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ORIGINAL RESEARCH

The Chemotherapy Medication of *Evodia lepta* (Spreng). Merr. on the Viability of Tongue Cancer Cells Through the PD-L1/MMP14/HSPA5 Pathway

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Background: Oral tongue squamous cell carcinoma (OTSCC), the most prevalent oral malignancy, lacks effective treatments.

Objective: Evaluate Evodia lepta (*E. lepta*) as a potential OTSCC therapeutic.

Methods: Cell viability (CCK-8) and protein expression (Western blot) were assessed in OTSCC (CAL27, TCA8113) and 3T3 cells after 24h treatment with *E. lepta* or cisplatin.

Results: Cisplatin significantly reduced the viability in all cells (IC_{50}: 3T3 = 9.5 μM; CAL27/TCA8113 = 3.5 μM). *E. lepta* selectively targeted OTSCC cells (IC_{50}: CAL27 = 80 μg/mL; TCA8113 = 60 μg/mL) with no 3T3 toxicity. Protein expression analysis revealed that *E. lepta* downregulated GPX4, ADRM1, MMP14, PD-L1, and HSPA5 in both CAL27 and 3T3 cells. Interestingly, the expression of p17 exhibited divergent regulation between cell types. In contrast, cisplatin treatment upregulated GPX4 and downregulated MMP14, PD-L1, and HSPA5 in CAL27 cells, with p17 regulation opposing that observed with *E. lepta*.

Conclusion: *E. lepta* selectively induces ferroptosis through GPX4 and HSPA5 downregulation, demonstrating multi-target effects including proteostasis disruption (ADRM1), metastasis inhibition (MMP14), and immune evasion suppression (PD-L1). Its GPX4 suppression contrasts with cisplatin's upregulation, suggesting utility in cisplatin-resistant OTSCC. PD-L1 reduction implies immunotherapeutic potential, meriting further study.

Keywords: *Evodia lepta*, cisplatin, tongue cancer, cytotoxicity, mechanism

Introduction

The "inflammation–cancer" hypothesis is a well-established framework for understanding tumorigenesis.[1–3] A central focus in oncology research is the elucidation of mechanisms by which chronic inflammation progresses to malignancy. Under physiological conditions, inflammatory cells support tissue repair and clear cellular debris; however, persistent or dysregulated inflammation leads to sustained production of growth factors and reactive oxygen species (ROS), which in turn promote genetic mutations and malignant transformation. These changes contribute to tumor initiation, progression, and metastasis.[2,4–7] Cisplatin remains a standard chemotherapeutic agent for various solid tumors, including lung and breast cancers. Despite its clinical efficacy, its use is frequently limited by severe side effects such as nephrotoxicity, ototoxicity, gastrointestinal disturbances, and myelosuppression.[8] In the context of oral cancers, particularly oral tongue squamous cell carcinoma (OTSCC)—the most prevalent subtype—therapeutic options remain suboptimal, underscoring the need for novel treatment strategies. Traditional Chinese medicines (TCMs) have attracted increasing attention as potential anticancer agents due to their broad pharmacological activities and favorable safety profiles.[9,10] According to TCM theory, the tongue reflects systemic organ health: the tip corresponds to the heart and lungs, the center to the spleen and stomach, the lateral regions to the liver and gallbladder, and the base to the kidneys.[11] Thus, anti-inflammatory herbs targeting the lung meridian may offer adjunctive benefits in managing OTSCC.

Melicope pteleifolia (Champ. ex Benth) T.G. Hartley, synonymously known as *Evodia lepta*, is a Rutaceae herb characterized by its bitter and cold nature. In traditional Chinese medicine (TCM), it is believed to act on the liver, lung,

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and stomach meridians, functioning to clear heat, eliminate toxins, promote qi circulation, relieve pain, and resolve dampness. Within the TCM classification, *E. lepta* is categorized as a heat-clearing and damp-drying herb. Clinically, it has been used to treat a range of conditions, including pharyngitis, tonsillitis, inflammatory arthralgia, malaria, jaundice, eczema, and traumatic injuries such as insect and snake bites. Pharmacological studies have identified multiple bioactive constituents in *E. lepta*, including alkaloids, coumarins, flavonoids, oligosaccharides, quinolines, and chromenes. These compounds exhibit a spectrum of biological activities, notably anti-inflammatory effects through the downregulation of pro-inflammatory cytokines such as TNF-α and IL-1β in inflammatory models. Additionally, *E. lepta* has demonstrated antibacterial, analgesic, antioxidant, antitumor, hepatoprotective, and metabolic regulatory properties, including modulation of lipid and glucose homeostasis.[12–35] Tumor immune evasion is often mediated by overexpression of programmed death-ligand 1 (PD-L1) on cancer cells, which binds to PD-1 on immune cells, leading to immune suppression. In lung adenocarcinoma, PD-L1 expression correlates with smoking history, tumor invasiveness, size, advanced TNM stage, lymph node metastasis, poor differentiation, and worse prognosis.[36,37] PD-L1 is regulated via the NF-κB/TNF-α signaling pathway,[38,39] suggesting that *E. lepta* may exert its antitumor effects in oral tongue squamous cell carcinoma (OTSCC) through this axis. Using established OTSCC cell models (TCA8113 and CAL27), we investigated the mechanistic actions of *E. lepta*. Previous studies have shown that ADRM1 knockdown inhibits NF-κB p65 nuclear translocation in leukemia cells;[40] hyperthermia suppresses tumorigenesis via the HSPA5/NF-κB/CD55 pathway in nasopharyngeal carcinoma;[41] and MMP14/TLR4/NF-κB signaling can reverse miR-7-mediated suppression of vascular inflammation.[42] Based on these findings, we hypothesize that *E. lepta* exerts antitumor effects in OTSCC via modulation of the MMP14–HSPA5–ADRM1–NF-κB–TNF-α–PD-L1 signaling axis. This proposed mechanism warrants further validation.

This study investigated the cytotoxic effects of *E. lepta* on oral tongue squamous cell carcinoma (OTSCC) cells using the CCK-8 assay and elucidated its underlying molecular mechanisms through Western blot analysis. Murine fibroblast 3T3 cells were included as non-malignant controls to assess treatment selectivity, while cisplatin was used as a standard chemotherapeutic comparator. Key proteins involved in cell death, invasion, drug resistance, and clinical prognosis were examined to identify potential therapeutic targets and signaling pathways modulated by *E. lepta*.

Materials and Methods

Reagents

Evodia lepta (Spreng). Merr. was collected from Zhangzhou (Fujian, China) in September 2021, under collection permission number JAT200735. The plant was taxonomically authenticated by Research Fellow Rongbing Chen at the Fujian Academy of Agricultural Sciences. The roots and stems were sliced and extracted with 50% ethanol for two weeks. The extract was subsequently dried into a paste at 55°C. The processed *E. lepta* extract was stored at 25°C in the Key Laboratory of Functional and Clinical Translational Medicine, Fujian Province University, Xiamen Medical College (Fujian, China). Prior to use, the extract was dissolved and diluted with sterile water. Other reagents used in this study included trypan blue (Sigma), RIPA lysis buffer (MCE), and various antibodies for Western blotting: β-actin (43 kDa), cytochrome c (12 kDa), matrix metalloproteinase 14 (MMP14, 66 kDa), programmed death-ligand 1 (PD-L1, 33 kDa), heat shock protein 70 family member 5 (HSPA5, 72 kDa), cleaved caspase-3 p17 subunit (32 kDa), and glutathione peroxidase 4 (GPX4, 22 kDa), all from HUABIO (China). Anti-adhesion regulating molecule 1 (ADRM1, 42 kDa) was obtained from Abcam. Additional materials included the PageRuler Prestained Protein Ladder (10–180 kDa, Thermo), enhanced chemiluminescence reagents (ECL, Bio-Rad), DMEM and PBS (Corning), fetal calf serum (Gemini), and trypsin (MCE). All antibodies were used according to the manufacturers' protocols.

Cell Culture

3T3, CAL27, and TCA8113 cell lines were purchased from FuHeng Biology (Shanghai, China). Cell line identity was confirmed by Short Tandem Repeat (STR) profiling, with verification documents provided in Chinese (manual available upon request). Cells were cultured and maintained in Dulbecco's Modified Eagle Medium (DMEM; MCE, China) supplemented with 10% fetal calf serum (FCS; Gemini, USA) at 37°C in a humidified incubator with 5% CO_2.

Cytotoxicity Test

3T3, CAL27 and TCA8113 cells were seeded in 96-well plates at a density of 1×10^5 cells/well in 100 μL per well; three replicate wells in each treatment group were established for all experiments. When cells in the logarithmic growth phase reached 50–70% confluence, the culture medium was replaced with fresh medium, and the cells were divided into the following groups: 1. blank group (complete cell-free medium); 2. control group (without drugs); and 3. experimental groups (with the addition of different concentrations of *E. lepta* or cisplatin at 10 μL per well); the final concentrations of *E. lepta* were 10, 20, 50, and 100 μg/mL, and those of cisplatin were 1, 2, 5, and 10 μM. After 24 h, 10 μL of CCK8 solution was added to each well, and the cells were incubated for 3 h. The optical density (OD) of 405/630 ($OD_{405/630}$) was determined using the SpectraMax i3 microplate reader, according to the manufacturer's instructions. The experiment was independently repeated 3 times.[43]

Cell survival rate = (experimental group - blank group) $OD_{405/630}$ value/(control group - blank group) $OD_{405/630}$ value × 100%.

Under microscopic observation, cells exhibiting an $OD_{405/630}$ value of approximately 0.2 were determined to be non-viable. Cell growth curves were generated by plotting optical density ($OD_{405/630}$) values against drug concentrations. The half-maximal inhibitory concentration (IC_{50}) was then estimated based on these curves. To further investigate the molecular changes associated with different stages of cytotoxicity, a drug concentration near the IC_{20} was selected for Western blot analysis and additional cytotoxicity assays.

Western Blot Analysis

CAL27 and 3T3 cell suspensions (2 mL of a 5×10^5 cells/mL suspension) were seeded in 6-well plates and incubated in a 37°C, 5% CO_2 incubator for 24 h; 100 μL of *E. lepta* or cisplatin was added separately to achieve the following final concentrations: *E. lepta*: 100 μg/mL for CAL27 cells and 250 μg/mL for 3T3 cells; cisplatin, 5, 10, and 50 μM for CAL27 cells and 50 μM for 3T3 cells. After incubation for 24 h, the cells in each group were harvested and washed twice with PBS. Then, RIPA cell lysis buffer (1 mmol/L PMSF, 1 mmol/L sodium orthovanadate, and 10 mg/L aprotinin) was added to the cells to extract total protein. The protein concentration was measured by the BCA method, and 40 μg of total protein was subjected to Western blot analysis. After 10% SDS–PAGE, membrane transfer and immunoblotting were performed. The membrane was incubated with primary antibodies. Then, the membrane was incubated with an HRP-conjugated secondary antibody. The protein bands were visualized with ECL reagent and imaged with a gel imaging system. The grayscale ratio of each target protein band with respect to the β-actin band was analyzed using Gel-Pro Analyzer software. All of the experiments were performed at least 3 times.[43]

Statistical Analysis

The data are presented as mean ± SD. Statistical analyses were conducted using GraphPad Prism 5 for Windows (GraphPad Software, San Diego, CA, USA). For data with normal distribution, one-way and two-way analysis of variance (ANOVA) was performed, followed by Dunnett's multiple comparison test to assess differences between treatment groups and controls. Count data were reported with two significant figures. A p-value < 0.05 was considered statistically significant.

Results

Viability of 3T3, CAL27, and TCA8113 Cells After Treatment with Various Concentrations of *E. lepta* and Cisplatin for 24 h

Cisplatin demonstrated concentration-dependent cytotoxicity in 3T3, CAL27, and TCA8113 cells, with calculated IC50 values of 9.5 μM, 3.5 μM, and 3.5 μM, respectively ($p < 0.05$; Table 1 and Figure 1). *E. lepta* extract similarly reduced viability in CAL27 and TCA8113 cells in a dose-responsive manner ($p < 0.05$), yielding IC50 values of approximately 80 μg/mL and 60 μg/mL. In contrast, 3T3 cell viability remained unaltered following *E. lepta* treatment (Table 2 and Figure 2), indicating significantly reduced cytotoxicity toward non-malignant cells compared to cisplatin.

Table 1 Viability of CAL27, TCA8113, and 3T3 Cells After 24-Hour Treatment with Various Concentrations of Cisplatin (Mean ± SD, n = 3)

Cell	Cisplatin (μM)				
	0	1	2	5	10
CAL27	1.20 ± 0.04	0.99 ± 0.07*	0.74 ± 0.05*	0.48 ± 0.07*	0.27 ± 0.03*
TCA8113	1.66 ± 0.01	1.21 ± 0.02*	1.05 ± 0.03*	0.76 ± 0.00*	0.29 ± 0.08*
3T3	1.46 ± 0.00	1.39 ± 0.02	1.30 ± 0.07*	1.16 ± 0.02*	0.60 ± 0.01*

Note: *$p < 0.05$ vs untreated group (0).

Changes in the Expression of Related Proteins in CAL27 and 3T3 Cells Treated with Various Concentrations of *E. lepta* or Cisplatin for 24 h

Malignant tumor cells are characterized by low differentiation, rapid growth, invasion and metastasis. Both *E. lepta* and cisplatin significantly decreased the survival rate of CAL27 and TCA8113 cells. Cell growth is related to the cell cycle and apoptosis, while the proteasome system is over-activated in the majority of tumors and affects the cell growth process. According to IC50 and cell viability results, to explore the changes in the expression of related proteins in CAL27 and 3T3 cells treated with different concentrations of *E. lepta* or cisplatin for 24 h, we measured the protein

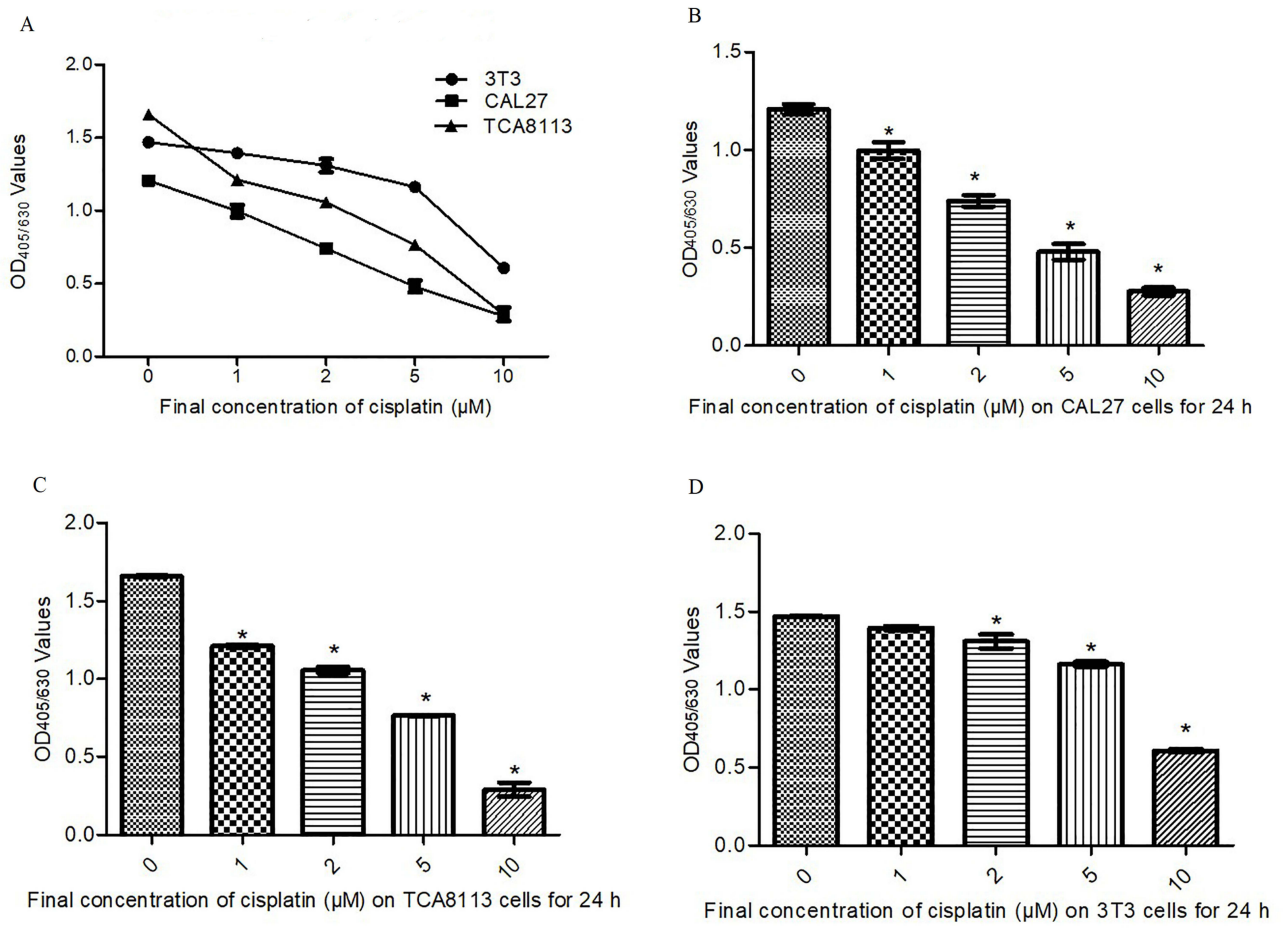

Figure 1 Cisplatin cytotoxicity in CAL27, TCA8113, and 3T3 cell lines. (**A**) Growth curves of CAL27, TCA8113 and 3T3 cells after 24h cisplatin treatment (0–10 μM, two-way ANOVA). (**B**) CAL27, (**C**) TCA8113, and (**D**) 3T3 viability dose-response. Data represent mean ± SD of three independent experiments; *$p < 0.05$ vs untreated control (one-way ANOVA with Dunnett's test). Full experimental details in Methods.

Table 2 Viability of CAL27, TCA8113, and 3T3 Cells After 24-Hour Treatment with Various Concentrations of *E. lepta* (Mean ± SD, n = 3)

Cell	*E. lepta* (μg/mL)				
	0	10	20	50	100
CAL27	1.20 ± 0.04	1.09 ± 0.04*	0.98 ± 0.07*	0.77 ± 0.04*	0.54 ± 0.06*
TCA8113	1.66 ± 0.01	1.39 ± 0.11*	1.05 ± 0.01*	0.89 ± 0.04*	0.53 ± 0.04*
3T3	1.47 ± 0.00	1.52 ± 0.04	1.52 ± 0.01	1.43 ± 0.06	1.49 ± 0.07

Note: *$p < 0.05$ vs untreated group (0).

expression levels of MMP14, PD-L1, HSPA5, ADRM1, p17, and GPX4, which are related to tumor cell growth and prognosis, through Western blotting.

CAL27 cells were treated with 20 μg/mL *E. lepta* for 24 h. The survival rate of CAL27 cells treated with *E. lepta* significantly decreased; some cells adhered weakly and even detached but remained viable. The above-mentioned proteins were differentially expressed between the fully adherent cells and the weakly adherent or floating cells. The total population of *E. lepta*-treated cells and the adherent cells alone were collected separately. The former group of cells was harvested and denoted as the *E. lepta*-20-all (E20a) group, while the adherent cells were harvested and denoted as the *E. lepta*-20 (E20) group. 3T3 cells were treated with 50 μg/mL *E. lepta* for 24 h, and only the adherent cells were harvested; these cells were denoted as the *E. lepta*-50 (E50) group. CAL27 cells were treated with 1 or 2 μM cisplatin

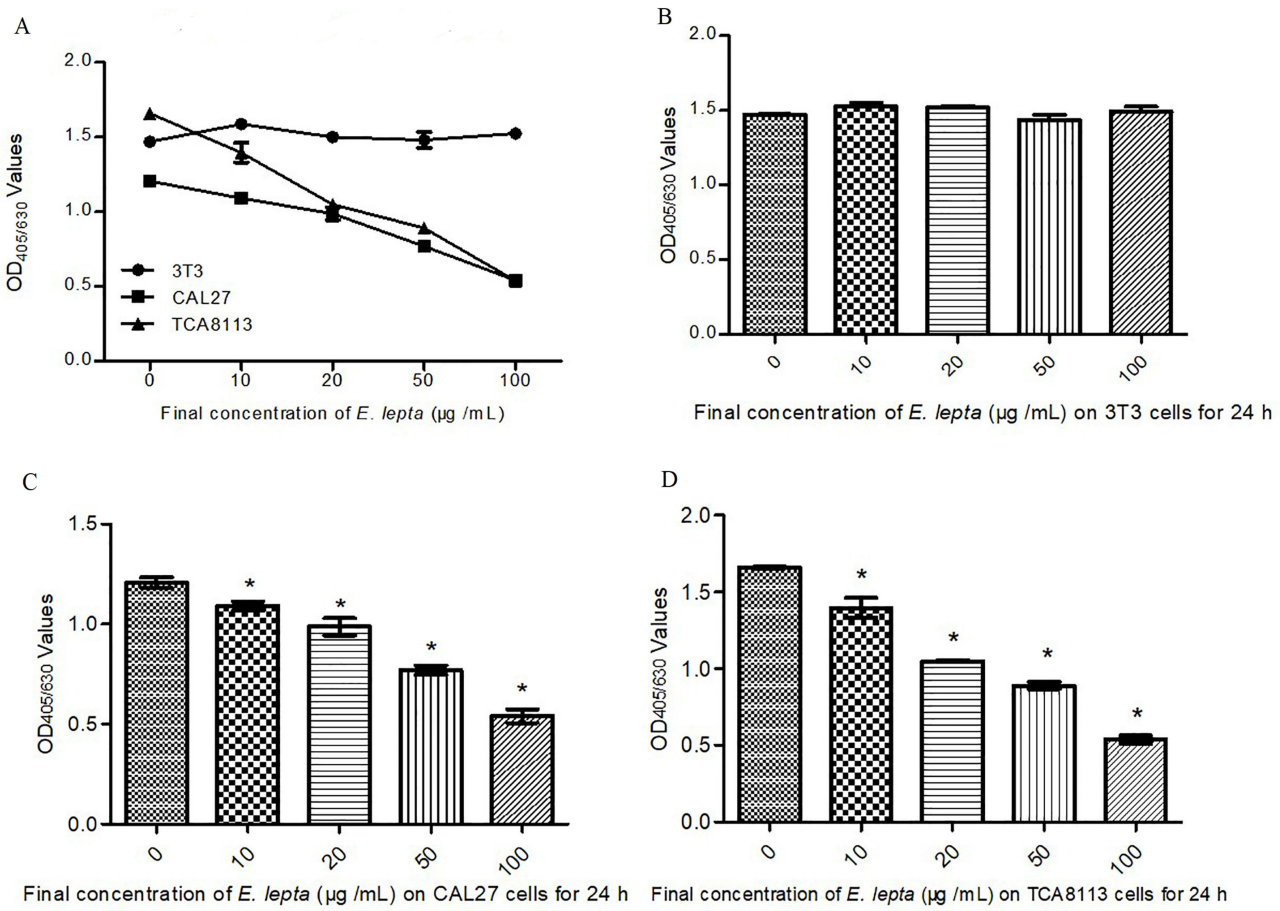

Figure 2 Dose-dependent effects of *E. lepta* on cell viability (**A**) Growth curves of CAL27, TCA8113 and 3T3 cells after 24h treatment with *E. lepta* (0–100 μg/mL, two-way ANOVA). (**B**) 3T3, (**C**) CAL27, and (**D**) TCA8113 viability dose-response. Data = mean ± SD (n = 3); *$p < 0.05$ vs untreated control (one-way ANOVA with Dunnett's test). See Methods for details.

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Table 3 Protein Expression Profiles in CAL27 and 3T3 Cells Treated with Increasing Concentrations of *E. lepta* or Cisplatin for 24 Hours. Results are Presented as Mean ± SD (n = 3)

	Mean ± SD					
	GPX4	**p17**	**ADRM1**	**HSPA5**	**PD-L1**	**MMP14**
CAL27-con	1	1	1	1	1	1
E. lepta-20	0.34 ± 0.02*	0.03 ± 0.00*	0.50 ± 0.00	0.42 ± 0.01*	0.08 ± 0.00*	0.46 ± 0.00*
E. lepta-20A	0.74 ± 0.02*	0.43 ± 0.01*	0.40 ± 0.01	0.78 ± 0.01*	0.66 ± 0.01*	0.71 ± 0.01*
Cisplatin-1	1.43 ± 0.00*	1.41 ± 0.02*	1.25 ± 0.04	0.67 ± 0.01*	0.81 ± 0.00*	0.86 ± 0.04
Cisplatin-2	1.87 ± 0.08*	3.55 ± 0.08*	0.53 ± 0.04	0.51 ± 0.02*	0.68 ± 0.03*	0.63 ± 0.01*
3T3-con	1	1	1	1	1	1
E. lepta-50	0.47 ± 0.04#	1.47 ± 0.19#	0.48 ± 0.04#	0.65 ± 0.07#	0.30 ± 0.03#	0.50 ± 0.06#
Cisplatin-10	0.61 ± 0.04#	1.06 ± 0.12	0.62 ± 0.04#	0.35 ± 0.03#	0.30 ± 0.02#	1.51 ± 0.20#

Notes: Set the control group value as 1. *$p < 0.05$ vs the CAL27 con group; #$p < 0.05$ vs the 3T3 con group.

and 3T3 cells were treated with 10 μM cisplatin for 24 h; the adherent cells from each group were harvested and denoted as the C1, C2, and C10 groups, respectively. The results are presented and shown in Table 3 and Figure 3.

For CAL27 cells, the expressions of the GPX4, p17, ADRM1, MMP14, PD-L1, and HSPA5 proteins were downregulated in E20a and E20 groups, and the degree of downregulation in E20a group was significantly greater than in E20 group for all proteins except ADRM1, which exhibited the opposite trend. The expressions of both GPX4 and p17 were upregulated in C1 and C2 groups, with a significantly greater degree of upregulation in C2 group than in C1 group. The expressions of MMP14, PD-L1, and HSPA5 were downregulated in C1 and C2 groups, with the degree of downregulation in the latter group significantly greater than that in the former group. ADRM1 protein expression was upregulated in C1 group and downregulated in C2 group. For 3T3 cells, the expressions of GPX4, ADRM1, PD-L1, and HSPA5 were downregulated and the expression of p17 was upregulated in both E50 and C10 groups; the expression of MMP14 was downregulated in E50 group and upregulated in C10 group. Additionally, *E. lepta* and cisplatin downregulated PD-L1 and HSPA5 in both CAL27 and 3T3 cells, while MMP14 was downregulated only in CAL27 cells. The changes in GPX4, p17, and ADRM1 expression were differentially related to the drug concentration and cell line.

Discussion

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer worldwide; half of head and neck cancers are oral squamous cell carcinoma (OSCC), and O SCC accounts for at least 90% of all malignant oral tumors.[44–46] Furthermore, OTSCC is one of the most common oral cancers, and its incidence has increased in recent years.[47] However, the survival rate of OTSCC patients has not significantly increased in the last decade. In the present study, cisplatin significantly decreased the viability of 3T3, CAL27 and TCA8113 cells ($p < 0.05$), while *E. lepta* significantly reduced the viability of CAL27 and TCA8113 cells ($p < 0.05$), with IC50 values of approximately 80 and 60 μg/mL, respectively, but had no significant effect on the 3T3 cells. Furthermore, *E. lepta* significantly downregulated the expression of GPX4, p17, ADRM1, MMP14, PD-L1, and HSPA5 in CAL27 cells. In 3T3 cells, a similar downregulation trend was observed for most of these proteins, with the exception of p17, which was upregulated. Cisplatin upregulated GPX4 and p17 and downregulated MMP14, PD-L1, and HSPA5 in CAL27 cells. It implies that *E. lepta* notably decreased the viability of CAL27 and TCA8113 OTSCC cells without injurious normal healthy cells, providing a new hope for OTSCC patients.

When apoptosis is initiated, caspase-3, a cysteine-aspartic protease, is activated by cleavage to generate a 17 kDa large subunit (ie, p17) and a 12 kDa small subunit (ie, p12). P17 and p12 dimerize to form the activated form of cleaved caspase-3.[48] In contrast to cisplatin, *E. lepta* downregulated p17 in CAL27 cells. The evidence has shown that apoptosis and ferroptosis contribute to cell death. The exact underlying mechanism remains to be further studied. ADRM1 is overexpressed in many types of solid tumors and promotes the proliferation and metastasis of cancer cells.[49,50] ADRM1 has demonstrated prognostic significance in bladder cancer (BC) and has predictive potential for both immunotherapy and chemotherapy responses involving immune-related pathways.[51] The inhibition of ADRM1

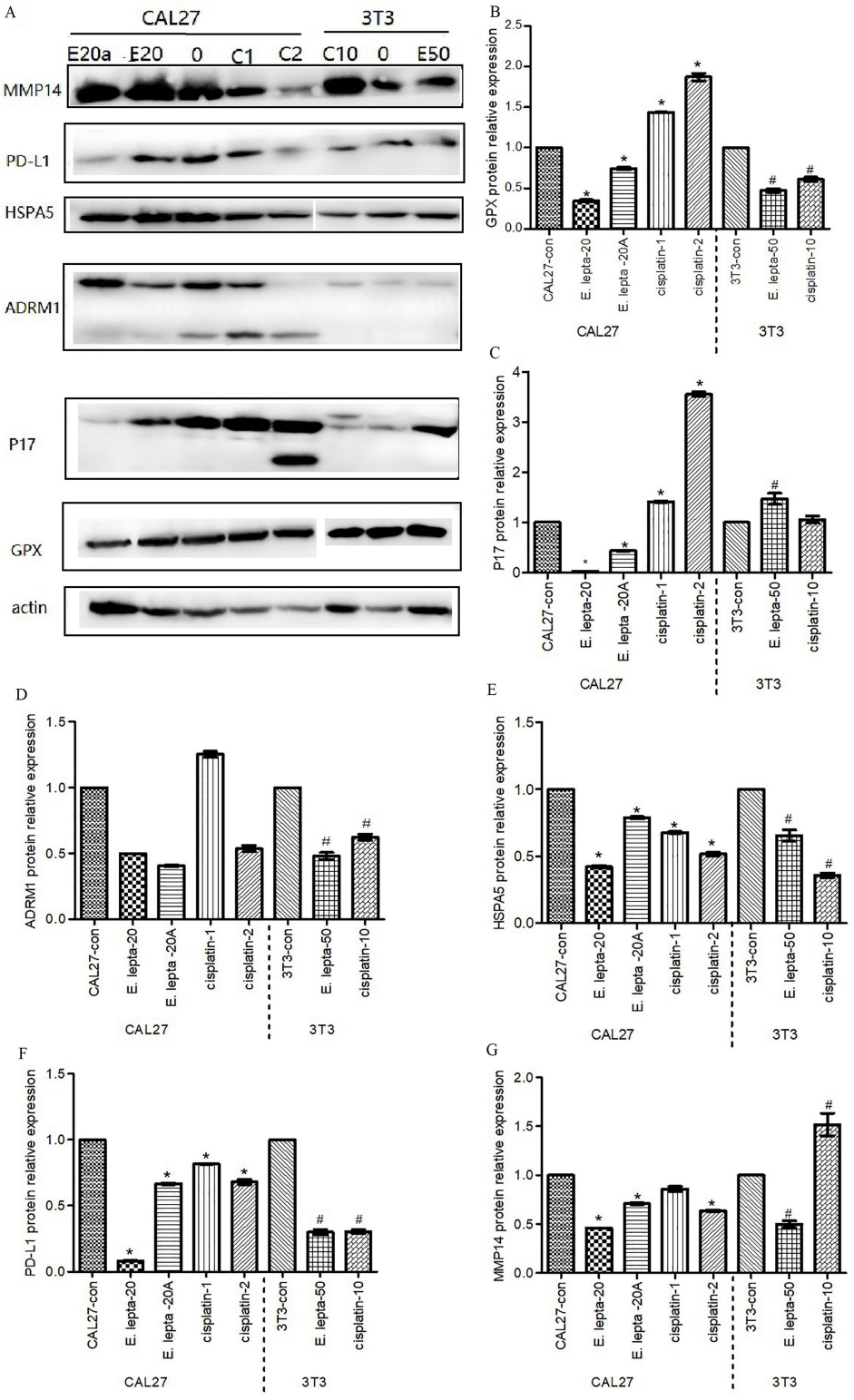

Figure 3 Protein expression profiles following *E. lepta* and cisplatin treatment. (**A**) Western blots of key proteins in CAL27 and 3T3 cells after 24h treatment with *E. lepta* (0, 20, 50 μg/mL) or cisplatin (0, 1, 2, 10 μM). Quantitative analysis of (**B**) GPX4, (**C**) p17, (**D**) ADRM1, (**E**) HSPA5, (**F**) PD-L1, and (**G**) MMP14 expression normalized to β-actin. Data = mean ± SD (n = 3); $^{*}p < 0.05$ vs CAL27 control, $^{\#}p < 0.05$ vs 3T3 control (one-way ANOVA with Dunnett's test). Full methods in Materials and Methods.

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suppresses cancer cell growth in vitro.[50] *E. lepta* displayed activity as an ADRM1 inhibitor in CAL27 cells and may thus be used for OTSCC treatment. In addition, PD-L1 upregulation in OTSCC is associated with a more advanced disease stage and a shorter disease-free survival time.[52] PD-L1 expression was downregulated in CAL27 cells treated with *E. lepta* and cisplatin, suggesting an improvement in prognosis and an increase in the disease-free survival time.

Upregulation of HSPA5 has been accompanying with poor prognosis in HNSCC patients.[53] As shown in Figure 3, both *E. lepta* and cisplatin may affect CAL27 cell metastasis and improve patient prognosis. HSPA5 is highly expressed in endometrial cancer and cervical cancer[54] and is associated with poor prognosis in cervical cancer patients; it may affect the proliferation and apoptosis of cervical cancer cells by upregulating the expression of the ferroptosis-related protein GPX4.[55] The GPX family is an important group of free radical scavenging molecules. One member of the GPX family, GPX4, has a strong ability to convert peroxides and can scavenge excess hydroperoxides to maintain ROS homeostasis, thus protecting the body from damage. Mitochondria are responsible for controlling cell death during the early stages of radiation exposure, while the GPX enzyme is essential for the maintenance of mitochondria-derived ROS levels.[56] The protein level of GPX4 was upregulated in gastric cancer tissues, and the β-catenin/TCF4 transcription complex can directly binds to the promoter region of GPX4 and induces its expression, resulting in the suppression of ferroptotic cell death. Concordantly, TCF4 deficiency promotes cisplatin-induced ferroptosis in vitro and in vivo.[57] In this study, GPX4 expression was significantly downregulated in CAL27 cells treated with *E. lepta*. This downregulation may inhibit intracellular free radical scavenging, which accelerates CAL27 cell apoptosis. In contrast, GPX4 upregulation in the surviving CAL27 cells treated with cisplatin was with respect to increased cisplatin resistance. As the cisplatin concentration decreased, the expression of GPX4 in CAL27 cells also decreased, but the underlying mechanism remains to be further studied. And GPx activity should be also determined in subsequent experiments.

MMP14 is an important factor that promotes angiogenic mimicry and is located mainly on the tumor cell membrane; it can regulate the biological behavior of cancer cells through the Wnt/β-catenin signaling pathway, promoting tumor proliferation, apoptosis inhibition, and cell invasion and metastasis. MMP14 is associated with TNM stage and lymphatic metastasis[58–61] and plays a critical role in the early diagnosis and prognosis of cancer.[62] Cetyltrimethylammonium bromide downregulates MMP14 and significantly inhibits the adhesion, migration, and invasion of OTSCC cells, independent of cell viability.[63] Treatment with either 50 or 100 mg/mL lactalbumin enzymatic hydrolysate (LAH), the hydrolysis product of alpha-lactalbumin, exhibited cytotoxicity in OTSCC cells, decreasing their proliferation and colony formation abilities, accompanied by downregulation of MMP14 protein expression.[64] Similarly, the MMP14 protein was downregulated in surviving CAL27 cells treated with 20 μg/mL *E. lepta* for 24 h. Importantly, MMPs play a pivotal role in tumor progression by facilitating the degradation of the basement membrane and subsequently promoting cancer metastasis.[65] Collectively, our results suggest that *E. lepta* may partially control the expression of MMPs, which play a key role in the metastasis of OTSCC cells.

Conclusions

This study presents the first evidence that *Evodia lepta* (*E. lepta*) selectively reduces the viability of OTSCC cells while sparing normal murine fibroblasts (3T3), highlighting its potential as a targeted anticancer agent. Notably, *E. lepta* and cisplatin appear to modulate overlapping molecular pathways in OTSCC cells. The cytotoxic effects of *E. lepta* are mediated, at least in part, through the ADRM1/MMP14/PD-L1/HSPA5 signaling axis, resembling the action of cisplatin. However, *E. lepta* uniquely preserves the viability of normal cells, possibly through modulation of the GPX4/p17 pathway or other yet-to-be-identified mechanisms, as supported by CCK-8 and Western blot data. Based on these findings, we propose two future research directions: 1. Combination Therapy Strategy: Co-administration of *E. lepta* with cisplatin in OTSCC models may enhance antitumor efficacy while reducing off-target toxicity. This strategy exploits their opposing effects on key regulators such as GPX4 and p17, potentially offering a synergistic or chemosensitizing benefit. 2. Mechanism-Driven in vivo Validation: Establishing CAL27 and TCA8113 xenograft models will allow the assessment of *E. lepta*'s tumor-selective activity, particularly its downregulation of GPX4, PD-L1, and HSPA5; differential p17 expression in tumor versus normal tissues; and its potential to suppress PD-L1 in vivo, thereby enhancing immune responsiveness. Importantly, systemic toxicity profiles should be compared to those of cisplatin monotherapy.

Overall, *E. lepta* demonstrates promising selective cytotoxicity against OTSCC, likely mediated through ferroptosis induction via GPX4 and HSPA5 downregulation. Its selectivity may be attributed to cell-type-specific regulation of p17, which differs between malignant and non-malignant cells. Furthermore, *E. lepta* exhibits broad antitumor activity by targeting proteostasis (ADRM1), metastatic potential (MMP14), and immune evasion (PD-L1). The contrast between *E. lepta*'s and cisplatin's regulatory effects on GPX4 and p17 underscores its potential utility in overcoming cisplatin resistance or serving as an adjuvant therapy. The immunomodulatory properties, particularly PD-L1 suppression, further strengthen its candidacy. These findings warrant further mechanistic exploration and validation through in vivo studies and clinical trials.

Data Sharing Statement

All data, models, and code generated or used during the study appear in the submitted article.

Ethics Approval and Consent to Participate

This research was approved by the Ethics Review Committees of Xiamen University.

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Disclosure

The authors declare that they have no conflicts of interest in this work.

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