotherapy followed by concurrent cisplatin chemoradiotherapy for organ preservation and cure in patients with advanced head and neck cancer: long-term follow-up. J Clin Oncol 22:3061–3069

Shneyvays V, Jacobson KA, Li AH, Nawrath H, Zinman T, Isaac A, Shainberg A (2000) Induction of apoptosis in rat cardiocytes by A3 adenosine receptor activation and its suppression by isoproterenol. Exp Cell Res 257:111–126

Thomadaki H, Tsiapalis CM, Scorilas A (2005) Polyadenylate polymerase modulations in human epithelioid cervix and breast cancer cell lines, treated with etoposide or cordycepin, follow cell cycle rather than apoptosis induction. Biol Chem 386:471–480

Wang BJ, Won SJ, Yu ZR, Su CL (2005) Free radical scavenging and apoptotic effects of cordycepin sinensis ractionated by supercritical carbon dioxide. Food Chem Toxicol 43:543–552

Yoshikawa N, Nakamura K, Yamaguchi Y, Kagota S, Shinozuka K, Kunitomo M (2004) Antitumour activity of cordycepin in mice. Clin Exp Pharmacol Physiol Suppl 2:S51–53

Zhou X, Meyer CU, Schmidtke P, Zepp F (2002) Effect of cordycepin on interleukin-10 production of human peripheral blood mononuclear cells. Eur J Pharmacol 453:309–317

Zieve GW, Roemer EJ (1988) Cordycepin rapidly collapses the intermediate filament networks into juxtanuclear caps in fibroblasts and epidermal cells. Exp Cell Res 177:19–26

Zieve GW, Feeney RJ, Roemer EJ (1987) Cordycepin disrupts the microtubule networks and arrests Nil 8 hamster fibroblasts at the onset of mitosis. Cell Motil Cytoskelet 7:337–446