Received: 8 November 2021 | Revised: 19 January 2022 | Accepted: 8 February 2022

DOI: 10.1111/jcmm.17248

ORIGINAL ARTICLE

WILEY

Curcumin analog HO-3867 triggers apoptotic pathways through activating JNK1/2 signalling in human oral squamous cell carcinoma cells

Chi-Wei Chen[1] | Ming-Ju Hsieh[2,3,4] | Po-Chung Ju[5,6] | Yi-Hsien Hsieh[7,8] | Chun-Wen Su[7,8] | Yen-Lin Chen[9,10] | Shun-Fa Yang[7,8] | Chiao-Wen Lin[9,10]

[1]Department of Life Science, College of Science and Engineering, National Dong Hwa University, Hualien, Taiwan

[2]Oral Cancer Research Center, Changhua Christian Hospital, Changhua, Taiwan

[3]Department of Post-Baccalaureate Medicine, College of Medicine, National Chung Hsing University, Taichung, Taiwan

[4]Graduate Institute of Biomedical Sciences, China Medical University, Taichung, Taiwan

[5]School of Medicine, Chung Shan Medical University, Taichung, Taiwan

[6]Department of Psychiatry, Chung Shan Medical University Hospital, Taichung, Taiwan

[7]Institute of Medicine, Chung Shan Medical University, Taichung, Taiwan

[8]Department of Medical Research, Chung Shan Medical University Hospital, Taichung, Taiwan

[9]Institute of Oral Sciences, Chung Shan Medical University, Taichung, Taiwan

[10]Department of Dentistry, Chung Shan Medical University Hospital, Taichung 402, Taiwan

Correspondence
Shun-Fa Yang, or Chiao-Wen Lin, Institute of Medicine, Chung Shan Medical University, Taichung 402, Taiwan.
Email: ysf@csmu.edu.tw (S.-F.Y.); cwlin@csmu.edu.tw (C.-W.L.)

Abstract

Human oral squamous cell carcinoma (OSCC) is the common head and neck malignancy in the world. While surgery, radiotherapy and chemotherapy are emerging as the standard treatment for OSCC patients, the outcome is limited to the recurrence and side effects. Therefore, patients with OSCC require alternative strategies for treatment. In this study, we aimed to explore the therapeutic effect and the mode of action of the novel curcumin analog, HO-3867, against human OSCC cells. We analysed the cytotoxicity of HO-3867 using MTT assay. In vitro mechanic studies were performed to determine whether MAPK pathway is involved in HO-3867 induced cell apoptosis. As the results, we found HO-3867 suppressed OSCC cells growth effectively. The flow cytometry data indicate that HO-3867 induce the sub-G1 phase. Moreover, we found that HO-3867 induced cell apoptosis by triggering formation of activated caspase 3, caspase 8, caspase 9 and PARP. After dissecting MAPK pathway, we found HO-3867 induced cell apoptosis via the c-Jun N-terminal kinase (JNK)1/2 pathway. Our results suggest that HO-3867 is an effective anticancer agent as its induction of cell apoptosis through JNK1/2 pathway in human oral cancer cells.

KEYWORDS
apoptosis, HO-3867, JNK1/2, oral squamous cell carcinoma

Chi-Wei Chen and Ming-Ju Hsieh equally contributed to this work as first authors.

1 | INTRODUCTION

Oral squamous cell carcinoma (OSCC) is the most common oral cavity cancer with over 90% of cases.[1] Betel nut chewing, smoking and drinking are the most common risk factors for oral cancer in Taiwan.[2–4] OSCC often develops regional and distant lymph node.[5,6] Although surgery, radiotherapy and chemotherapy have applied for the treatments of OSCC, the prognosis of OSCC is still poor due to the recurrence and resistance to treatments.[7,8] According to its high incidence and mortality, development of new and effective treatments for OSCC is an urgent and unmet goal.

Programmed cell death (apoptosis) is a process of eliminating cells to maintain cell population and the normal growth of an organism during development, ageing and DNA damage.[9,10] On the contrary, defects in apoptosis result in neoplastic cells survival as dysregulated cell proliferation, increased cell motility and tumour progression.[11] Apoptosis is triggered through intrinsic, extrinsic and endoplasmic reticulum (ER) pathways.[12,13] Caspases drive apoptosis through activation of caspases initiator 8, 9 and 10 (initiators), caspases 3 and 7 (executioners), and caspases 1, 4 and 5 (inflammatorys).[10,14] The inhibitors of apoptosis proteins are known as the inhibitor of apoptosis protein (IAP) family, including cellular inhibitors of apoptosis 1, 2 (cIAP-1 and cIAP-2), X-linked inhibitor of apoptosis (XIAP) and survivin prohibit death receptor-mediated apoptosis through binding caspases.[15–19] The mitogen-activated protein kinase (MAPKs) (ERKs, JNKs and p38 signalling) also mediate progression of apoptosis; however, the role of MAPKs is relied on status of activated MAPKs, cell types, stimuli or cell stress.[20] Among them, activation of apoptotic via JNKs is through transcriptionally upregulating pro-apoptotic genes or phosphorylating mitochondrial pro- and anti-apoptotic proteins.[21]

Curcumin analog HO-3867 is an antioxidant and antiproliferative compound as it is also known as an antitumour agent through blocking the Janus kinase/signal transducer and activator of transcription (JAK/STAT3) pathway and downregulation focal adhesion kinase and fatty acid synthase in human breast cancer, ovarian cancer and human pancreatic cancer cells alone or in combined with cisplatin or doxorubicin.[22–31] In addition, HO-3867 is also found to activate phosphatase and tensin homolog (PTEN) in human smooth muscle cells and in lung and heart tissues.[32,33] A more recent study found that HO-3867 transcriptionally converts mutant p53 protein to active wild-type p53 in cancer cells.[34] Nevertheless, HO-3867 is revealed to rescue suppression of placenta-specific protein 1 (PLAC1) level in ovarian cancer cells.[35]

As OSCC has over 40% mutant rate of TP53[8,36] and mutant TP53 leads cancer progression,[37] we aimed to explore whether HO-3867 is capable of suppressing OSCC cell growth. We analysed its therapeutic effect on OSCC and to discover the inside mechanisms involved in HO-3867 induced apoptosis and attempted to define its underlying mechanisms.

2 | MATERIALS AND METHODS

2.1 | Cell culture and HO-3867 treatment

Being purchased from the American Type Culture Collection (Manassas, VA, USA) and the Japanese Collection of Research Bioresources (Osaka, Japan), the human OSCC SCC-9 and HSC-3 cells were supplemented with 10% FBS, 5 mL glutamine and 1% penicillin/streptomycin, and cultured in DMEM. HO-3867 was dissolved initially in 100% DMSO to achieve a 100 mM stock solution of HO-3867, and appropriate amounts of stock solution were subsequently added into the culture medium to achieve the indicated concentrations.

2.2 | Microculture tetrazolium (MTT) assay

To obtain information regarding the effect of apoptosis induced by HO-3867, we subjected 6.5×10^4/mL SCC-9 and HSC-3 cells in 24-well plates for 16 h and treated them with different concentrations (0, 2.5, 5, 10 and 20 μM) of HO-3867 to assay the cell viability via MTT assay as described previously.[38,39]

2.3 | Flow cytometric analysis

To estimate the proportion of SCC-9 and HSC-3 cells in different phases of the cell cycle affected by HO-3867, cellular DNA contents were measured via flow cytometry as stated previously.[40] Briefly, we cultured 7.0×10^5 SCC-9 and HSC-3 cells in 6-cm dishes and treated them with different concentrations (0, 2.5, 5, 10 and 20 μM) of HO-3867 for 24 h. After staining with PI, 7.0×10^5 SCC-9 and HSC-3 cells in one Eppendorf tube, the cell cycle was analysed on a BD AccuriTM C6 Plus personal flow cytometer (BD Biosciences, San Jose, CA, USA).

2.4 | Annexin V-FITC apoptosis staining assay

We cultured 7.0×10^5 SCC-9 and HSC-3 cells in one 6-cm dish and treated them with different concentrations (0, 2.5, 5, 10 and 20 μM) of HO-3867 for 24 h. Subsequently, SCC-9 and HSC-3 cells were harvested with trypsinization together with floating non-viable cells. The FITC Annexin V Apoptosis Detection Kit I was performed as reported previously.[41]

2.5 | Human apoptosis array (ARY009, R&D systems)

Human apoptosis array (ARY009, R&D systems) Kit was used to evaluate protein lysates of 1.5×10^6 SCC-9 cells/dish from vehicle- or

20 μM HO-3867-treated for 24 h according to the manufacturer's protocols (R&D Systems, Minneapolis, MN, USA).

2.6 | Protein extraction and western blot analysis

To investigate the molecular mechanism further, the initiator and effector caspases and signalling pathways were detected using Western blot analysis. As described previously, 7.0 x 10^5/dish SCC-9 and HSC-3 cells were cultured in 6 cm plates for 16 h and treated with different concentrations (0, 2.5, 5, 10 and 20 μM) of HO-3867 for 24 h, and the total cell lysates of SCC-9 and HSC-3 cells were prepared.[42,43] Blots were then incubated with a horseradish peroxidase goat anti-rabbit or anti-mouse.

2.7 | Statistical analysis

The SigmaStat 2.0 software package (Jandel Scientific, San Rafael, CA, USA) was applied for statistical analyses. Differences between untreated and HO-3867-treated groups were calculated by Student's *t*-test, and a p value of <0.05 was considered statistically significant. Each experiment was done in triplicate at least ($n \geq 3$) were performed.

3 | RESULTS

3.1 | Cytotoxicity of HO-3867 in human oral squamous cell carcinoma SCC-9 and HSC-3 cells

Curcumin and its analogs have been shown their anticancer effects, including suppression of oral squamous cell carcinoma (Table 1). The main goal of this study is to examine whether the novel curcumin analog HO-3867 exhibits antitumour activity (Figure 1A). We first performed cytotoxicity assay in human oral cancer SCC-9 and HSC-3 cells using MTT assay. We found HO-3867 effectively suppressed SCC-9 and HSC-3 cells growth at the dose region from 10 to 20 μM (Figure 1B). Moreover, cell proliferation was assessed by using the CCK-8 method in SCC-9 and HSC-3 cells. As shown in Figure 1C, treatment of cells with HO-3867 for 24 h significantly decreased the proportion of viable cells in a concentration-dependent manner. Our results show that HO-3867 inhibits the cell growth and cell proliferation in human oral cancer SCC-9 and HSC-3 cells in vitro.

3.2 | HO-3867 induces apoptosis and sub-G1 fraction arrest of SCC-9 and HSC-3 cells

Given that HO-3867 potently suppressed cell viability in SCC-9 and HSC-3 cells, we assumed that HO-3867 may affect with cell cycle progression. To examine this hypothesis, we tested the cell cycle progression of oral cancer SCC-9 and HSC-3 cells by FACS. Compared to the vehicle control, HO-3867-treated SCC-9 and HSC-3 cells exhibited a sub-G1 phase accumulation at the dose of 20 μM (57.7% in SCC-9 cells and 41.7% in HSC-3 cells) (Figure 2A–C).

3.3 | HO-3867 increases cleaved caspase 3 and decreases cIAP-1 and XIAP in SCC-9 and HSC-3 cells

Increased sub-G1 phase cells in HO-3867 cells suggest apoptotic pathway may be induced by the treatment of HO-3867. To test this possibility, we measured and apoptotic cell populations in HO-3867-treated SCC-9 and HSC-3 cells. Examining the Annexin V positive cells by flow cytometry assay, we found that there were significant inductions of Annexin V positive cells in both lines treated with HO-3867 (Figure 3A). Remarkedly, at the dose of 20 μM, HO-3867 induced extremely high amount of cell apoptosis by over 40% in SCC-9 cells and over 80% in HSC-3 cells (Figure 3B,C).

3.4 | Analysis of activating extrinsic and intrinsic apoptotic processes by HO-3867 in SCC-9 and HSC-3 cells

The next question is how apoptosis was activated in HO-3867-treated cells. Since curcumin can increase apoptotic levels through multiple signalling, such as TNF and caspase 8,[44] we hypothesized HO-3867 may affect signalling associated with apoptosis or cell survival. To test this hypothesis, we first examined human apoptosis array (ARY009, R&D systems) in SCC-9 cells. The human apoptosis array contains 35 proteins that associated with apoptotic process as our previous reports.[45] We identified a serial change in the protein amounts (Figure 4A) We found that cleaved caspase-3 was increased by approximately 2.3-fold in HO-3867-treated SCC-9 cells compared to the vehicle control (Figure 4B). Nevertheless, we also found that XIAP, cIAP and Survivin were reduced by 60% upon HO-3867 (Figure 4B). These results indicate that HO-3867 induces apoptotic pathway through activating cleaved caspase-3 in human oral cancer cells.

The clarify the capability of HO-3867 triggering apoptotic pathway through activating cleaved caspase proteins, we measured both total and cleaved forms of apoptotic proteins, including caspase 8, caspase 9, caspase 3 and PARP in HO-3867-treated SCC-9 and HSC-3 cells. Significantly, treatment of HO-3867 decreased the pro form of caspase 8, caspase 9, caspase 3 and PARP in SCC-9 cells (Figure 5A). Treatment of HO-3867 increased the active form of caspase 8, caspase 9, caspase 3 and PARP in SCC-9 cells (Figure 5B). Consistently, in HSC-3 cells, HO-3867 reduced the pro form of caspase 8, caspase 9, caspase 3 and PARP, and enhanced the active form of caspase 8, caspase 9, caspase 3 and PARP (Figure 5C,D) These results imply that HO-3867 induces apoptotic pathway through caspase pathway in human oral cancer cells.

TABLE 1 Molecular actions of curcumin analog on human OSCC cells

Curcumin analog	Cell line	Mechanism of action	Testing dose	References
Curcumin	YD10B	↑reactive oxygen species (ROS) production and autophagy ↑LC3-II formation and PARP cleavage	1–40 μM	[70]
FLLL-32	SCC-9 HSC-3	↓cell viability ↑apoptosis via caspase-3/-8/-9 and p38 MAPK signalling pathway ↑HO-1	1–16 μM	[50]
PAC (3,5-Bis (4-hydroxy-3-methoxybenzylidene)-N-methyl-4-piperidone)	CA9-22 gingival epithelial cells (GEC)	↓cell proliferation and colony formation ↑cytotoxicity, intracellular ROS, intracellular glutathione (GSH) activity ↑autophagy by targeting LC3B and p62 ↓epithelial-to-mesenchymal transition and inhibits cell migration ↓mitochondrial membrane potential ↑apoptosis via ERK1/2, p38/JNK, NF-κB and Wnt cellular signalling pathways	1–10 μM	[72]
EF-24 (diphenyl difluoroketone)	CAL-27	↓cell viability ↑apoptosis via caspase-3and 9 ↓phosphorylated forms of MEK1 and ERK	0.1–30 μM	[73]
EF-24 (diphenyl difluoroketone)	KB	↓cell viability ↑nuclear condensation and fragmentation ↑apoptosis via caspase-3/-7/-9	0.1–100 μM	[74]
DMC (Demethoxycurcumin)	SCC-9 HSC-3	↓cell viability ↑G2/M phase arrest ↑apoptosis via caspase-3/-8/-9 and PARP ↓cIAP1/XIAP and activating the p38 MAPK-HO-1 axis	12.5–50 μM	[49]
DBA (Dibenzylideneacetone)	Human mucoepidermoid carcinoma (MC3 and YD15)	↓cell viability ↑apoptosis by inhibition of specificity protein 1 (Sp1) protein stability ↑Bim and truncated Bid (t-Bid) via Sp1 Anti-tumorigenic activity of DBA (20 mg/kg/day) in an athymic nude mouse xenograft model	5–15 μM	[75]
DBA (Dibenzylideneacetone)	HSC-4 HSC-2 YD-10B SCC-15	↓cell viability ↑apoptosis through Sp1 degradation ↑increased Bax expression	2.5–10 μM	[71]
trienone 11 (1,7-bis(3-hydroxyphenyl)-1,4,6-heptatrien-3-one)	CLS-354	↑apoptotic cell death via ROS and caspase-3/7, -8, and -9 activations Activates ROS to mediate caspase activation and eventually apoptosis via the intrinsic pathway	0.01–80 μM	[76]

TABLE 1 (Continued)

Curcumin analog	Cell line	Mechanism of action	Testing dose	References
H-4073	UM-SCC-74A UM-SCC-1 UM-SCC-74B UM-SCC-38 UM-SCC-47 CAL27	↓cell proliferation, migration, survival and angiogenesis cell proliferation via JAK/STAT3, FAK, Akt and VEGF signalling pathways ↓tumour growth and angiogenesis in SCID mouse xenograft model	2.5–20 μM	[77]

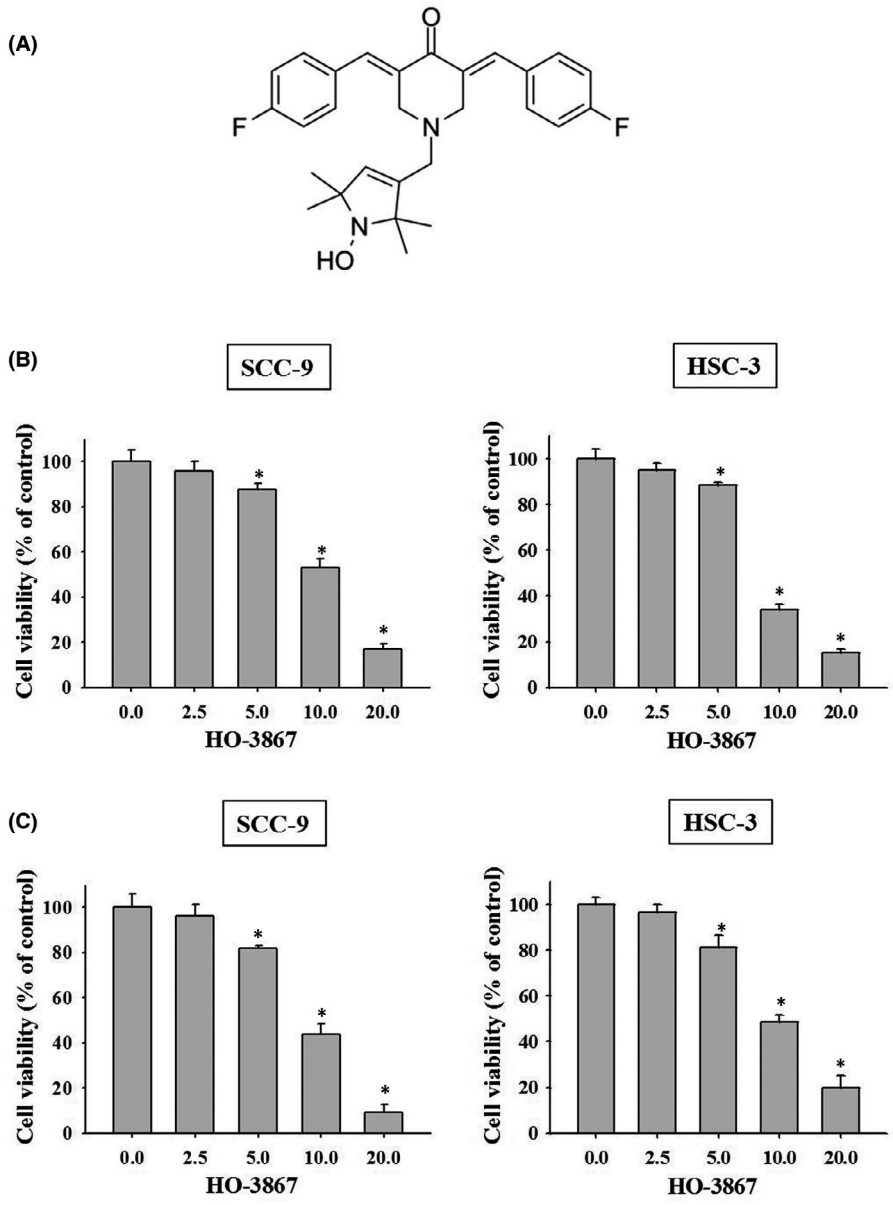

FIGURE 1 Effects of HO-3867 on the cell viability of SCC-9 and HSC-3 cells. (A) The structure of curcumin analog HO-3867. (B, C) The viability of SCC-9 and HSC-3 cells treated with HO-3867 (0, 2.5, 5, 10 and 20 μM) for 24 h was detected by MTT assay and CCK-8 assay, and the effects are illustrated after quantitative analysis. $^*p < 0.05$, compared with the vehicle group

3.5 | HO-3867 activates extrinsic and intrinsic apoptotic processes via JNK1/2 pathways in SCC-9 and HSC-3 cells

Mitogen-activated protein kinase pathway is known to mediate the apoptotic pathway.[20] To determine whether the treatment of HO-3867 could activate MAPK signalling in human oral cancer cells, we detected the phosphorylated levels of ERK1/2, JNK1/2 and p38 in human oral cancer cells SCC-9 and HSC-3 cells using immune blot. Upon the treatment of HO-3867 at the dosages of 2.5–20 μM, phosphorylated ERK1/2 (p-ERK1/2), p38 (p-p38) and phosphorylated JNK1/2 (p-JNK1/2) were enriched (Figure 6A,B), indicating HO-3867 activates MAPKs pathway in human oral cancer cells. To further digest which MAPK signalling is response to HO-3867 induced

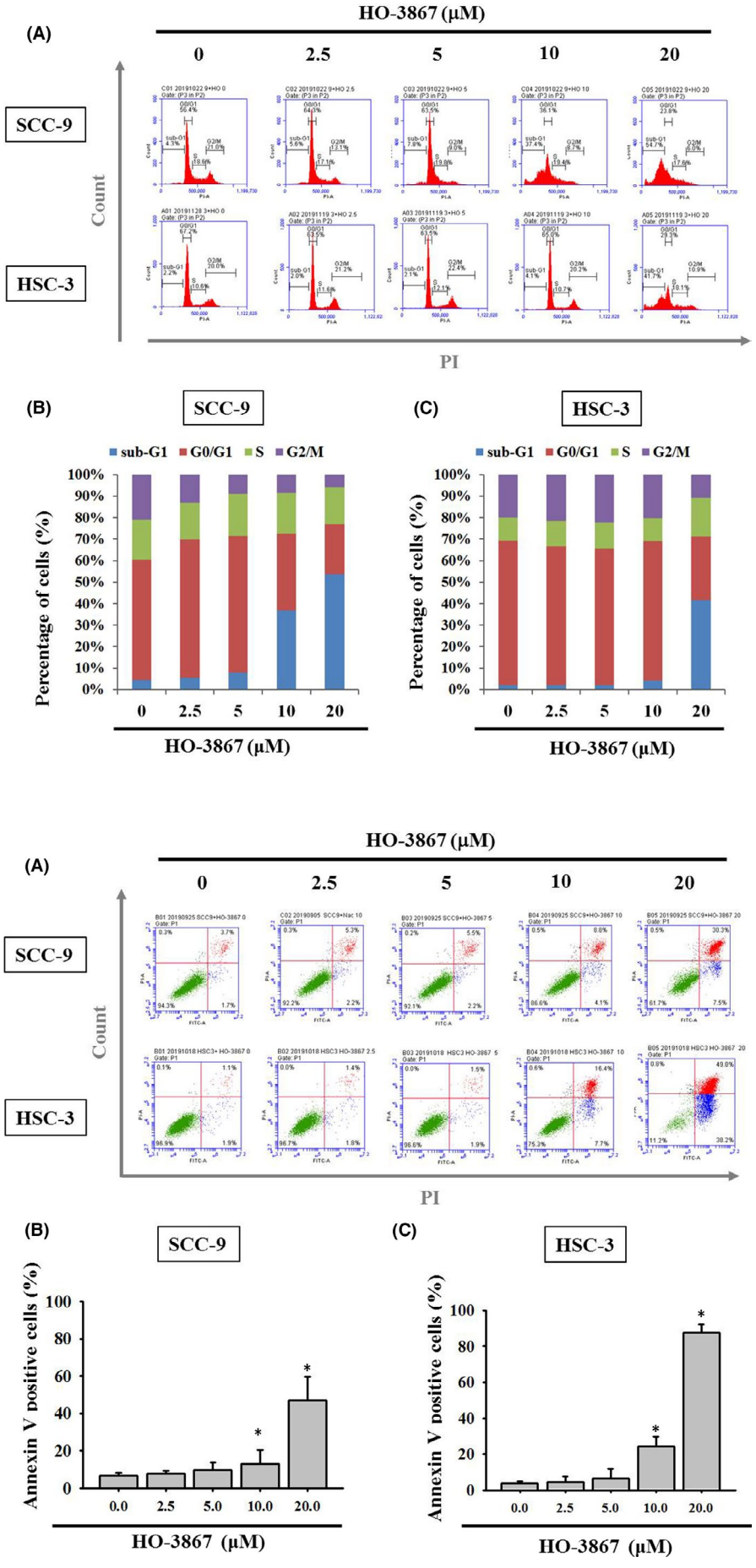

FIGURE 2 Effects of HO-3867 on the cell cycle of SCC-9 and HSC-3 cells. SCC-9 and HSC-3 cells were treated with HO-3867 (0, 2.5, 5, 10 and 20 μM) for 24 h and then subjected to flow cytometry after (A) PI staining to analyse DNA contents. (B, C) The cell cycle profile of (B) SCC-9 cells and (C) HSC-3 cells in flow cytometry was quantified

FIGURE 3 Effects of HO-3867 on the cell apoptosis in SCC-9 and HSC-3 cells. Oral cancer SCC-9 and oral cancer HSC-3 cells were treated with HO-3867 (0, 2.5, 5, 10 and 20 μM) for 24 h and then subjected to (A) flow cytometry to analyse DNA contents. (B, C) Subsequently quantitative analyses of apoptosis of (B) SCC-9 cells and (C) HSC-3 cells were summed up. *$p < 0.05$, compared with the vehicle group

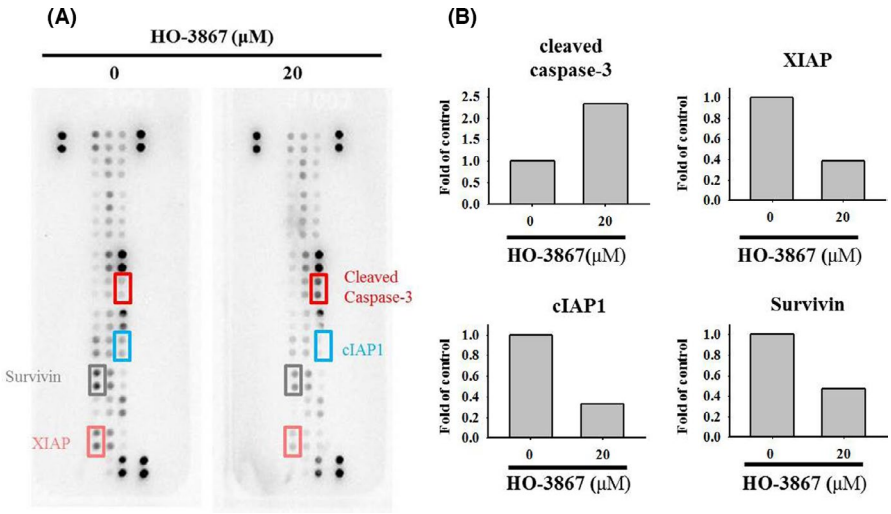

FIGURE 4 Effects of HO-3867 on the human apoptosis array and IAPs expression in SCC-9 cells. (A) After treatment of 20 μM HO-3867 for 24 h in SCC-9 cells, the human apoptosis array (ARY009, R&D systems) were employed and the increased cleaved caspase-3 protein and decreased XIAP, cIAP1 and survivin proteins were exposed to quantitative analysis. (B) Intensity qualifications of cleaved caspase-3, XIAP, cIAP1 and survivin in HO-3867-treated SCC-9 cells

apoptosis, we next measure apoptotic signalling in HO-3867-treated human oral cancer cells SCC-9 and HSC-3 cells under inhibitions of ERK1/2, JNK1/2 or p38. The inhibitors were U0126,[46] JNK-IN-8[47] and SB203580[48] for blocking the phosphorylation of ERK1/2, JNK1/2 and p38 respectively. According to previous studies,[49,50] after 2-hour pretreatments of U0126 (10 μM), JNK-IN-8 (10 μM) and SB203580 (10 μM), cells were administered with HO-3867 (20 μM) for another 24 h. We found the treatment of JNK-IN-8 was able to attenuate the formation of the active form of caspase 3, caspase 8, caspase 9 and PARP in SCC-9 cells (Figure 7A). Moreover, as shown in Figure 7B, inhibition of JNK1/2 pathway using JNK-IN-8 (10 μM) effectively reduced the apoptotic pathway as the active form of caspase 3, caspase 8, caspase 9 and PARP were reduced in HSC-3 cells (Figure 7B). Altogether, using inhibitors of JNK1/2 (JNK-IN-8), HO-3867 increases in cleaved caspases 3, 8 and 9 are rescued, but they could not be affected by co-treatment with the U0126 (ERK1/2 inhibitor) and p38 inhibitor (SB203580). HO-3867 induces apoptotic pathways in OSCC SCC-9 and HSC-3 cells through activating JNK1/2 signalling.

4 | DISCUSSION

As HO-3867 is a versatile antitumour agent with targeting STAT3, PTEN and p53 in various cancer types,[22–31] we attempted to examine whether HO-3867 suppress OSCC and to analyse how HO-3867 triggered cell death. In the present study, we detected that HO-3867 exhibited great therapeutic effects on OSCC cells, including inducing G2/M cell cycle arrest and apoptotic cell death. Upregulation of the p-JNK1/2 and downregulation of cIAP1/XIAP/Survivin were critical for HO-3867-induced apoptotic cell death in OSCC cells.

It is exciting to find that JNK1/2 signalling is elevated in HO-3867-treated OSCC cells. While it is reported that the JNK1/2 signalling and IAPs mediate cell apoptosis,[51] our study has revealed a novel avenue of modulating JNK1/2 signalling and IAPs using a single compound, HO-3867. Although inhibitors directly targeting either JNK1/2 or IAPs are not clinical used, development of curcumin and its analogs into clinical is actively processing,[52] as curcumin functions as an anticancer agent in in vitro, in vivo studies and clinical trials.[53] However, the detail mechanism of how HO-3867 activates JNK1/2 and attenuates IAPs in OSCC still unknown.

Over decades, drugs targeting p53, STAT3, ERK1/2, JNK1/2 and p38 are investigated for anticancer propose,[54–59] such as COTI2 for reactivation of mutant p53 to a form with WT properties,[60] LLL12B blocking STAT3,[61] LY3214996 targeting ERK1/2,[62] AS602801 suppressing JNK[63] and BIRB796 targeting p38.[64] Therefore, as HO-3867 is reported to target p53,[34] STAT3,[29] JNK1/2 and IAPs, HO-3867 would be a potential therapeutic approach for treatment of OSCC or other types of cancers that may have dysregulation of p53, STAT3, JNK1/2 and IAPs.

As a versatile compound, HO-3867 has been examined its potential to be a treatment for many diseases, such as breast cancer,[34] ovarian cancer,[22,25,26,30,35,65] pancreatic cancer[29,31] and endometrial cancer.[28] HO-3867 is found not only induce apoptosis in ovarian cancer cells[25,65] but also to repress the migration and invasion of ovarian cancer cells by inhibiting the expression or activity of FAS, FAK, VEGF and their downstream protein levels.[25] Moreover, HO-3867 has been evaluated for the treatment of pulmonary hypertension,[66] pulmonary hypertension secondary to left-heart failure[33] and arterial restenosis.[32] Together, these researches indicate that HO-3867 has highly potential to be developed into an anticancer agent or a regimen for other diseases.

Curcumin and its analogs have been shown their anticancer effects in vitro and in vivo,[67] including suppression of oral squamous cell carcinoma.[68] Through targeting EGFR mediated AKT, ERK1/2 and STAT3 pathways, curcumin inhibits SCC-25 cell growth at the dosage range of 10–80 μM.[69] Moreover, curcumin promoted apoptosis by inducing cleaved caspase 3 and cleaved PARP in YD10B OSCC cells at the dose of 10 μM.[70] Interestingly, several analogs of curcumin are identified their antitumour activity through induction of apoptosis in OSCC.[71,72] In HSC-4 and HSC-2 human oral cancer cells lines, Dibenzylideneacetone inhibits cell viability by triggering apoptosis at the dose of 5–10 μM.[71] PAC (3,5-Bis (4-hydroxy-3-methoxybenzylidene)-N-methyl-4-pip

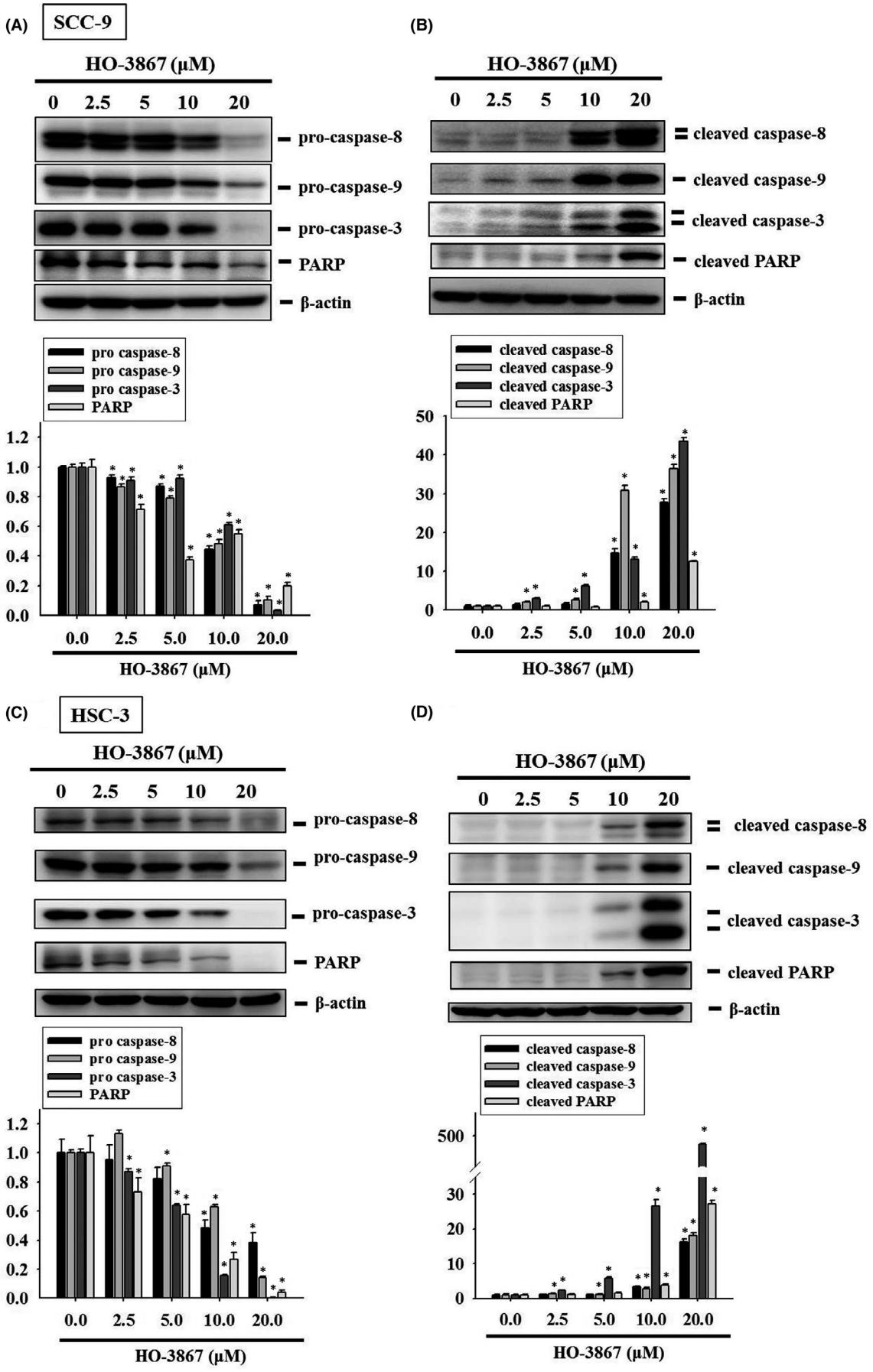
(A)
SCC-9
HO-3867 (μM)
0 2.5 5 10 20
pro-caspase-8
pro-caspase-9
pro-caspase-3
PARP
β-actin
pro caspase-8
pro caspase-9
pro caspase-3
PARP
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0.0 2.5 5.0 10.0 20.0
HO-3867 (μM)
(B)
HO-3867 (μM)
0 2.5 5 10 20
cleaved caspase-8
cleaved caspase-9
cleaved caspase-3
cleaved PARP
β-actin
cleaved caspase-8
cleaved caspase-9
cleaved caspase-3
cleaved PARP
50
40
30
20
10
0
0.0 2.5 5.0 10.0 20.0
HO-3867 (μM)
(C)
HSC-3
HO-3867 (μM)
0 2.5 5 10 20
pro-caspase-8
pro-caspase-9
pro-caspase-3
PARP
β-actin
pro caspase-8
pro caspase-9
pro caspase-3
PARP
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0.0 2.5 5.0 10.0 20.0
HO-3867 (μM)
(D)
HO-3867 (μM)
0 2.5 5 10 20
cleaved caspase-8
cleaved caspase-9
cleaved caspase-3
cleaved PARP
β-actin
cleaved caspase-8
cleaved caspase-9
cleaved caspase-3
cleaved PARP
500
30
20
10
0
0.0 2.5 5.0 10.0 20.0
HO-3867 (μM)

FIGURE 5 Effects of HO-3867 on the activation of caspases -3, -8, and -9 in SCC-9 and HSC-3 cells. Western blot analysis for (A) caspase-3, caspase-8 caspase-9 and PARP as well as (B) their active forms after various concentrations (0, 2.5, 5, 10 and 20 μM) of HO-3867 treatment for 24 h in SCC-9 and (C, D) HSC-3 cells were measured. All of them were subjected to quantitative analysis. *$p < 0.05$, compared with the control group

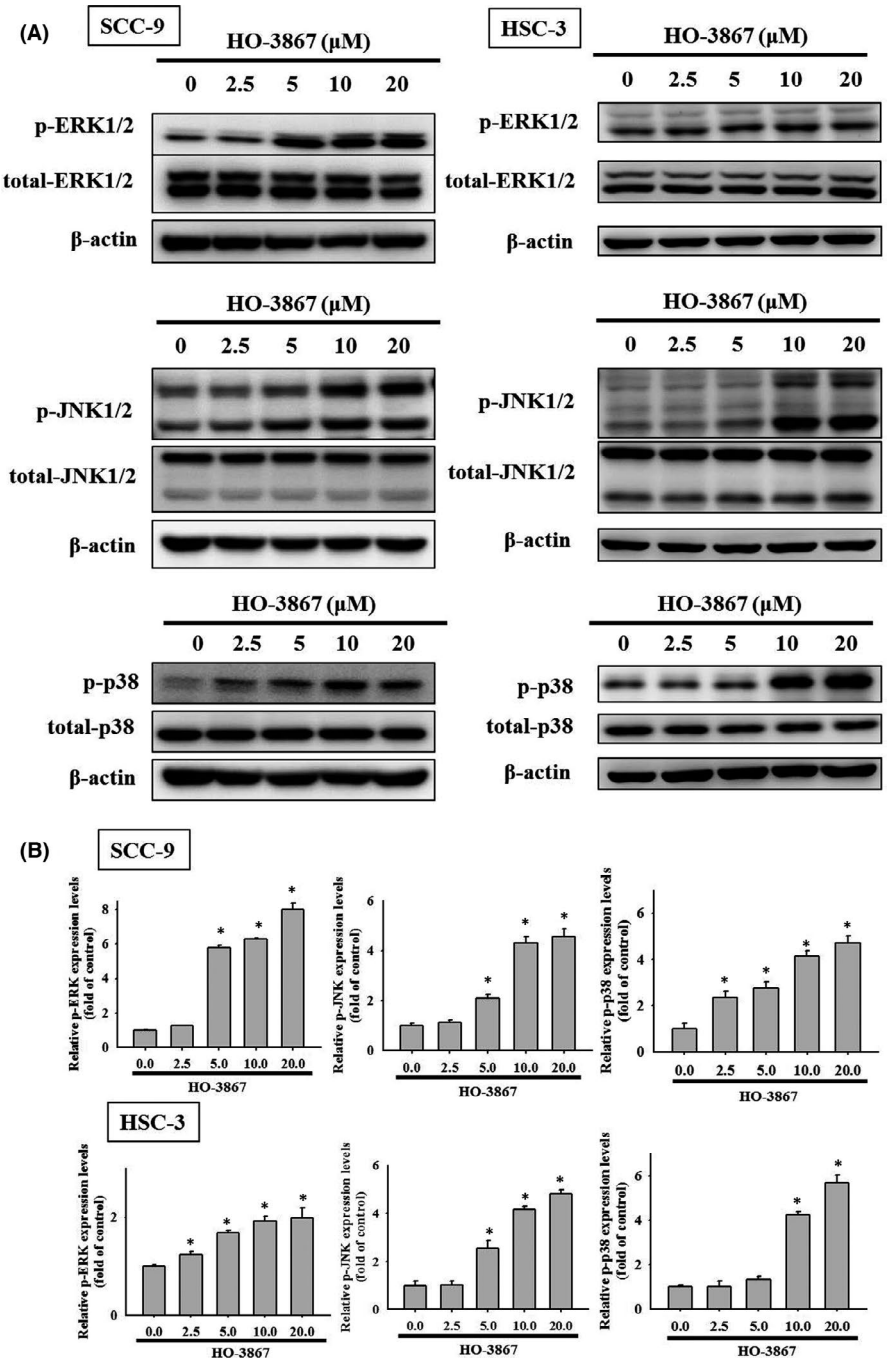

FIGURE 6 Effects of HO-3867 on the phosphorylation of MAPK pathway in SCC-9 and HSC-3 cells. (A) Expressions of ERK1/2, JNK1/2 and p38, as well as their phosphorylation after various concentrations (0, 2.5, 5, 10 and 20 μM) of HO-3867 treatment for 24 h in SCC-9 and HSC-3 cells, were measured via Western blot analysis. (B) They were subjected to quantitative analysis. *$p < 0.05$, compared with the vehicle group

eridone) is recently reported to reduce cell survival through promote apoptosis and autophagy by activating NF-κB, MAPK, Wnt, caspase-3/9 and PARP1 at the dose of 5 μM in oral cancer CA9-22 cells.[72] In this study, we show that the curcumin analog HO-3867 exhibits anti-OSCC activity by inducing apoptosis at the similar dose range (2.5–20 μM), suggesting that HO-3867 is comparably potent to OSCC as curcumin and other analogs.

In conclusion, we have revealed that the curcumin analog HO-3867 suppresses OSCC growth via inducing cell cycle arrest and apoptosis. As inhibition of apoptosis is a hallmark of cancer progression, we

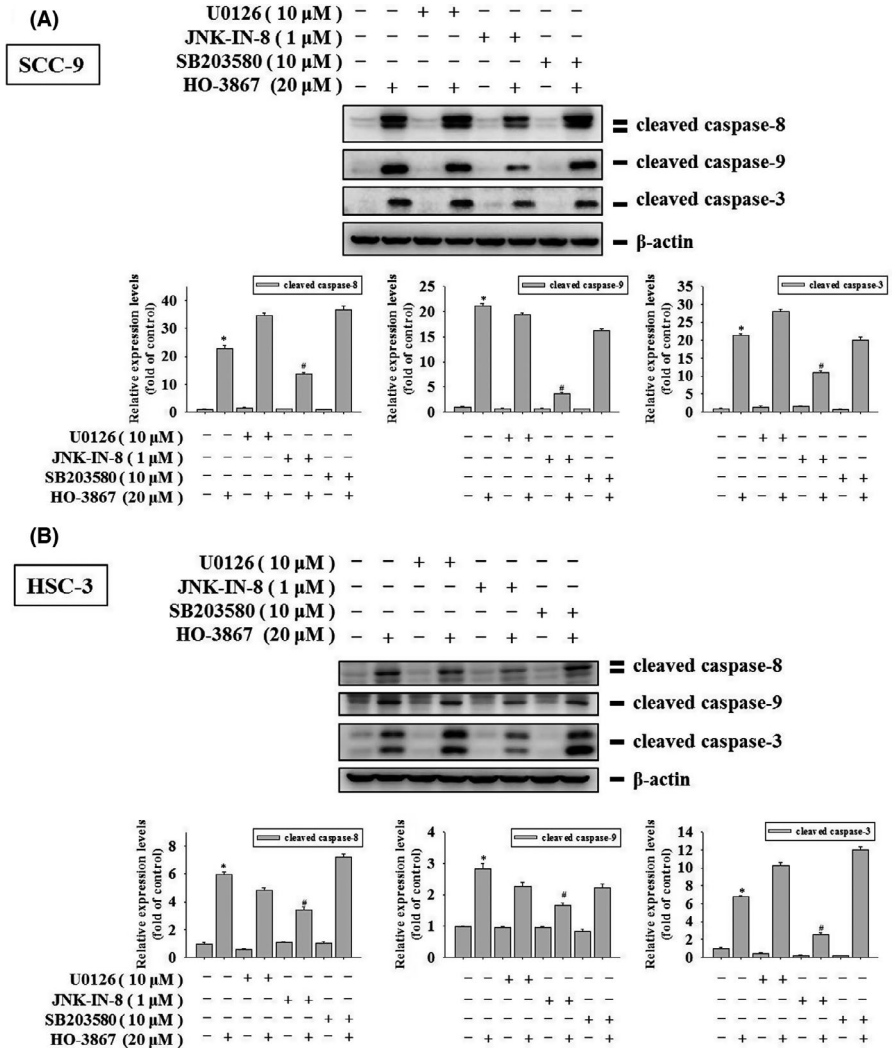

FIGURE 7 Effects of HO-3867 and inhibitors of ERK1/2, JNK1/2 and p38 on cleaved caspase-3, caspase-8 and caspase-9 expressions of SCC-9 and HSC-3 cells. Expressions of cleaved caspases 3, 8 and 9 after pretreatment with or without 10 μM of U0126 (ERK1/2 inhibitor), 1 μM of JNK-IN-8 (JNK1/2 inhibitor) and 10 μM of SB203580 (p38 inhibitor) for 1 h followed by 20 μM or without HO-3867 treatment for an additional 24 h in (A) SCC-9 and (B) HSC-3 cells were measured through Western blot analysis. Next, they were subjected to quantitative analysis. $^{*}p < 0.05$, compared with the vehicle group. $\#p < 0.05$, compared with the HO-3867-treated group

found HO-3867 triggers OSCC cell apoptosis via promoting cleaved caspase-3 through JNK1/2 signalling. As the results, HO-3867 has high potential to improve the outcome of treatment of OSCC.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

Chi-Wei Chen: Conceptualization (equal); Writing – original draft (equal); Writing – review & editing (equal). **Ming-Ju Hsieh:** Methodology (equal); Resources (equal). **Po-Chung Ju:** Methodology (equal). **Yi-Hsien Hsieh:** Methodology (equal). **Chun-Wen Su:** Methodology (equal). **Yen-Lin Chen:** Investigation (equal); Writing – review & editing (equal). **Shun-Fa Yang:** Conceptualization (equal); Writing – original draft (equal); Writing – review & editing (equal). **Chiao-Wen Lin:** Conceptualization (equal); Writing – original draft (equal); Writing – review & editing (equal).

DATA AVAILABILITY STATEMENT

The data used to support the findings of the present study are available from the corresponding author upon request.

ORCID

Yi-Hsien Hsieh https://orcid.org/0000-0003-4942-1888
Shun-Fa Yang https://orcid.org/0000-0002-0365-7927

REFERENCES

1. Thompson L. World Health Organization classification of tumours: pathology and genetics of head and neck tumours. *Ear Nose Throat J*. 2006;85(2):74.
2. Jeng JH, Chang MC, Hahn LJ. Role of areca nut in betel quid-associated chemical carcinogenesis: current awareness and future perspectives. *Oral Oncol*. 2001;37(6):477-492.
3. Shen YW, Shih YH, Fuh LJ, Shieh TM. Oral submucous fibrosis: A review on biomarkers, pathogenic mechanisms, and treatments. *Int J Mol Sci*. 2020;21(19):7231.
4. Anand R, Dhingra C, Prasad S, Menon I. Betel nut chewing and its deleterious effects on oral cavity. *J Cancer Res Ther*. 2014;10(3):499-505.
5. Su CW, Lin CW, Yang WE, Yang SF. TIMP-3 as a therapeutic target for cancer. *Ther Adv Med Oncol*. 2019;11:1758835919864247.
6. Su SC, Yeh CM, Lin CW, et al. A novel melatonin-regulated lncRNA suppresses TPA-induced oral cancer cell motility through replenishing PRUNE2 expression. *J Pineal Res*. 2021;71(3):e12760.
7. Su SC, Chang LC, Huang HD, et al. Oral microbial dysbiosis and its performance in predicting oral cancer. *Carcinogenesis*. 2021;42(1):127-135.

8. Su SC, Lin CW, Liu YF, et al. Exome sequencing of oral squamous cell carcinoma reveals molecular subgroups and novel therapeutic opportunities. *Theranostics*. 2017;7(5):1088-1099.
9. Norbury CJ, Hickson ID. Cellular responses to DNA damage. *Annu Rev Pharmacol Toxicol*. 2001;41:367-401.
10. Elmore S. Apoptosis: a review of programmed cell death. *Toxicol Pathol*. 2007;35(4):495-516.
11. Reed JC. Apoptosis-targeted therapies for cancer. *Cancer Cell*. 2003;3(1):17-22.
12. Igney FH, Krammer PH. Death and anti-death: tumour resistance to apoptosis. *Nat Rev Cancer*. 2002;2(4):277-288.
13. Szegezdi E, Logue SE, Gorman AM, Samali A. Mediators of endoplasmic reticulum stress-induced apoptosis. *EMBO Rep*. 2006;7(9):880-885.
14. Cohen GM. Caspases: the executioners of apoptosis. *Biochem J*. 1997;326(Pt 1):1-16.
15. Varfolomeev E, Goncharov T, Fedorova AV, et al. c-IAP1 and c-IAP2 are critical mediators of tumor necrosis factor alpha (TNFalpha)-induced NF-kappaB activation. *J Biol Chem*. 2008;283(36):24295-24299.
16. Salvesen GS, Duckett CS. IAP proteins: blocking the road to death's door. *Nat Rev Mol Cell Biol*. 2002;3(6):401-410.
17. Eckelman BP, Salvesen GS, Scott FL. Human inhibitor of apoptosis proteins: why XIAP is the black sheep of the family. *EMBO Rep*. 2006;7(10):988-994.
18. Srinivasula SM, Hegde R, Saleh A, et al. A conserved XIAP-interaction motif in caspase-9 and Smac/DIABLO regulates caspase activity and apoptosis. *Nature*. 2001;410(6824):112-116.
19. Altieri DC. Survivin and apoptosis control. *Adv Cancer Res*. 2003;88:31-52.
20. Wada T, Penninger JM. Mitogen-activated protein kinases in apoptosis regulation. *Oncogene*. 2004;23(16):2838-2849.
21. Dhanasekaran DN, Reddy EP. JNK signaling in apoptosis. *Oncogene*. 2008;27(48):6245-6251.
22. Selvendiran K, Ahmed S, Dayton A, et al. HO-3867, a curcumin analog, sensitizes cisplatin-resistant ovarian carcinoma, leading to therapeutic synergy through STAT3 inhibition. *Cancer Biol Ther*. 2011;12(9):837-845.
23. Dayton A, Selvendiran K, Kuppusamy ML, et al. Cellular uptake, retention and bioabsorption of HO-3867, a fluorinated curcumin analog with potential antitumor properties. *Cancer Biol Ther*. 2010;10(10):1027-1032.
24. Dayton A, Selvendiran K, Meduru S, et al. Amelioration of doxorubicin-induced cardiotoxicity by an anticancer-antioxidant dual-function compound, HO-3867. *J Pharmacol Exp Ther*. 2011;339(2):350-357.
25. Selvendiran K, Ahmed S, Dayton A, et al. HO-3867, a synthetic compound, inhibits the migration and invasion of ovarian carcinoma cells through downregulation of fatty acid synthase and focal adhesion kinase. *Mol Cancer Res*. 2010;8(9):1188-1197.
26. Selvendiran K, Tong L, Bratasz A, et al. Anticancer efficacy of a difluorodiarylidenyl piperidone (HO-3867) in human ovarian cancer cells and tumor xenografts. *Mol Cancer Ther*. 2010;9(5):1169-1179.
27. Tierney BJ, McCann GA, Cohn DE, et al. HO-3867, a STAT3 inhibitor induces apoptosis by inactivation of STAT3 activity in BRCA1-mutated ovarian cancer cells. *Cancer Biol Ther*. 2012;13(9):766-775.
28. Tierney BJ, McCann GA, Naidu S, et al. Aberrantly activated pSTAT3-Ser727 in human endometrial cancer is suppressed by HO-3867, a novel STAT3 inhibitor. *Gynecol Oncol*. 2014;135(1):133-141.
29. Hu Y, Zhao C, Zheng H, et al. A novel STAT3 inhibitor HO-3867 induces cell apoptosis by reactive oxygen species-dependent endoplasmic reticulum stress in human pancreatic cancer cells. *Anticancer Drugs*. 2017;28(4):392-400.
30. Rath KS, Naidu SK, Lata P, et al. HO-3867, a safe STAT3 inhibitor, is selectively cytotoxic to ovarian cancer. *Cancer Res*. 2014;74(8):2316-2327.
31. Mast JM, Tse D, Shee K, et al. Diarylidenylpiperidones, H-4073 and HO-3867, induce G2/M cell-cycle arrest, apoptosis and inhibit STAT3 phosphorylation in human pancreatic cancer cells. *Cell Biochem Biophys*. 2019;77(2):109-119.
32. Selvendiran K, Kuppusamy ML, Bratasz A, et al. Inhibition of vascular smooth-muscle cell proliferation and arterial restenosis by HO-3867, a novel synthetic curcuminoid, through up-regulation of PTEN expression. *J Pharmacol Exp Ther*. 2009;329(3):959-966.
33. Ravi Y, Sai-Sudhakar CB, Kuppusamy P. PTEN as a therapeutic target in pulmonary hypertension secondary to left-heart failure: effect of HO-3867 and supplemental oxygenation. *Cell Biochem Biophys*. 2021;79(3):593-607.
34. Madan E, Parker TM, Bauer MR, et al. The curcumin analog HO-3867 selectively kills cancer cells by converting mutant p53 protein to transcriptionally active wildtype p53. *J Biol Chem*. 2018;293(12):4262-4276.
35. Devor EJ, Schickling BM, Lapierre JR, et al. The synthetic curcumin analog HO-3867 rescues suppression of PLAC1 expression in ovarian cancer cells. *Pharmaceuticals (Basel)*. 2021;14(9):942.
36. Cancer Genome Atlas Research N, Weinstein JN, Collisson EA, et al. The Cancer Genome Atlas Pan-Cancer analysis project. *Nat Genet*. 2013;45(10):1113-1120.
37. Chiang YT, Chien YC, Lin YH, et al. The function of the mutant p53-R175H in cancer. *Cancers*. 2021;13(16):4088.
38. Hsieh YS, Chu SC, Yang SF, et al. Silibinin suppresses human osteosarcoma MG-63 cell invasion by inhibiting the ERK-dependent c-Jun/AP-1 induction of MMP-2. *Carcinogenesis*. 2007;28(5):977-987.
39. Lu KH, Yang HW, Su CW, et al. Phyllanthus urinaria suppresses human osteosarcoma cell invasion and migration by transcriptionally inhibiting u-PA via ERK and Akt signaling pathways. *Food Chem Toxicol*. 2013;52:193-199.
40. Chen YT, Lin CW, Su CW, et al. Magnolol triggers caspase-mediated apoptotic cell death in human oral cancer cells through JNK1/2 and p38 pathways. *Biomedicines*. 2021;9(10):1295.
41. Yang JS, Lin RC, Hsieh YH, et al. CLEFMA activates the extrinsic and intrinsic apoptotic processes through JNK1/2 and p38 pathways in human osteosarcoma cells. *Molecules*. 2019;24(18):3280.
42. Liao MY, Chuang CY, Hsieh MJ, et al. Antimetastatic effects of Eclipta prostrata extract on oral cancer cells. *Environ Toxicol*. 2018;33(9):923-930.
43. Yeh CM, Hsieh MJ, Yang JS, et al. Geraniin inhibits oral cancer cell migration by suppressing matrix metalloproteinase-2 activation through the FAK/Src and ERK pathways. *Environ Toxicol*. 2019;34(10):1085-1093.
44. Ismail NI, Othman I, Abas F, H. Lajis N, Naidu R. Mechanism of apoptosis induced by curcumin in colorectal cancer. *Int J Mol Sci*. 2019;20(10):2454.
45. Lu PW, Lin RC, Yang JS, et al. GO-Y078, a curcumin analog, induces both apoptotic pathways in human osteosarcoma cells via activation of JNK and p38 signaling. *Pharmaceuticals (Basel)*. 2021;14(6):497.
46. Duncia JV, Santella JB 3rd, Higley CA, et al. MEK inhibitors: the chemistry and biological activity of U0126, its analogs, and cyclization products. *Bioorg Med Chem Lett*. 1998;8(20):2839-2844.
47. Zhang T, Inesta-Vaquera F, Niepel M, et al. Discovery of potent and selective covalent inhibitors of JNK. *Chem Biol*. 2012;19(1):140-154.
48. Lali FV, Hunt AE, Turner SJ, Foxwell BM. The pyridinyl imidazole inhibitor SB203580 blocks phosphoinositide-dependent protein kinase activity, protein kinase B phosphorylation, and retinoblastoma hyperphosphorylation in interleukin-2-stimulated T cells independently of p38 mitogen-activated protein kinase. *J Biol Chem*. 2000;275(10):7395-7402.
49. Chien MH, Yang WE, Yang YC, et al. Dual targeting of the p38 MAPK-HO-1 axis and cIAP1/XIAP by demethoxycurcumin triggers caspase-mediated apoptotic cell death in oral squamous cell carcinoma cells. *Cancers (Basel)*. 2020;12(3):703.

50. Su CW, Chuang CY, Chen YT, et al. FLLL32 triggers caspase-mediated apoptotic cell death in human oral cancer cells by regulating the p38 pathway. *Int J Mol Sci*. 2021;22(21):11860.
51. Sanna MG, da Silva CJ, Ducrey O, et al. IAP suppression of apoptosis involves distinct mechanisms: the TAK1/JNK1 signaling cascade and caspase inhibition. *Mol Cell Biol*. 2002;22(6):1754-1766.
52. Tomeh MA, Hadianamrei R, Zhao X. A review of curcumin and its derivatives as anticancer agents. *Int J Mol Sci*. 2019;20(5):1033.
53. Kotha RR, Luthria DL. Curcumin: biological, pharmaceutical, nutraceutical, and analytical aspects. *Molecules*. 2019;24(16).
54. Lee S, Rauch J, Kolch W. Targeting MAPK signaling in cancer: mechanisms of drug resistance and sensitivity. *Int J Mol Sci*. 2020;21(3):1102.
55. Johnston PA, Grandis JR. STAT3 signaling: anticancer strategies and challenges. *Mol Interv*. 2011;11(1):18-26.
56. Duffy MJ, Synnott NC, O'Grady S, Crown J. Targeting p53 for the treatment of cancer. *Semin Cancer Biol*. 2022;79:58–67.
57. Huang J. Current developments of targeting the p53 signaling pathway for cancer treatment. *Pharmacol Ther*. 2021;220:107720.
58. Lopes EA, Gomes S, Saraiva L, Santos MMM. Small molecules targeting mutant P53: a promising approach for cancer treatment. *Curr Med Chem*. 2019;26(41):7323-7336.
59. Tzatsos A, Papavassiliou AG. Molecular "rehabilitation" by rational drug targeting: the challenge of P53 in cancer treatment. *Anticancer Res*. 1999;19(5B):4353-4356.
60. Lindemann A, Patel AA, Silver NL, et al. COTI-2, a novel thiosemicarbazone derivative, exhibits antitumor activity in hnscc through p53-dependent and -independent mechanisms. *Clin Cancer Res*. 2019;25(18):5650-5662.
61. Chen X, Pan L, Wei J, et al. LLL12B, a small molecule STAT3 inhibitor, induces growth arrest, apoptosis, and enhances cisplatin-mediated cytotoxicity in medulloblastoma cells. *Sci Rep*. 2021;11(1):6517.
62. Bhagwat SV, McMillen WT, Cai S, et al. ERK inhibitor LY3214996 targets ERK pathway-driven cancers: a therapeutic approach toward precision medicine. *Mol Cancer Ther*. 2020;19(2):325-336.
63. Okada M, Kuramoto K, Takeda H, et al. The novel JNK inhibitor AS602801 inhibits cancer stem cells in vitro and in vivo. *Oncotarget*. 2016;7(19):27021-27032.
64. Zhao L, Wang Y, Xu Y, et al. BIRB796, an inhibitor of p38 mitogen-activated protein kinase, inhibits proliferation and invasion in glioblastoma cells. *ACS Omega*. 2021;6(17):11466-11473.
65. Bixel K, Saini U, Kumar Bid H, et al. Targeting STAT3 by HO3867 induces apoptosis in ovarian clear cell carcinoma. *Int J Cancer*. 2017;141(9):1856-1866.
66. Peng H, Zhou L, Li H, et al. The therapeutic effect and mechanism of Rapamycin combined with HO-3867 on monocrotaline-induced pulmonary hypertension in rats. *Eur J Pharm Sci*. 2021;170:106102.
67. Mansouri K, Rasoulpoor S, Daneshkhah A, et al. Clinical effects of curcumin in enhancing cancer therapy: A systematic review. *BMC Cancer*. 2020;20(1):791.
68. Davoodvandi A, Farshadi M, Zare N, et al. Antimetastatic effects of curcumin in oral and gastrointestinal cancers. *Front Pharmacol*. 2021;12:668567.
69. Zhen L, Fan D, Yi X, et al. Curcumin inhibits oral squamous cell carcinoma proliferation and invasion via EGFR signaling pathways. *Int J Clin Exp Pathol*. 2014;7(10):6438-6446.
70. Kim JY, Cho TJ, Woo BH, et al. Curcumin-induced autophagy contributes to the decreased survival of oral cancer cells. *Arch Oral Biol*. 2012;57(8):1018-1025.
71. Yu HJ, Shin JA, Nam JS, Kang BS, Cho SD. Apoptotic effect of dibenzylideneacetone on oral cancer cells via modulation of specificity protein 1 and Bax. *Oral Dis*. 2013;19(8):767-774.
72. Semlali A, Contant C, Al-Otaibi B, Al-Jammaz I, Chandad F. The curcumin analog (PAC) suppressed cell survival and induced apoptosis and autophagy in oral cancer cells. *Sci Rep*. 2021;11(1):11701.
73. Lin C, Tu C, Ma Y, et al. Curcumin analog EF24 induces apoptosis and downregulates the mitogen activated protein kinase/extracellular signal-regulated signaling pathway in oral squamous cell carcinoma. *Mol Med Rep*. 2017;16(4):4927-4933.
74. Jeon H-S, Jo M-H, Kim H-J, et al. Anticancer activities of diphenyl difluoroketone, a novel curcumin analog, on KB human oral cancer cells. *J Korean Soc Appl Biol Chem*. 2012;55(4):451-456.
75. Lee HE, Choi ES, Jung JY, et al. Inhibition of specificity protein 1 by dibenzylideneacetone, a curcumin analogue, induces apoptosis in mucoepidermoid carcinomas and tumor xenografts through Bim and truncated Bid. *Oral Oncol*. 2014;50(3):189-195.
76. Utaipan T, Boonyanuphong P, Chuprajob T, Suksamrarn A, Chunglok W. A trienone analog of curcumin, 1,7-bis(3-hydroxyphenyl)-1,4,6-heptatrien-3-one, possesses ROS- and caspase-mediated apoptosis in human oral squamous cell carcinoma cells in vitro. *Appl Biol Chem*. 2020;63(1):7.
77. Kumar B, Yadav A, Hideg K, et al. A novel curcumin analog (H-4073) enhances the therapeutic efficacy of cisplatin treatment in head and neck cancer. *PLoS One*. 2014;9(3):e93208.

How to cite this article: Chen C-W, Hsieh M-J, Ju P-C, et al. Curcumin analog HO-3867 triggers apoptotic pathways through activating JNK1/2 signalling in human oral squamous cell carcinoma cells. *J Cell Mol Med*. 2022;26:2273–2284. doi:10.1111/jcmm.17248