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Original Article

# Cordycepin attenuates migration and invasion of HSC-4 oral squamous carcinoma cells through autophagy-dependent FAK/Akt and MMP2/MMP9 suppression

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## KEYWORDS

Cordycepin;  
Autophagy;  
Migration;  
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MMP2;  
LC3

**Abstract** *Background/purpose:* Cordycepin has been proposed anti-cancer effects, however, it is unclear whether and how cordycepin affects oral squamous carcinoma cell (OSCC) migration and invasion. This study aimed to investigate the effect of cordycepin on migration and invasion of OSCC (HSC-4 cells), and its underlying mechanism.

*Materials and methods:* Cell viability was measured with MTT assay. Migrative and invasive abilities were determined by scratch wound healing, agarose spot and transwell invasion assays, respectively. Monodansylcadaverine (MDC) staining, immunofluorescence staining of LC3 and RT-PCR evaluated the gene expression of LC3 and p62 were applied to investigate autophagy. MMP2 and MMP9 gene expression and activity were examined by RT-PCR and gelatin zymography. Expression of caspase 3, cleaved caspase 3, FAK, p-FAK, Akt and p-Akt was determined by Western blot.

*Results:* Cordycepin significantly inhibited HSC-4 cell migration and invasion in a concentration-dependent manner. Cordycepin treatment caused an induction of autophagy, as evidenced by increased MDC fluorescence intensity and MDC positive cells, and upregulated expression level of LC3 gene. In addition, inhibition of autophagy by chloroquine (CQ) significantly abolished

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cordycepin-inhibited HSC-4 cell migration and invasion, demonstrating that cordycepin-inhibited migration and invasion was mediated by autophagy. Mechanistic studies showed that cordycepin significantly suppressed FAK and Akt phosphorylation, and MMP2 and MMP9 activities. Conversely, CQ pre-incubation significantly restored its expression and activity in cordycepin-treated cells. **Conclusion:** Cordycepin induces autophagy to suppress FAK and Akt phosphorylation, and MMP2 and MMP9 activity, which responsible for the attenuation of HSC-4 cell migration and invasion. © 2022 Association for Dental Sciences of the Republic of China. Publishing services by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

## Introduction

Around 30% of all head and neck cancers occur in the oral cavity, and most oral cancers are composed of squamous carcinoma.<sup>1,2</sup> Like other epithelial cancers, metastasis is the most important prognostic factor of oral squamous carcinoma cells (OSCC).<sup>2</sup> Therefore, therapeutically targeting OSCC metastasis is still of potential therapeutic importance. Cordycepin (3'-deoxyadenosine), a major bioactive component of *Cordyceps sinensis*, has been shown to inhibit the growth of many cancers through triggering apoptosis, arresting cell cycle and targeting cancer stem cells.<sup>3–5</sup> Additionally, cordycepin inhibits migration and invasion of tumor cells. However, it is unclear whether and how cordycepin affects OSCC migration and invasion.

Recent studies have shown that cordycepin promotes autophagy in OSCC and others.<sup>5–8</sup> Autophagy occurs in stressful conditions, such as nutrient deprivation and are the common phenomena after cancer treatment.<sup>9,10</sup> Autophagy plays a paradoxical role in the promotion and suppression of tumours.<sup>9–11</sup> With regard to cancer metastasis, only a few studies exist implicating a role of autophagy in migration and invasion of cancers. Matrix metalloproteases (MMPs) have been required for cancer migration and invasion.<sup>12</sup> In OSCC, MMP2 and MMP9 are highly elevated and are associated with increased tumour progression.<sup>13–15</sup> Focal adhesion kinase (FAK) is a non-receptor tyrosine kinase that contributes to the regulation of cell adhesion, metastasis and survival.<sup>16</sup> FAK has been suggested to regulate the expression and activity of MMP2 and MMP9.<sup>17,18</sup> Akt/protein kinase B, a core member of phosphatidylinositol-3-kinase (PI3K), is one of downstream effector of FAK that plays important role in proliferation, invasion and migration.<sup>19,20</sup> Accumulated evidences have shown that PI3K/Akt signaling promotes metastasis through upregulation of MMP2 and MMP9.<sup>21,22</sup> However, the involvement of these signaling in cordycepin-inhibited cancer cell migration is scarce.

Here, we found an inhibitory effect of cordycepin on OSCC migration and invasion. High dose of cordycepin inhibits migration and invasion via stress-induced cell death whereas low dose attenuates the migration and invasion through autophagy-dependent FAK/Akt/MMP2 and MMP9 pathway suppression.

## Material and methods

### Cell culture

Human squamous carcinoma (HSC-4) cells were obtained from JCRB Cell Bank (JCRB0624). The cells were cultured in DMEM supplemented with 10% FBS and 100 U/ml penicillin/streptomycin (all from HyClone, Logan, UT, USA) in a 5% CO<sub>2</sub> humidified atmosphere.

### Cell viability assay

After HSC-4 cells ( $1.5 \times 10^4$  cells/well) being treated with cordycepin (0–800  $\mu$ M), the MTT solution (0.5 mg/ml) was added and incubated for 3 h. Later, DMSO (100  $\mu$ l) was used to dissolve the formazan crystals. The absorbance was measured at 570 nm using microplate reader (BMG Labtech, Ortenberg, Germany).

### Wound healing assay

After HSC-4 cells ( $2 \times 10^5$  cells/24-well plate) being incubated with serum deprivation or diluted cordycepin, a sterile tip was used to vertically scratch through the cell monolayer. The detached cells were removed by PBS. Fresh medium was added and incubated for 24 h. The wound areas at 0 and 24 h after treatment were imaged and analysed using ImageJ.

### Invasion assay

Boyden chamber Transwell assay with Matrigel-coated Transwell inserts (pore size of 4  $\mu$ m) was used to assess invasion. HSC-4 cells, after being incubated with serum deprivation or cordycepin were reseeded at  $6 \times 10^4$  cells/well onto the Transwell insert and cultured for 24 h. Later, the migrated cells were fixed with 4% paraformaldehyde and stained with 0.5% crystal violet. Images of stained cells were taken and the results were calculated from five randomly independent fields.

### Anchorage-dependent colony formation assay

HSC-4 cells were seeded onto 24-well culture plates at  $0.1 \times 10^3$  cells/well and incubated for 24 h. The cells then were incubated with cordycepin (0–100  $\mu$ M) in 10% FBS containing medium for 2 weeks. Culture medium was replaced every 3–5 days. After 2 weeks, the cells were fixed with 4% paraformaldehyde and stained with 0.5% crystal violet. The generated colonies were imaged, and the number and size of the colonies were analysed by ImageJ.

### Agarose spot invasion assay

Invasive ability of HSC-4 cells was evaluated by agarose spot method as previously described.<sup>23</sup> Low-melting agarose (Sigma–Aldrich, St. Louis, MO, USA) was placed into 24-well culture plate. HSC-4 cells, after being incubated with serum deprivation or cordycepin, were reseeded at density of  $3 \times 10^4$  cells/well on the top of agarose spot. After 24 h, the invaded cells were imaged and the cell-invaded zone area in agarose spot was measured using ImageJ. The invaded area was relatively compared to the control untreated cells and presented as percentage of control.

### Immunofluorescence staining

After being treated, HSC-4 cells were fixed with 4% paraformaldehyde and blocked with 3% BSA for 30 min at 4 °C. The cells were then incubated overnight with an anti-rabbit LC3 primary antibody (Millipore, Massachusetts, USA) at 4 °C. Afterwards, the cells were incubated with an Alexa Fluor 532 goat anti-rabbit IgG secondary antibody (Thermo Scientific, Rockford, IL, USA) for 1 h at 4 °C. The coverslips were mounted with PermaFluor Aqueous Medium (Thermo Scientific).

### Monodansylcadaverine (MDC) staining

MDC was used to label autophagic vacuole membranes as previously described.<sup>24</sup> After being incubated with serum deprivation or diluted cordycepin, HSC-4 cells were fixed with 4% paraformaldehyde, washed with PBS twice and incubated with 50  $\mu$ M MDC for 10 min in the dark. The fluorescence intensity and single cell fluorescence counts were observed and measured by ImageJ.

### Gelatin zymography

Conditioned media of cordycepin treatment were collected and concentrated. Equal amount of protein was subjected to SDS-PAGE containing 0.1% gelatin. The gels were then washed in washing buffer for 30 min, incubated with incubation buffer at 37 °C overnight, and stained with staining solution containing 0.1% Coomassie brilliant blue for 30 min. The gelatinolytic activity was visualized by destaining solution until the band can clearly be seen and analyzed by ImageJ.

### RNA isolation and reverse transcription (RT)-PCR

Total RNA was extracted using NucleoSpin RNA Plus kit (Macherey–Nagel, Dueren Germany). RNA (1  $\mu$ g) was used for complementary DNA (cDNA) synthesis using ReverTra Ace® qPCR RT Master Mix with gDNA Remover (Toyobo Co., Ltd., Japan). PCR was performed on a BioRad/C1000Touch Thermocycle (BioRad, Temecula, CA, USA) to amplify cDNA with specific primers (Supplementary Table 1). The amplified cDNA products were subjected to a 1.5% agarose gel electrophoretic separation and visualised by safe red staining.

### Western blot analysis

Protein expressions of caspase 3, cleaved caspase 3, Akt, p-Akt (Cell Signaling Technologies, Beverly, MA, USA), Fak and p-Fak (Abcam, Cambridge, UK) were determined after the cells being incubated with cordycepin. Total proteins were extracted using RIPA buffer. Equal amount of protein was subjected to SDS-PAGE, transferred to PVDF membrane (Millipore), and incubated with appropriate primary and secondary antibodies. The membranes were developed using X-ray film. The densitometry of protein bands was quantified by ImageJ.

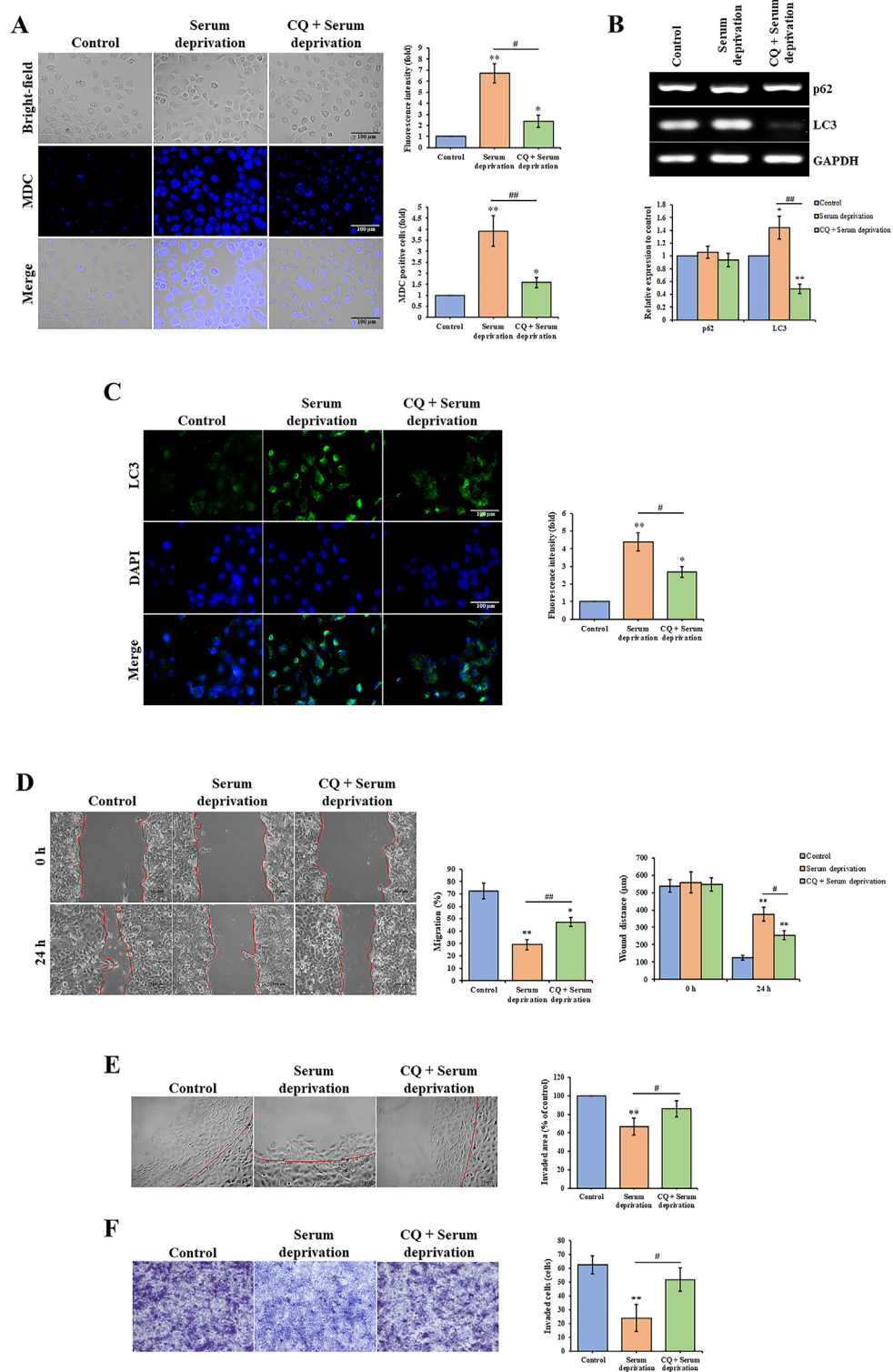
### Statistical analysis

All data are expressed as mean  $\pm$  standard deviation (SD). Multiple comparisons among all experimental groups were analysed by ANOVA followed by Duncan's post hoc test. *P*-value of <0.05 was considered as a statistically significant difference.

## Results

### Induction of autophagy impairs migration and invasion in oral squamous carcinoma cells

To investigate the involvement of autophagy in cell migration and invasion, serum deprivation (serum-free media) was performed to induce autophagy in HSC-4 cells. As shown in Fig. 1A–C, the intensity of MDC-stained autophagic vacuoles, the number of MDC-positive cells, LC3 gene expression and LC3 immunofluorescence intensity were distinctly increased in serum-free medium whereas chloroquine (CQ), an inhibitor of autophagy, pre-incubation significantly reduced MDC staining, expression level of LC3 and immunofluorescence intensity of LC3 gene compared to serum-free medium. In addition, HSC-4 cells in serum-free medium displayed a significant decrease in percentage of migration, invaded area and number of invaded cells whereas the wound area distance was significantly increased compared to control cells compared to untreated cells. In contrast, CQ pre-treatment led to significantly abolish the effect of autophagy on the percentage of migration, the invaded area and the invaded cells (Fig. 1D–F, respectively). These results demonstrate that the induction of autophagy impaired the migration and invasion capacity of HSC-4 cells.



**Figure 1** Autophagy induction impairs migration and invasion in HSC-4 cells. (A) MDC staining, (B) mRNA expression of p62 and LC3 genes, and (C) LC3 immunofluorescence staining in HSC-4 cells pre-treated with 50 µM CQ for 1 h, followed by incubation in serum-free medium (serum deprivation) for 24 h. (D) Migratory ability assessed by wound healing assay, (E and F) invasive ability examined by the agarose spot and Transwell invasion assays in HSC-4 cells pre-treated with 50 µM CQ for 1 h, followed by incubation in serum-free medium for 24 h. The data represent mean ± SD where n = 3: \**P* < 0.05, \*\**P* < 0.01 versus control cells, #*P* < 0.05, ###*P* < 0.01 versus serum deprivation. CQ; chloroquine.



## Cordycepin reduces migration and invasion in oral squamous carcinoma cells

In order to investigate the pharmacological potential of cordycepin on cell migration and invasion, we firstly determined the cytotoxic dose dependence of cordycepin. Cordycepin at concentrations of 25–50  $\mu\text{M}$  did not show any cytotoxic effect (Fig. 2A). Consistently, 25 and 50  $\mu\text{M}$  cordycepin did not affect cleaved caspase 3/caspase 3 ratio, while 100  $\mu\text{M}$  cordycepin significantly increased the ratio of cleaved caspase 3/caspase 3 (Fig. 2B). Therefore, a non-toxic concentration within this range and a toxic dose (100  $\mu\text{M}$ ) of cordycepin were employed in further experiments. *In vitro* migration and invasion assays were used to investigate the inhibitory effect of cordycepin on cell migration and invasion. As shown in Fig. 2C–E, cordycepin at both non-toxic and toxic concentrations significantly decreased the percentage of migration, the invaded area and the number of invaded cells whereas the wound area distance was significantly increased compared to untreated cells. These results suggest that cordycepin had a direct inhibitory potential on migration and invasion, and an indirect effect via triggering cell death.

Furthermore, the potency of cordycepin on growth ability was also examined. The results showed that cordycepin at 50–100  $\mu\text{M}$  significantly decreased cell proliferation as well as colony number and size compared to control cells (Fig. 3A and B). These data demonstrate that cordycepin has an inhibitory effect on the growth of HSC-4 cells.

## Autophagy involves in cordycepin-inhibited migration and invasion

Previous studies have reported that cordycepin induces autophagy<sup>5,6</sup> and autophagy induction results in impaired cell migration and invasion (Fig. 1). To evaluate whether autophagy induction by cordycepin treatment is involved in the anti-migratory and invasive potential of cordycepin, HSC-4 cells were pre-incubated with or without 50  $\mu\text{M}$  CQ prior to treatment with 50  $\mu\text{M}$  cordycepin, and then cell migration and invasion were assessed. The results show that cordycepin caused a significant decrease in the percentage of migration, the percentage of invaded area and the number of invaded cells, whereas the wound area distance was significantly increased compared to control cells. On the other hand, CQ pre-treatment significantly reduced the effect of cordycepin on cell migration and invasion (Fig. 4A–C). In addition, autophagic vacuoles, and autophagy-related gene expression were also assessed. It was noted that the intensity of MDC-stained autophagic vacuoles, the number of MDC-positive cells and the mRNA expression of LC3, but not p62, in cordycepin-treated cells were significantly increased, while CQ significantly prevented the effects of cordycepin (Fig. 5A and B). Similarly, immunofluorescence analysis of LC3 revealed that fluorescence intensity of LC3 protein was prominent in cordycepin-treated cells. With CQ pre-incubation, LC3 fluorescence intensity was significantly decreased compared to cordycepin treatment (Fig. 5C). These data demonstrate that cordycepin exerts inhibitory effects on

cell migration and invasion through the induction of autophagy.

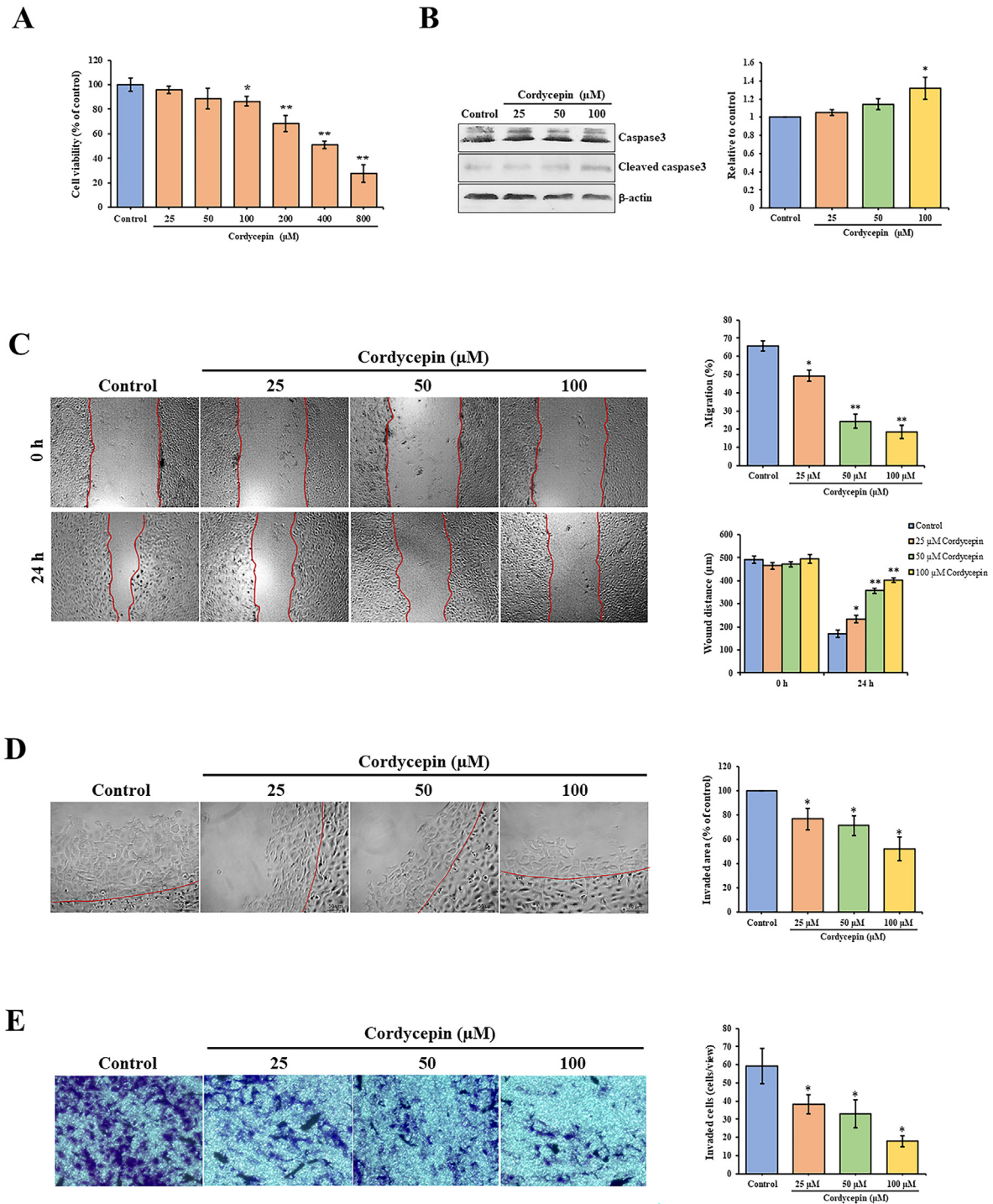
## Inducing autophagy by cordycepin suppresses FAK/Akt phosphorylation and MMP2-MMP9 activity

To further investigate how autophagy induction by cordycepin attenuates cell migration and invasion, the expression of FAK, Akt and their phosphorylated levels was investigated. Cordycepin at 50 and 100  $\mu\text{M}$  significantly decreased the ratio of p-FAK/FAK and p-Akt/Akt (Fig. 6A). However, CQ pre-treatment significantly inhibited the effects of cordycepin on decreased the ratio of p-FAK/FAK and p-Akt/Akt (Fig. 6B). Further, MMP2 and MMP9 gene expression and activity were determined. As presented in Fig. 6C, MMP2 gene expression was significantly downregulated with 50 and 100  $\mu\text{M}$  cordycepin treatment compared to control cells, while MMP9 gene expression was not affected by cordycepin. On the other hand, CQ pre-treatment significantly inhibited cordycepin-downregulated MMP2 gene expression (Fig. 6D). Furthermore, the activity of both MMPs was significantly suppressed in cordycepin-treated cells whereas CQ pre-incubation significantly abolished cordycepin's effect on MMP2 activity (Fig. 6E). The results suggest that cordycepin inhibits migration and invasion of HSC-4 cells via autophagy-dependent FAK/Akt/MMP2 and MMP9 pathway suppression.

## Discussion

Studies investigating the role of cordycepin on OSCC migration and invasion are infrequent, and have only been described in one report demonstrating that cordycepin reduces OSCC migration by upregulated E-cadherin and downregulated N-cadherin.<sup>25</sup> In this study, we confirmed the anti-proliferative effect of cordycepin, and further elucidated that cordycepin could potently inhibit migration and invasion of HSC-4 cells. We also demonstrated that cordycepin initiates autophagy, which further enhances the suppression of FAK and Akt phosphorylation, and MMP2 and MMP9 activity. Inhibition of autophagy by CQ is most likely associated with abolished cordycepin-inhibited HSC-4 migration and invasion.

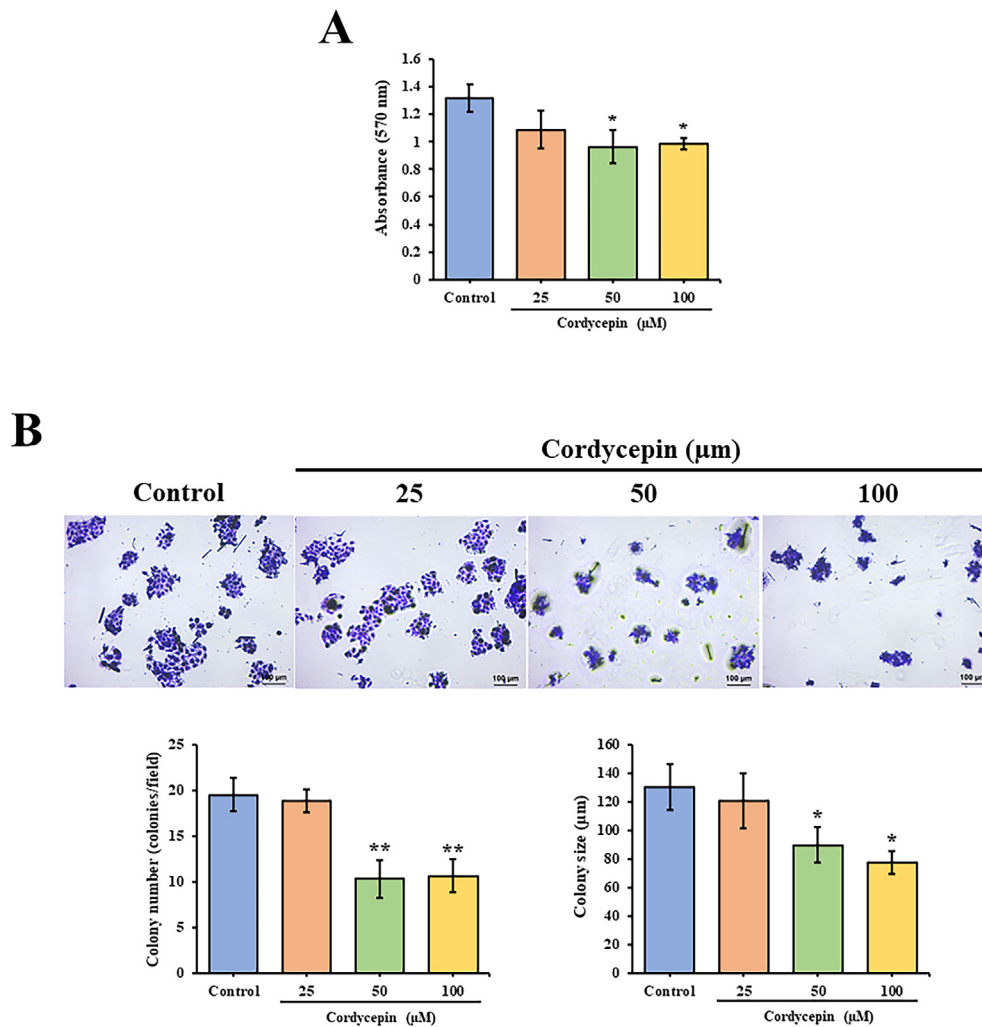
We have demonstrated the effects of cordycepin in non-toxic (25–50  $\mu\text{M}$ ) and toxic (100  $\mu\text{M}$ ) concentrations to suggest direct inhibitory potential on migration and invasion, and indirect effect via triggering cell stress. Both concentrations significantly reduced migration and invasion, however, the toxic one produced more impact, but not significant. It is likely that the toxic dose trigger stress-induced apoptosis, as evidenced by increased the ratio of cleaved caspase 3/caspase 3. Escaping apoptosis serves as an important process for success of metastatic processes, particularly in the resistance to apoptotic cell death induced by loss of cell–cell and cell-ECM contacts.<sup>26,27</sup> Similar to the findings of previous studies, a 100  $\mu\text{M}$  of cordycepin induced cell apoptosis in OSCCs such as OEC-M1 and OC3 cells,<sup>28,29</sup> and induced apoptosis by cordycepin inhibited EMT in SAS and OEC-M1 cells.<sup>25</sup>



**Figure 2** Cordycepin reduces migration and invasion in HSC-4 cells. (A) Cell viability assessed by the MTT assay in HSC-4 cells treated with diluted cordycepin (0–800 μM) for 24 h. (B) Expression of cleaved caspase 3/caspase 3 ratio, (C) Migratory ability determined by the wound healing assay, (D and E) invasive capacity examined by the agarose spot and Transwell invasion assays in HSC-4 cells treated with diluted cordycepin (0–100 μM) for 24 h. The data represent mean ± SD where n = 3: \*P < 0.05, \*\*P < 0.01 versus control cells.

On the other hand, the non-toxic dose (50 μM) of cordycepin impaired migration and invasion in HSC-4 cells by inducing autophagy. Many studies have demonstrated that cordycepin can activate autophagy in oral squamous,<sup>7</sup> ovarian<sup>5</sup> and breast cancer cells<sup>8</sup> through AMPK/mTOR

pathway. Serum starvation is previously used as a positive control for autophagy studies. Serum deprivation causes LC3-mediated autophagic flux via AMPK pathway.<sup>30</sup> Like several cell models, in OSCC, using serum or nutrient starvation results in induction of autophagy.<sup>30,31</sup> Autophagy



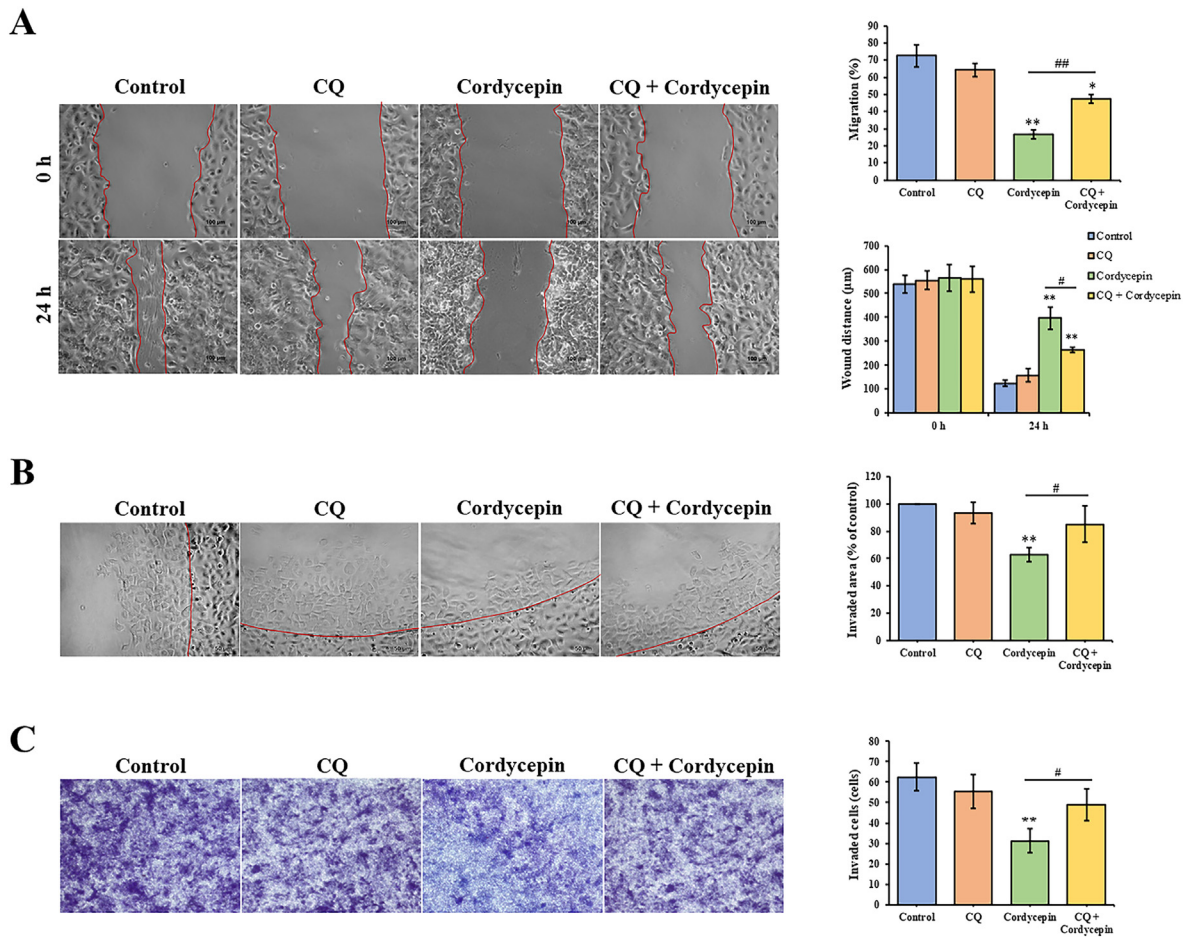
**Figure 3** Cordycepin reduces the proliferation and growth of HSC-4 cells. (A) Cell proliferation assessed by the MTT assay of HSC-4 cells treated with diluted cordycepin (0–100 μM) for 24 h. (B) Growth ability examined by the anchorage-dependent growth assay in HSC-4 cells treated with diluted cordycepin (0–100 μM) for 24 h. The data represent mean ± SD where n = 3: \*P < 0.05 versus control cells.

occurs in numerous stressful conditions, such as the presence of damaged proteins and organelles, nutrient deprivation and is a common phenomenon after cancer treatment.<sup>9,10</sup> Autophagy plays a paradoxical role in the promotion and suppression of tumours.<sup>9–11</sup> Autophagy has been demonstrated to influence cancer migration and invasion.<sup>32</sup> One study has shown that inhibition of autophagy suppresses migration and invasion of hepatic carcinoma cells,<sup>33</sup> suggesting a metastasis-promoting role of autophagy. Conversely, autophagy induction attenuates migration and invasion in glioblastoma, breast cancer cells and OSCC,<sup>34–36</sup> whereas autophagy inhibition augments OSCC migration and invasion,<sup>37</sup> indicating a metastasis-suppressing function of autophagy. Similarly, our findings showed that autophagy in HSC-4 cells, induced by cordycepin treatment or serum deprivation, reduced migratory and invasive ability. In contrast, the CQ-mediated

autophagy inhibition could restore the migration and invasion. Therefore, autophagy has a multifaceted role in cancer metastasis, which might have different functions in different types of cancer, for instance, an anti-metastatic function in OSCC.

Aberrant hyperactivation of FAK and Akt is implicated in metastasis of multiple cancers, including OSCC.<sup>38–40</sup> Selective inhibition of both kinases provides a potential strategy for cancer treatment.<sup>38–40</sup> In this study, we have shown that cordycepin treatment induced autophagy-dependent FAK/Akt suppression. Autophagy participates in protein degradation through lysosome pathway. Inhibiting the fusion of autophagosome to lysosome will inhibit protein degradation.<sup>41,42</sup> CQ has been proposed as autophagy inhibitor in preventing the autophagosome fusion with lysosome.<sup>41</sup> Our studies found that autophagy inhibition by CQ significantly prevented p-FAK and p-Akt





**Figure 4** Autophagy is involved in cordycepin-inhibited migration and invasion. (A) Migratory ability assessed by the wound healing assay, (B and C). Invasive ability examined by the agarose spot and Transwell invasion assays in HSC-4 cells pre-treated with 50  $\mu$ M CQ for 1 h, followed by 50  $\mu$ M cordycepin for 24 h. The data represent mean  $\pm$  SD where  $n = 3$ ; \* $P < 0.05$ , \*\* $P < 0.01$  versus control cells, # $P < 0.05$ , ## $P < 0.01$  versus cordycepin. CQ; chloroquine.

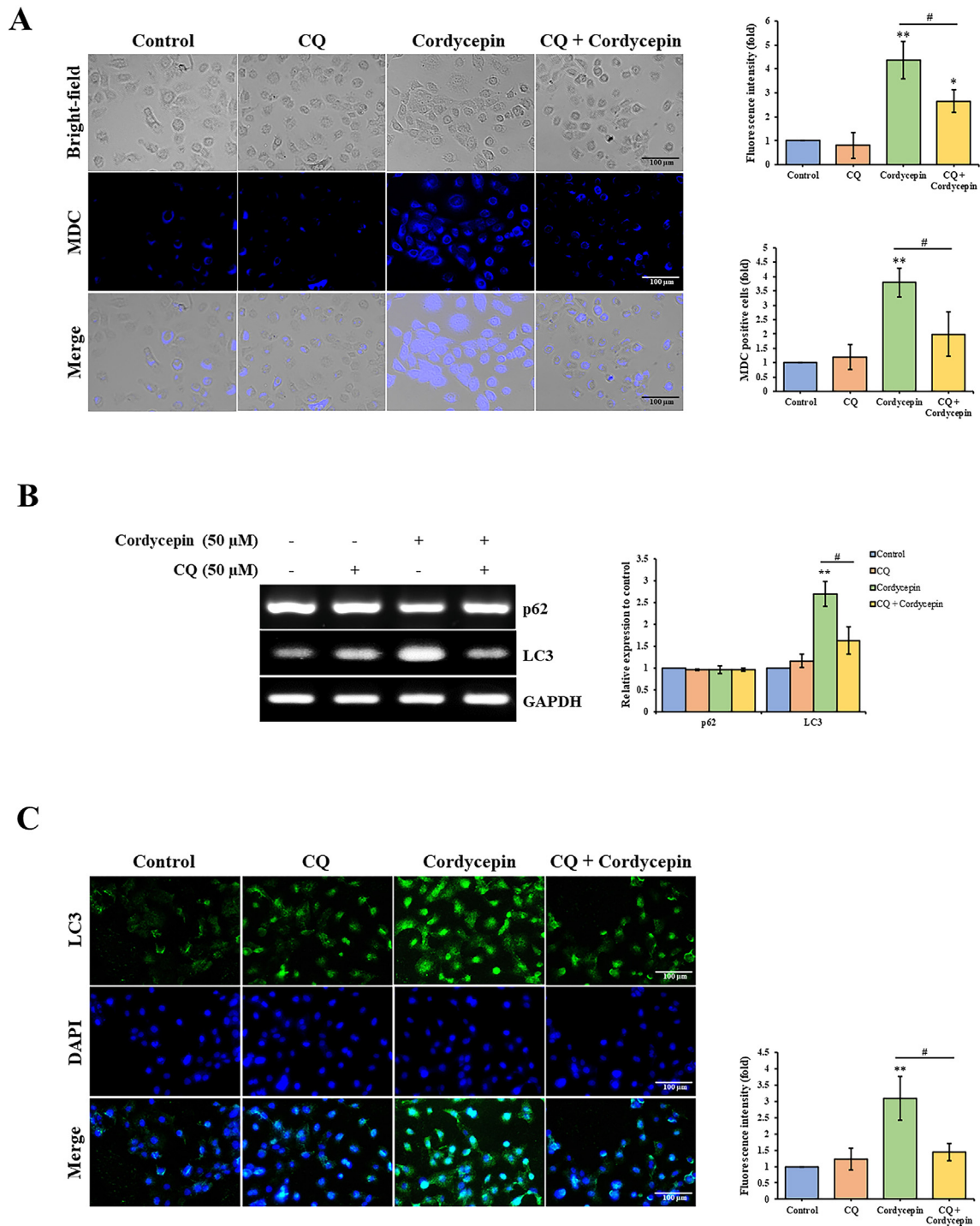
reduction induced by cordycepin, and reversed the impacts of cordycepin on HSC-4 cell migration and invasion. Thus, the present findings support the notion that curcumin induces autophagy-dependent FAK/Akt suppression leading to suppression of cell migration and invasion.

Like FAK and Akt results, cordycepin treatment significantly decreased activity of MMP2 and MMP9, while CQ-inhibited autophagy led to abolish cordycepin-suppressed activity of both MMPs. Previous data showed that inducing autophagy reduces MMP2 and MMP9 expression and activity through autophagic degradation and inhibits invasiveness.<sup>15,43</sup> In contrast, inhibition of autophagy can increase invasiveness with produced more MMP2 and MMP9 levels.<sup>44</sup> It might be possible that cordycepin may reduce MMP2 and MMP9 activities via autophagic degradation. In addition, the reduction of FAK and Akt phosphorylation in cordycepin

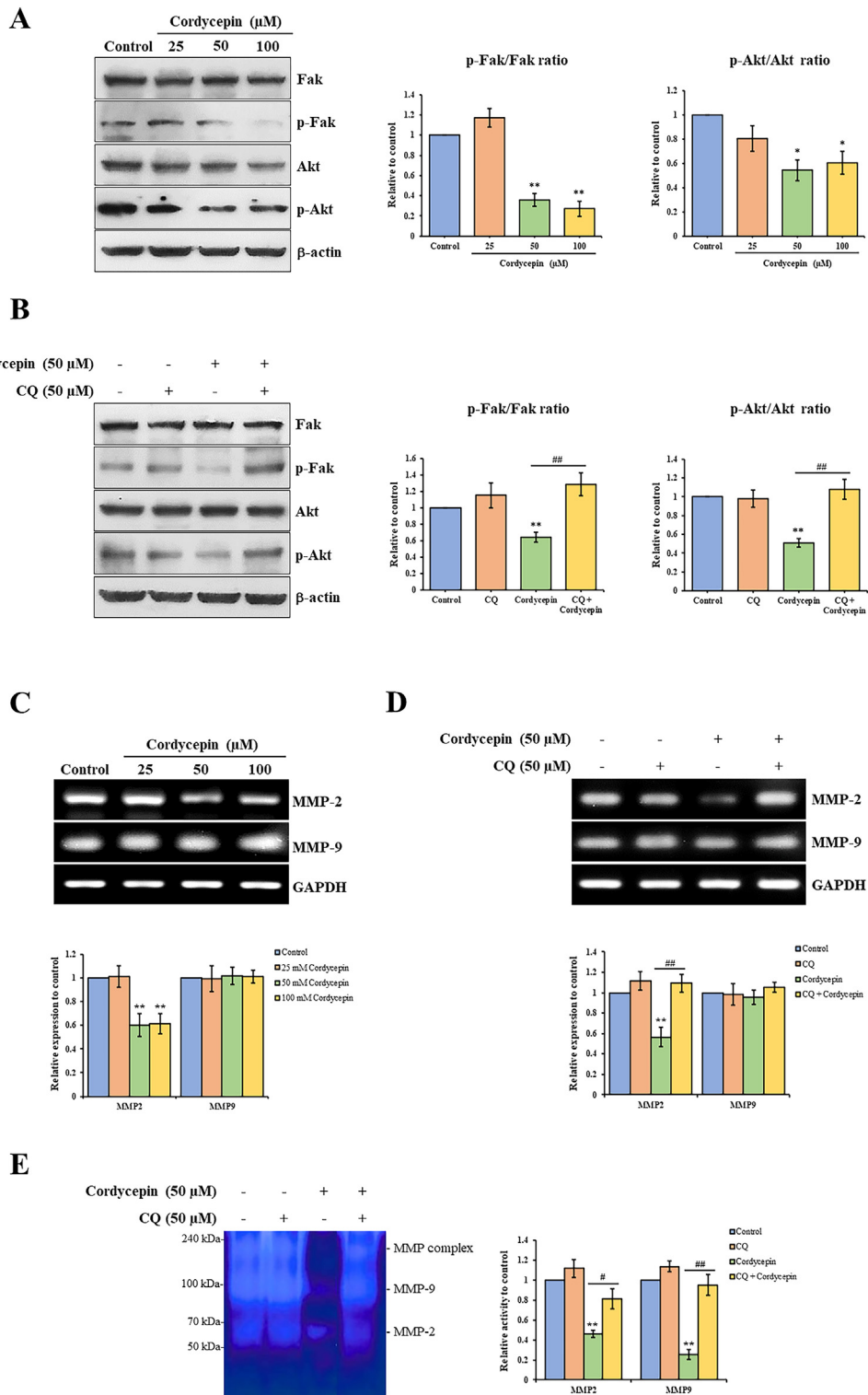
treatment may involve in the reduced MMP2 and MMP9 activities. Because FAK/Akt signaling has been proposed to regulate MMP2 and MMP9 expressions and activities in many tumors, in which pharmacological or genetic inhibition of FAK or Akt reduced the production of both MMP2 and MMP9.<sup>17,18,21,22,45</sup>

To the best of our knowledge, this is the first report to reveal an inhibitory effect of cordycepin at both toxic and non-toxic concentrations on HSC-4 cell migration and invasion. The toxic concentration inhibits migration and invasion via stress-induced cell death. On the other hand, the non-toxic dose induces autophagy to suppress FAK and Akt phosphorylation, and MMP2 and MMP9 activity, which responsible for attenuated HSC-4 cell migration and invasion. These findings contribute to a better understanding of the effect and mechanism of cordycepin that may





**Figure 5** Effect of cordycepin on autophagy induction. (A) MDC staining in HSC-4 cells pre-treated with 50  $\mu$ M CQ for 1 h, followed by 50  $\mu$ M cordycepin for 24 h. (B) mRNA expression of p62 and LC3 in HSC-4 cells pre-treated with 50  $\mu$ M CQ for 1 h, followed by 50  $\mu$ M cordycepin for 24 h. (C) Fluorescence intensity of LC3 protein of HSC-4 cells pre-treated with 50  $\mu$ M CQ for 1 h, followed by 50  $\mu$ M cordycepin for 24 h. The data represent mean  $\pm$  SD where  $n = 3$ : \* $P < 0.05$ , \*\* $P < 0.01$  versus control cells, # $P < 0.05$ , ## $P < 0.01$  versus cordycepin. CQ; chloroquine.



**Figure 6** Inducing autophagy by cordycepin attenuates migration and invasion by reducing MMP2 expression. (A) Expression of p-FAK/FAK and p-Akt/Akt ratio in HSC-4 cells treated with diluted cordycepin (0–100  $\mu\text{M}$ ). (B) Expression of p-FAK/FAK and p-Akt/Akt ratio in HSC-4 cells pre-treated with 50  $\mu\text{M}$  CQ for 1 h, followed by 50  $\mu\text{M}$  cordycepin for 24 h. (C) mRNA expression of MMP2 and MMP9 in HSC-4 cells treated diluted cordycepin (0–100  $\mu\text{M}$ ) for 24 h. (D). mRNA expression of MMP2 and MMP9, (E). MMP2 and MMP9 activity determined by gelatin zymography in HSC-4 cells pre-treated with 50  $\mu\text{M}$  CQ for 1 h, followed by 50  $\mu\text{M}$  cordycepin for 24 h. The data represent mean  $\pm$  SD where  $n = 3$ . \* $P < 0.05$ , \*\* $P < 0.01$  versus control cells, # $P < 0.05$ , ## $P < 0.01$  versus cordycepin. CQ; chloroquine.

eventually support the use of cordycepin as an alternative treatment for OSCC in the future.

## Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jds.2022.03.002>.

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