

Evodiamine triggers ferroptosis by inhibiting the c-Fos/SLC7A11 axis to suppress tumor growth in head and neck squamous cell carcinoma

Cheng-Lung Hsu

hsu2221@adm.cgmh.org.tw

chang gung memorial hospital, Linkou, Taiwan <https://orcid.org/0000-0002-8197-3079>

Chiao-Yun Lin

Linkou Chang Gung Memorial Hospital <https://orcid.org/0000-0002-1256-7018>

Chen-Yang Huang

Yu-Li Chen

chang gung memorial hospital, Linkou, Taiwan

Jai-Shin Liu

Kar-Wai Lui

Yin-Kai Chao

Li-Yu Lee

Chia-Hsun Hsieh

Hsien-Chi Fan

An-Chi Lin

Shiang-Fu Huang

Chien-Yu Lin

Hung-Ming Wang

Chia-Lung Tsai

Chwan-Fwu Lin

Hsin-Pai Li

Chang Gung Memorial Hospital

Tsong-Long Hwang

Chang Gung University of Science and Technology <https://orcid.org/0000-0002-5780-3977>

Article

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Abstract

Head and neck squamous cell carcinoma (HNSCC) is associated with poor prognosis and limited therapeutic options in advanced disease. Here, we investigated the anticancer activity of evodiamine (EVO), a major bioactive compound of *Wu Zhu Yu* (WZY), with a focus on redox regulation and ferroptosis. EVO exhibited potent cytotoxicity in HNSCC cell lines and significantly suppressed tumor growth in patient-derived xenograft (PDX) mouse models, with additive effects observed in combination with docetaxel. Transcriptomic analysis identified SLC7A11 and c-Fos as significantly downregulated following EVO treatment, which was validated at both mRNA and protein levels. Mechanistically, EVO disrupted redox homeostasis by increasing reactive oxygen species (ROS), promoting lipid peroxidation, reducing the GSH/GSSG ratio, and inducing mitochondrial damage, which is consistent with ferroptosis. EVO also enhanced the antitumor efficacy of the GPX4 inhibitor RSL3. Our molecular docking model supports EVO directly engages xCT and competitively occupies the PX8-binding pocket. Clinically, elevated SLC7A11 expression correlated with advanced buccal cancer and poor prognosis. Furthermore, c-Fos was able to transcriptionally transactivate *SLC7A11*; however, this transactivation was suppressed by EVO. These findings identify EVO as a novel inducer of ferroptosis through targeting the c-Fos/SLC7A11 axis and disrupting redox balance in HNSCC.

1. Introduction

(1) HNSCC

Head and neck squamous cell carcinoma (HNSCC) is a heterogeneous group of malignancies arising from the mucosal epithelium of the upper aerodigestive tract, including the oral cavity, pharynx, and larynx, and accounts for more than 90% of all head and neck cancers ¹. Globally, HNSCC represents a major public health burden with substantial morbidity and mortality. The principal etiological risk factors include tobacco use, excessive alcohol consumption, and infection with high-risk human papillomavirus (HPV), particularly HPV-16, which is strongly associated with oropharyngeal squamous cell carcinoma ¹, ². Locally advanced disease (stage III–IV) usually requires multimodality treatment, including surgery followed by adjuvant RT or concurrent chemoradiotherapy (CRT), with platinum-based chemotherapy serving as the standard radiosensitizer ². For patients with recurrent or metastatic HNSCC, systemic therapy remains the cornerstone of treatment, and the emergence of immune checkpoint inhibitors targeting programmed cell death-1 (PD-1), such as pembrolizumab or nivolumab, has significantly improved survival outcomes and has become incorporated into current therapeutic strategies ^{1, 3}.

(2) Ferroptosis

Ferroptosis is a distinct form of regulated cell death characterized by iron-dependent lipid peroxidation and the accumulation of reactive oxygen species (ROS), leading to oxidative damage of cellular membranes ⁴. Unlike apoptosis or necrosis, ferroptosis is primarily driven by dysregulated iron

metabolism and impaired lipid peroxide detoxification systems, particularly involving depletion of glutathione (GSH) and inactivation of glutathione peroxidase 4 (GPX4), a key antioxidant enzyme that protects cells from lipid peroxidation^{4,5}. Notably, growing evidence suggests that ferroptosis plays a critical role in the pathogenesis and treatment response of head and neck squamous cell carcinoma (HNSCC)⁶. Several studies have demonstrated that dysregulation of ferroptosis-related genes and pathways contributes to tumor growth, metastasis, and therapeutic resistance in HNSCC^{7,8}. Furthermore, induction of ferroptosis has been shown to enhance the antitumor efficacy of conventional therapies such as chemotherapy, radiotherapy, and immunotherapy, highlighting its potential as a novel therapeutic strategy for HNSCC management⁹. Therefore, targeting ferroptosis-associated pathways may provide new opportunities for improving treatment outcomes in patients with HNSCC.

(3) Evodiamine

Evodiamine (EVO) from *Tetradium ruticarpum* (Chinese name: Wu Zhu Yu, (WZY))¹⁰ is a quinazolinocarboline alkaloid and has attracted increasing attention for its potent anticancer activities in recent years¹¹. Numerous studies have demonstrated that EVO exerts inhibitory effects on multiple types of cancer by regulating diverse cellular processes, including cell proliferation, apoptosis, cell cycle arrest, and metastasis¹¹. Mechanistically, EVO modulates several key oncogenic signaling pathways, such as PI3K/AKT, STAT3, and p53-related pathways, thereby suppressing tumor cell growth and survival¹². In addition, EVO has been shown to induce oxidative stress and mitochondrial dysfunction, leading to programmed cell death in cancer cells. Moreover, EVO has been reported to inhibit tumor invasion, stemness, and immune evasion in several malignancies, suggesting its potential role as a multi-target therapeutic compound in cancer treatment¹³.

2. Materials and methods

2.1. Drugs

Evodiamine (EVO) and Rutaecarpine were obtained from MedChemExpress (Monmouth Junction, NJ, USA). Erastin, (1S,3R)-RSL 3 (RSL3), and N-acetylcysteine (NAC) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Docetaxel (Taxotere®, Sanofi-Aventis Deutschland GmbH, Frankfurt am Main, Germany).

2.2. Patients

Between January 2024 and December 2025, 20 histologically diagnosed squamous cell carcinoma locally advanced/metastatic head and neck cancer patients were enrolled for patient-derived xenograft study. Tumor sample collection was performed in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board of Chang Gung Memorial Hospital (IRB No.202301109B0). Written informed consent was obtained from all patients.

2.3. Cancer cell lines and animal studies

Squamous cell carcinoma 4 (SCC4) was purchased from the American Type Culture Collection (Manassas, VA, USA). The PDX establishment procedure has been described previously^{14,15}. We had successfully established 5 HNSCC PDXs and the patients' clinical information were listed in Supplemental Table 1. HNSCC-1 and HNSCC-2 cell lines were cultured and derived from our HNSCC PDX-1 and PDX-2 tumors, respectively. Mice were maintained and handled in accordance with the Chang Gung Memorial Hospital (CGMH) Institutional Animal Care and Use Committee (IACUC) guidelines. All animal experiments were approved by the CGMH IACUC (IACUC No. 2023072501).

2.4. Cell viability assay and treatment combination analysis

HNSCC cell lines with or without treatment were collected to assess cell viability using MTT. For clonogenic assay, 1000–2000 cells/well were plated in triplicate in 6-well plate. Cells were treated with indicated drugs for 24 h and then allowed to recover in drug-free medium for 14 days. Cells were then washed with 1X PBS and stained with a 0.5% crystal violet (Solarbio) solution in 25% methanol for 10 min. Plates were then scanned to obtain pictures.

2.5. Bulk RNA sequencing and differential expression gene analysis

Details of RNA extraction and sequencing were described previously¹⁴. RNA was extracted from the cells by using TRIzol/chloroform method. The raw fastq reads were aligned to a human–mouse hybrid genome (GRCh38 and GRCm39 build) by using the STAR software. The reads aligned to the mouse genome were removed from downstream analysis. Transcript abundance was evaluated using the STAR software with the default parameters. Differentially expressed gene (DEG) analysis and gene ontology enrichment were performed after calibration with apegln package.

2.6. Lipid peroxidation assay

Cells were seeded in triplicate in 6- or 12-well plates, allowed to adhere for 24 h, and treated with the indicated compounds for the specified durations. Cells were then trypsinized, washed with PBS, and stained with C11-BODIPY 581/591 (2.5 μ M; Cayman Chemical, #27086) in serum-free medium at 37°C for 30 min in the dark. After washing, cells were immediately analyzed using a BD FACSLyric flow cytometer. Oxidation of C11-BODIPY shifts fluorescence emission from ~ 590 nm to ~ 510 nm. Live cells were gated by forward and side scatter, and lipid peroxidation was quantified as the percentage of cells with increased FL1 (FITC) fluorescence relative to controls.

2.7. Glutathione and malondialdehyde (MDA) measurement

Cells were seeded in 6-cm culture dishes and harvested after 24 h. GSH/GSSG levels were measured using a GSH/GSSG Ratio Detection Assay Kit (ab138881, Abcam, Cambridge, UK), and MDA levels were determined using a Lipid Peroxidation MDA Assay Kit (ab238537, Abcam, Cambridge, UK), according to the manufacturers' instructions.

2.8. Transmission electron microscopy

Briefly, cells subjected to the indicated treatments were fixed in 3% glutaraldehyde and 2% paraformaldehyde in 0.1 M cacodylate buffer (pH 7.4) at 4°C, followed by post-fixation with 1% OsO₄ for 2 h. Samples were dehydrated through a graded ethanol series, embedded in EPON-812, and sectioned into 80-nm ultrathin slices. Sections were stained with uranyl acetate and lead citrate and examined using a Hitachi HT-7800 transmission electron microscope (Hitachi, Tokyo, Japan).

2.9. EVO-SLC7A11 (xCT) docking model

Molecular docking calculations were conducted using AutoDock Vina, with ligand preparation performed in AutoDockTools 1.5.6. The SLC7A11 protein structure (PDB: 7EPZ) and the EVO (CID: 442088) and PX8 (CID: 447938) structures were retrieved from the Protein Data Bank and PubChem, respectively. All 3D structural models were visualized using PyMOL (v.3.1.6.1).

2.10. SLC7A11 promoter activity and Chromatin immunoprecipitation (ChIP) assays

2.10.1. SLC7A11 promoter reporter plasmid construction

SLC7A11 promoter region (– 1747 and + 8) was amplified by PCR. The sequences of the primers were as follows: 5'-AGCTCTCGAGACAAACCAGCTCAGCTTCCT-3' (sense), 5'-AGCTGGTACCTTGCTGTTCCCTGAGATTCC-3' (antisense). The promoter fragment was digested with *Xho*I and *Kpn*I, and subcloned into the corresponding sites of the pGL3-basic luciferase reporter vector (Promega, Madison, WI, USA).

2.10.2. SLC7A11 promoter reporter luciferase assay

Cells were co-transfected with 1 µg *SLC7A11* promoter–luciferase reporter plasmid, 0.5 µg pCMV β-galactosidase plasmid, and 1 µg vector or c-Fos expression plasmid (Addgene #187905, Cambridge, MA, USA) using Lipofectamine 2000 (Thermo Fisher Scientific, USA). Cell lysates were prepared with Reporter Lysis Buffer (Promega, E3971), and luciferase activity was measured using Luciferase Assay Reagent (Promega, E1500). β-Galactosidase activity was determined by a colorimetric ONPG or CPRG assay and used to normalize luciferase activity.

2.10.3. Chromatin immunoprecipitation (ChIP) assays

Chromatin immunoprecipitation was performed using a CUT&RUN assay kit (Cell Signaling Technology, Cat. No. 86652) according to the manufacturer's instructions. Antibodies against c-Fos (Cell Signaling Technology, Cat. No. 2250) and rabbit IgG (Bioss, Cat. No. bs-0295P) were used as the experimental and

negative controls, respectively. Enrichment of the SLC7A11 promoter were assessed using primers targeting the c-Fos binding region – 1746¹⁶ and – 1400 to -1200¹⁷.

2.11. Antibodies

The following antibodies were used: SLC7A11 for Western blot (ab307601) and immunohistochemistry (IHC) (ab37185), GPX4 (ab125066), and FACL4 (ab155282) were from Abcam (Cambridge Biomedical Campus, Cambridge, United Kingdom); Tubulin (AF7010) was from Affinity Biosciences; and c-Fos for Western blot and IHC (66590-1-Ig) were from Proteintech (Rosemont, IL, USA).

2.12. Statistical analysis

The median survival time of patients with NPC was estimated as the first observed event (death) at which the Kaplan–Meier survival probability was equal to 1.5. Furthermore, overall survival was calculated as the period from recurrence or metastasis to death, estimated by Kaplan–Meier plotting, and compared via log-rank test. Two-tailed p-values below 0.05 were considered statistically significant.

3. Results

3.1 Anticancer efficacy screening of WZY water and ethanol extracts in HNSCC

We established five patient-derived xenografts (PDXs) and two corresponding cell lines from advanced HNSCC patients (Supplementary Table 1). To identify active anticancer components of the herbal formula Ji-Ming-San, Wu Zhu Yu (WZY) was extracted using water and pure ethanol. The anticancer activities of these extracts were evaluated in HNSCC cell lines by MTT assay (Table 1 and Supplementary Data 2). Among the tested extracts, the pure ethanol extract of WZY exhibited the strongest anticancer activity.

Table 1
Wu Zhu Yu MMT assay

SCC-4		HNSCC-C-1		HNSCC-C-2	
Water extraction					
mg/ml	average ± sd(%)	mg/ml	average ± sd(%)	mg/ml	average ± sd(%)
1	20.4 ± 0.6	1	6.6 ± 0.9	1	50.9 ± 1.3
0.1	61.9 ± 2.7	0.1	72.5 ± 0.5	0.1	94.5 ± 3.4
0.01	100.6 ± 1.0	0.01	96.7 ± 3.6	0.01	100.4 ± 1.8
Ethanol extraction					
mg/ml	average ± sd	mg/ml	average ± sd	mg/ml	average ± sd
1	7.4 ± 0.3	1	7.9 ± 0.3	1	15.0 ± 0.4
0.1	39.3 ± 0.9	0.1	9.0 ± 0.3	0.1	17.1 ± 1.0
0.01	36.9 ± 1.1	0.01	12.8 ± 0.6	0.01	63.9 ± 0.7

3.2 Anticancer efficacy of EVO in HNSCC cell lines and PDX models

Evodiamine (EVO) and Rutaecarpine are the major active anticancer component in WZY, exhibiting its effects via multiple mechanisms¹². We tested the anticancer efficacy of EVO and Rutaecarpine in HNSCC cell lines and the IC₅₀ ranged between 1.49 x10⁻⁷~3.92x10⁻⁷ M for EVO and 3.04 x10⁻⁶~7.69 x10⁻⁶ for Rutaecarpine (Fig. 1A and 1B). We further tested EVO antitumor growth effect in HNSCC PDX mouse models. EVO had antitumor growth effect in both HNSCC PDX mouse models and had prominent additive antitumor and cancer related leukocytosis effect when combined with Taxotere (TAX) (Fig. 1C ~ 1F). In HNSCC PDX-2, EVO also demonstrated effective antitumor growth effect (Fig. 1G ~ 1I). Taking together, our data indicated that EVO is a promising anticancer candidate for HNSCC.

3.3 Transcriptome analysis of EVO treatment in the HNSCC PDX tumors and their derived cell lines

To understand the signaling pathways and molecular mechanisms that underlie the antitumor effect of EVO in HNSCC PDX mouse models, RNA-seq was performed. Transcriptome analysis revealed that *c-Fos* and *SLC7A11* (*Solute Carrier Family 7 Member 11*) were the two most significantly downregulated genes in the EVO-treated HNSCC PDX-1 (Fig. 2A and 2B). We further confirmed this mRNA downregulation via RT-qPCR (Fig. 2C). In HNSCC PDXs and cell lines, we also found SCL7A11 was highly expressed (Fig. 2D and 2E). We also confirmed the overexpression of SLC7A11 protein in the HNSCC PDX-1 tumor, but not the non-tumor region, via IHC staining, as indicated in Fig. 2F.

3.4 EVO induces lipid ROS accumulation, ferroptosis, mitochondrial damage, and inhibits colony formation in HNSCC

SLC7A11 (xCT), a cystine–glutamate antiporter, promotes glutathione biosynthesis to suppress ROS accumulation, lipid peroxidation, and ferroptosis. We therefore hypothesized that EVO may impair SLC7A11-mediated antioxidant defense.

Lipid peroxidation was first assessed using the C11-BODIPY 581/591 probe in HNSCC cells treated with EVO or erastin (SLC7A11 inhibitor). Both treatments induced a rightward fluorescence shift, indicating increased lipid ROS, with a stronger effect observed for erastin. Consistently, EVO increased intracellular malondialdehyde (MDA) levels in HNSCC-1 cells (Fig. 3B). In addition, the GSH/GSSG ratio was significantly reduced by 60–80% following EVO or erastin treatment (Fig. 3C).

Increased lipid ROS was further confirmed by Liperfluor staining and confocal microscopy after EVO treatment (Fig. 3D). Transmission electron microscopy revealed characteristic ferroptotic mitochondrial alterations, including reduced cristae in EVO-treated HNSCC-1 cells compared with controls (Fig. 3E). Finally, EVO or erastin significantly reduced colony formation in HNSCC cells (Fig. 3F).

Collectively, these results demonstrate that EVO induces lipid ROS accumulation and ferroptosis, disrupts mitochondrial integrity, and suppresses HNSCC cell proliferation.

3.5 EVO modulates ferroptosis regulators and sensitizes cells to ferroptosis induction

We further characterized the concentration- and time-dependent effects of EVO in HNSCC. EVO exhibited an optimal inhibitory effect on SLC7A11 protein expression at approximately 1 μ M after 24 hours of treatment. A rebound phenomenon was observed following prolonged treatment, as shown in Fig. 4A and 4B. Additionally, EVO demonstrated a potential additive effect with RSL3, a GPX4 inhibitor and ferroptosis inducer, in suppressing HNSCC cell growth, as illustrated in Fig. 4C and 4D.

3.6 EVO targets SLC7A11 in silico and in vitro

EVO is a small lipophilic molecule structurally similar to erastin, a known SLC7A11 inhibitor. Cellular thermal shift assay (CETSA) showed that both EVO and erastin increased xCT thermal stability, indicating direct target engagement (Fig. 5A–B).

To further characterize binding, molecular docking (AutoDock Vina) predicted that EVO binds a hydrophobic pocket within the xCT cytodomain (Gly349, Pro352, Gly353, Phe467, Lys472, Phe477, Ser481) with an affinity of -7.5 kcal/mol. This site overlaps with the binding position of distearoyl phosphatidic acid (PX8), with EVO showing higher predicted affinity (-7.5 vs. -5.0 kcal/mol). These

findings suggest that EVO may competitively displace PX8, destabilizing the xCT cytodomain and inhibiting SLC7A11 function.

3.7 SLC7A11 expression predicts poor prognosis in HNSCC

Since EVO may inhibit HNSCC cell growth mainly go through c-Fos/SLC7A11 pathway, we were interested to know whether SLC7A11 expression in HNSCC may influence patients' survival. We observed that SLC7A11 was highly expressed in advanced buccal cancer (Fig. 6A), and higher SLC7A11 expression (grade 2 and 3) was significantly correlated with poorer overall survival ($p = 0.017$) (Fig. 6B).

3.8 c-Fos regulates SLC7A11 in HNSCC

c-Fos, a core component of the activator protein-1 (AP-1) transcription factor complex, has been widely implicated in tumor progression by regulating genes involved in proliferation, survival, and stress responses. Previous studies have shown that c-Fos may either downregulate¹⁶ or upregulate¹⁷ SLC7A11 expression in different cancer models. Our IHC staining results showed that c-Fos protein was highly expressed in HNSCC PDX tumor (Fig. 7A) suggesting c-Fos may serve as transcription activator for *SLC7A11*. To demonstrate c-Fos transactivate *SLC7A11* in HNSCC. Furthermore, the *SLC7A11* promoter luciferase reporter assay demonstrated that EVO treatment in Fig. 7B. Through ChIP assay, we found the major c-Fos binding site at SLC7A11 promoter at -1829~-1602 site but not at -1200~-1015 area as shown in Fig. 7C. We also found c-Fos protein expression may correlate with SLC7A11 and *N*-acetylcysteine may attenuate EVO inhibitory effect in ferroptosis pathway in HNSCC as shown in Fig. 7D.

4. Discussion

4.1. Evodiamine induces ferroptosis by disrupting the SLC7A11-dependent redox axis in HNSCC

Ferroptosis is a key mechanism underlying the anticancer activity of evodiamine (EVO)^{4,5}. Prior studies show that EVO induces ferroptosis via GPX4 suppression, lipid peroxide accumulation, and disruption of TRIM26-mediated GPX4 stabilization, leading to ROS elevation and glutathione (GSH) depletion¹⁸. EVO has also been linked to tumor microenvironment modulation, potentially intersecting with ferroptosis-associated metabolic pathways¹⁹.

Here, we demonstrate that EVO induces ferroptosis in HNSCC by targeting SLC7A11, a central regulator of cystine uptake and redox homeostasis. EVO increased ROS accumulation, lipid peroxidation, and mitochondrial damage, consistent with ferroptotic cell death. It also enhanced sensitivity to chemotherapy and the GPX4 inhibitor RSL3, indicating reduced ferroptotic threshold.

The mechanism of SLC7A11 suppression by EVO remains to be fully defined and may involve both transcriptional and post-translational regulation. Further in vivo and clinical validation is warranted.

Overall, EVO emerges as a ferroptosis-inducing agent targeting tumor redox homeostasis in HNSCC.

4.2. EVO suppresses the c-Fos/SLC7A11 axis in a context-dependent manner

SLC7A11 transcription is tightly regulated by stress-responsive factors, including NRF2, ATF4, and p53^{20, 21}, with emerging evidence implicating AP-1 signaling²². We identify c-Fos as a positive regulator of SLC7A11 in HNSCC, where its suppression by EVO reduces SLC7A11 expression and promotes ferroptosis.

This contrasts with reports of c-FOS-mediated repression of SLC7A11 in other cancer types¹⁶, highlighting the context-dependent nature of AP-1 regulation. AP-1 complexes can act as transcriptional activators or repressors depending on dimer composition and chromatin context²³.

Our data support a model in which c-Fos directly activates SLC7A11 transcription in HNSCC, maintaining redox balance. EVO disrupts this axis, shifting cells toward oxidative stress and ferroptosis. Targeting the c-Fos/SLC7A11 pathway may therefore enhance ferroptotic susceptibility.

4.3. EVO engages xCT and converges on system xc⁻ inhibition

Our findings suggest that EVO functionally targets xCT (SLC7A11), converging on system xc⁻ inhibition. CETSA demonstrated stabilization of xCT upon EVO treatment, supporting target engagement.

Docking analysis predicted EVO binding within a hydrophobic pocket of the xCT cytodomain overlapping the PX8 binding site, with favorable affinity. Unlike erastin, which likely targets the transmembrane domain^{4, 24}, EVO may interact with a distinct structural interface.

Although further validation is required, these results support a model in which EVO impairs xCT function, limiting cystine uptake and GSH synthesis, thereby promoting lipid peroxidation and ferroptosis.

4.4. SLC7A11 expression is a poorly prognosis factor in HNSCC

SLC7A11 expression is a significant adverse prognostic factor in head and neck cancer, consistent with its role in maintaining redox balance and preventing ferroptosis²⁰. Elevated SLC7A11 may help tumor cells withstand oxidative stress within the tumor microenvironment, promoting survival, progression, and resistance to radiotherapy and chemotherapy²⁵. Increased expression has also been linked to aggressive clinicopathological features and poorer overall survival across multiple cancer types²⁶. Consistent with these findings, our data show that SLC7A11 is significantly overexpressed in advanced buccal cancer and is associated with poor prognosis. These results support SLC7A11 as a potential prognostic biomarker and therapeutic target, although further studies are needed to confirm its clinical value.

Abbreviations

ChIP: Chromatin immunoprecipitation

EVO: evodiamine

GPX4: glutathione peroxidase 4

GSH: glutathione

HNSCC: Head and neck squamous cell carcinoma

MDA: malondialdehyde

PDX: patient-derived xenograft

PX8: distearoyl phosphatidic acid

RSL3: (1S,3R)-RSL 3

WZY: Wu Zhu Yu

Declarations

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Author Contributions

CLH, HPL, and TLH designed the experiments, interpreted the data, prepared the figures, secured funding, and wrote the manuscript. CYL, CYH, and YLC performed the experiment and prepared the figures. JSL provided technical support for 3D docking. ACL and HCF performed the animal and cell line experiments. KWL, YKC, LYL, CHH, SFH, CYL, HMW, CLT, and CFL collected HNSCC clinical specimens and analyzed the clinical data.

Competing Interests

The authors have no competing interest to declare.

Ethics declarations:

This study was approved by the Institutional Review Board of CGMH (CGMH IRB No.201900135A3). Mice were maintained and handled in accordance with the Chang Gung Memorial Hospital (CGMH) Institutional Animal Care & Use Committee (IACUC) guidelines. Animal experiments were approved by the CGMH IACUC No. 2023072501.

Consent for publication: Not applicable.

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Figures

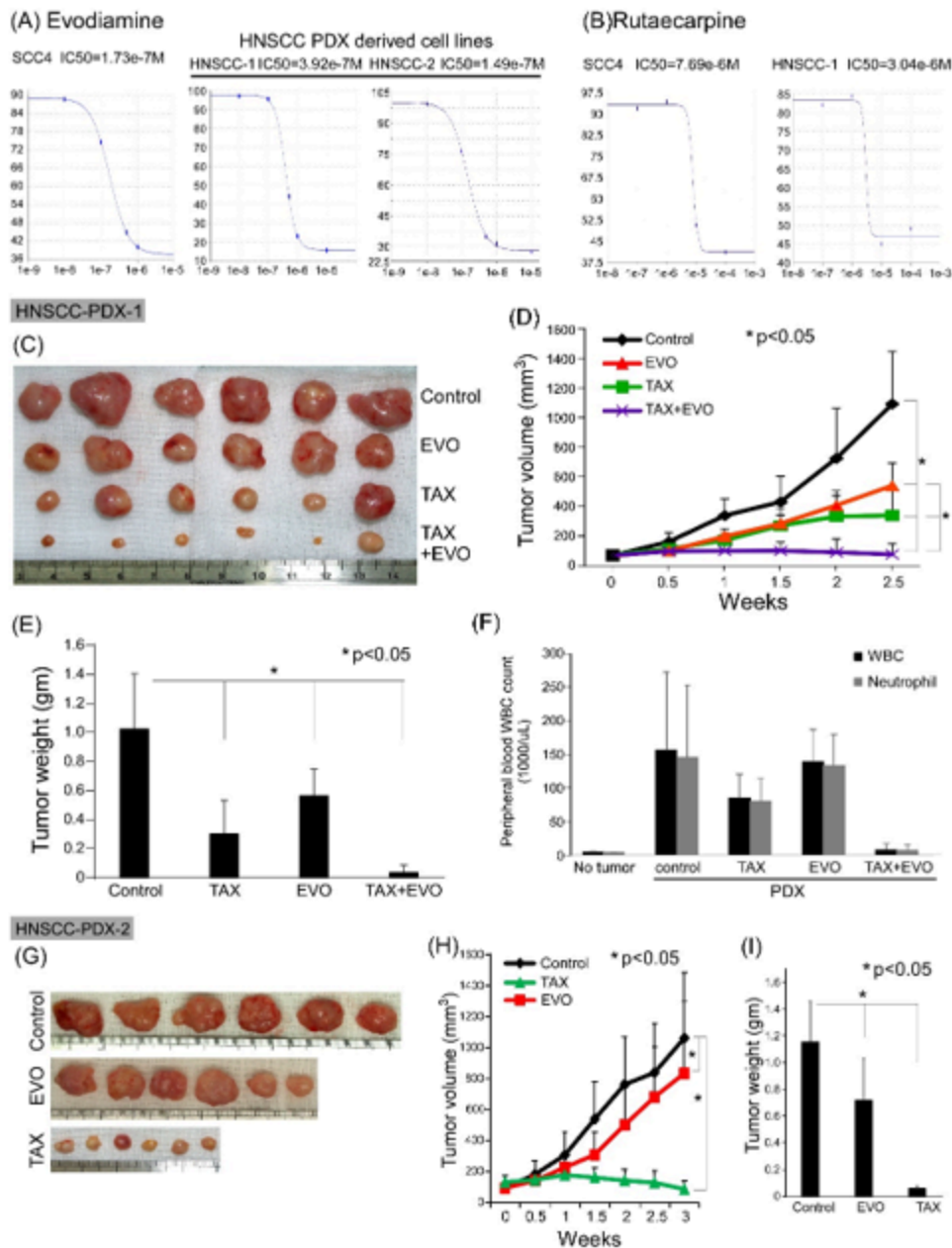


Figure 1

Figure 1

Evaluation of evodiamine (EVO) in HNSCC-PDX models and cell lines. (A) IC₅₀ values of EVO in HNSCC cell lines. (B) IC₅₀ values of rutaecarpine in HNSCC cell lines. Evaluation of EVO in HNSCC-PDX models: (C–F) HNSCC-PDX-1, including (C) representative gross tumors, (D) tumor volume, (E) tumor weight, and (F) peripheral blood white blood cell counts. (G–I) HNSCC-PDX-2, including (G) representative gross tumors, (H) tumor volume, and (I) tumor weight. Abbreviations: EVO, evodiamine; TAX, Taxotere.

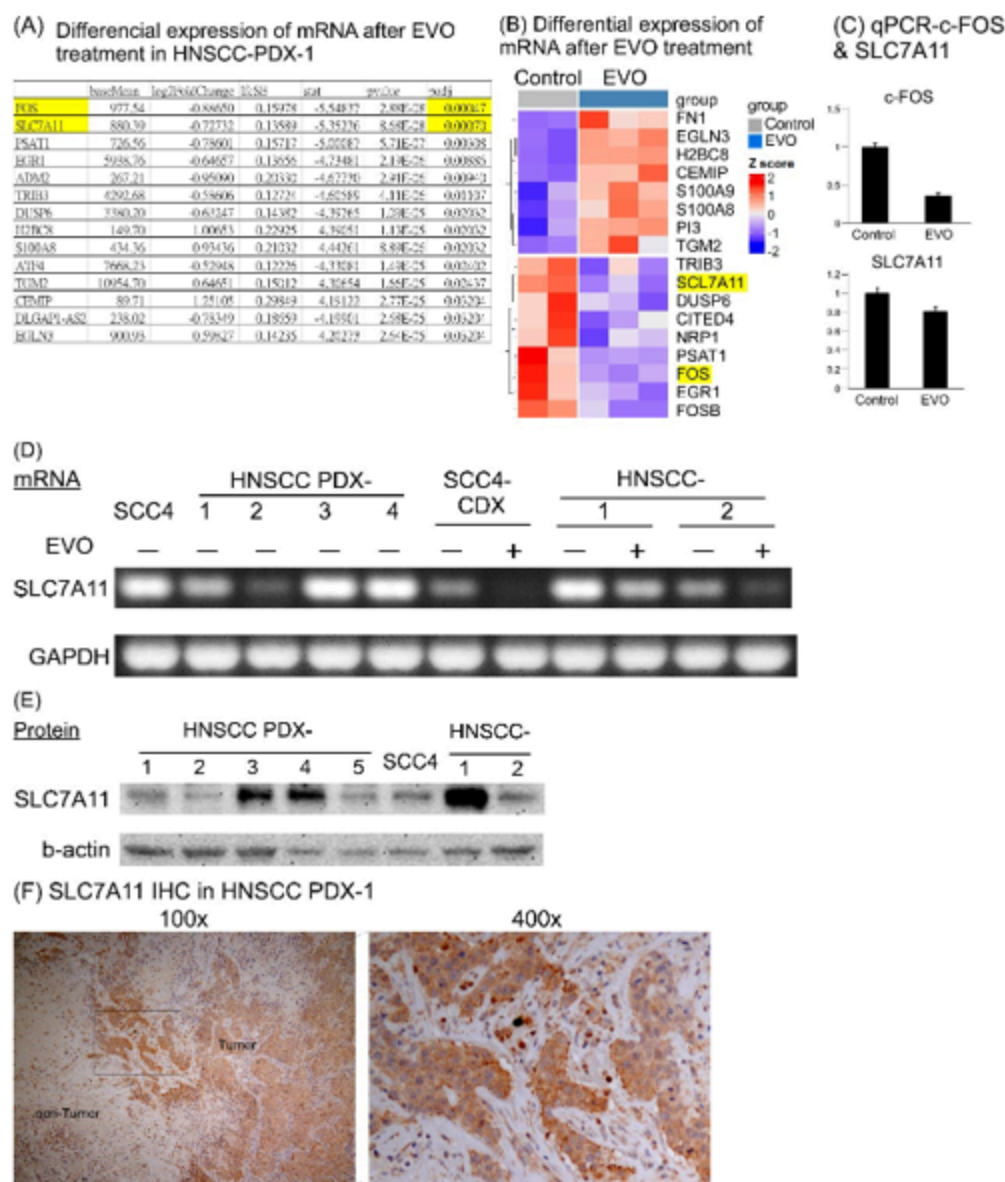


Figure 2

Figure 2

EVO treatment transcriptome analysis and SLC7A11 expression in HNSCC cell lines and PDX. (A) EVO treatment transcriptome analysis in HNSCC PDX-1 (B) Differential gene expression in EVO treatment (C) mRNA qPCR analysis in HNSCC PDX-1 after EVO treatment. SLC7A11 expression in HNSCC PDXs and cell lines in (D) mRNA expression, in (E) Protein, and in (F) IHC.

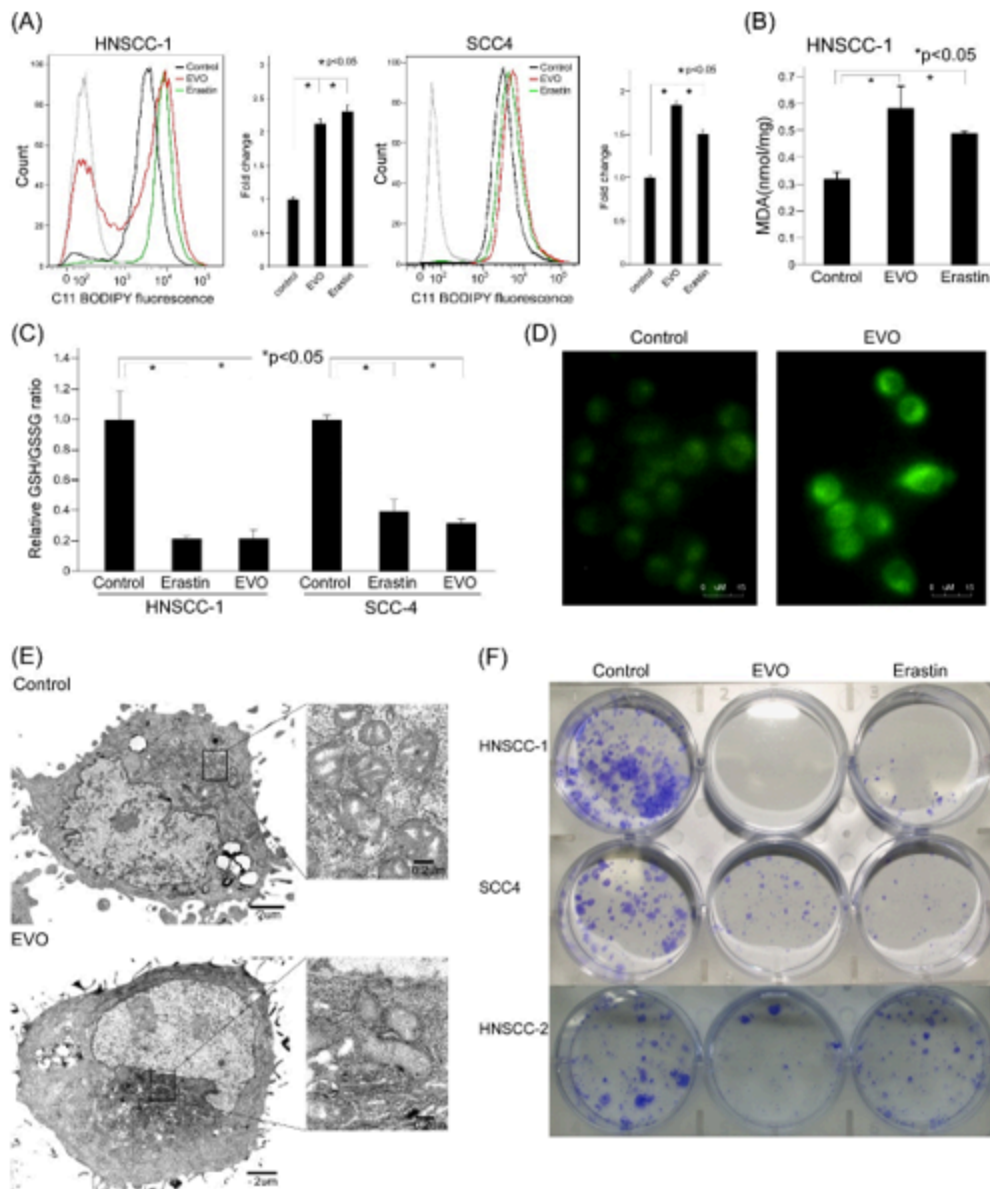


Figure 3.

Figure 3

EVO induce ROS and ferroptosis. (A) EVO induce intracellular ROS accumulation as assessed by flow cytometry. Cells were treated with evodiamine (red line) or erastin (green line), followed by staining with a ROS-sensitive fluorescent probe and flow cytometric analysis. C11-BODIPY581/591 (5uM) probe was used to detected lipid peroxidation level in HNSCC-1 and SCC4 cells in treating Erastin and EVO. (B) MDA assay in HNSCC-1 cell line. (C) GSH/GSSG ratio after EVO treatment in HNSCC cell lines. (D) Lipid ROS detected by confocal microscopy performed at ddH₂O or EVO (1 uM) in HNSCC-1 stained with Liperfluor probe (10 uM). (E) EVO treatment, mitochondria cristae decreased in HNSCC-1. (F) EVO inhibit HNSCC cell lines colony formation. Abbreviation: EVO, evodiamine.

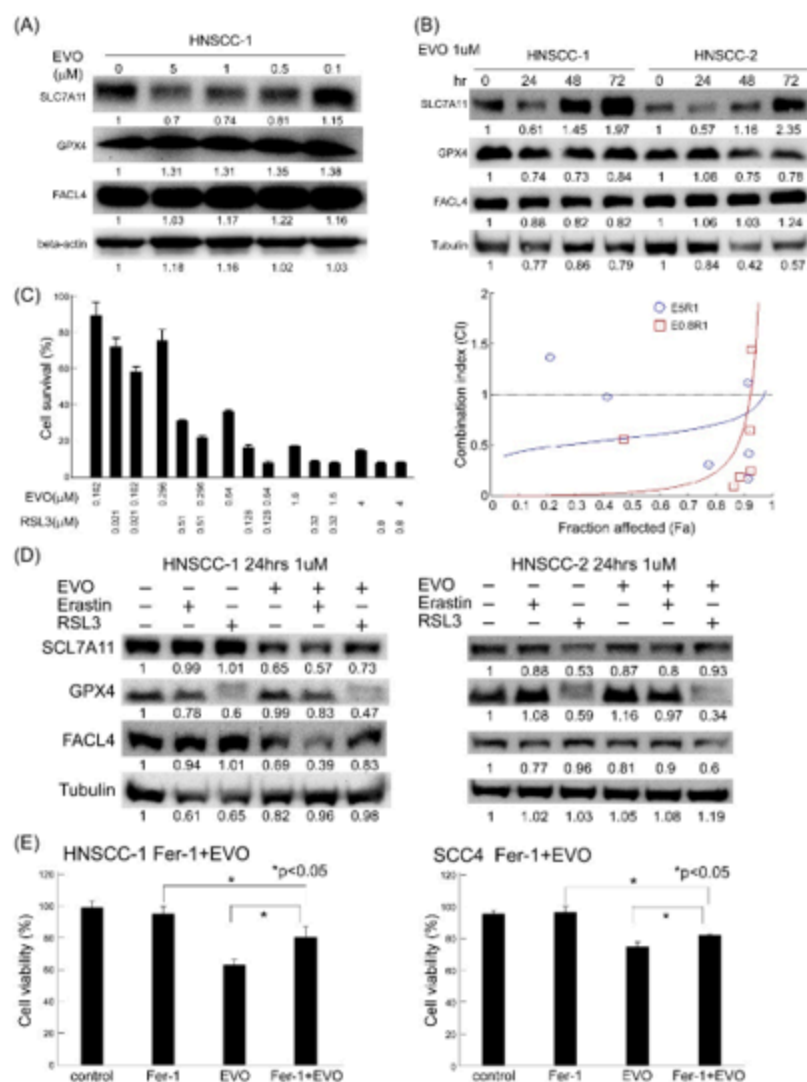


Figure 4

EVO may sensitize ferroptosis inducer to induce ferroptosis in HNSCC cell lines. EVO inducing ferroptosis index protein expression changes of concentration effect in (A) and time course analysis in (B). (C) EVO may sensitize RSL3 to induce ferroptosis in HNSCC-1. (D) Combination of EVO and ferroptosis inducers effect in ferroptosis index proteins changes. (E) Ferrostatin-1 may partly rescue EVO induced ferroptosis.

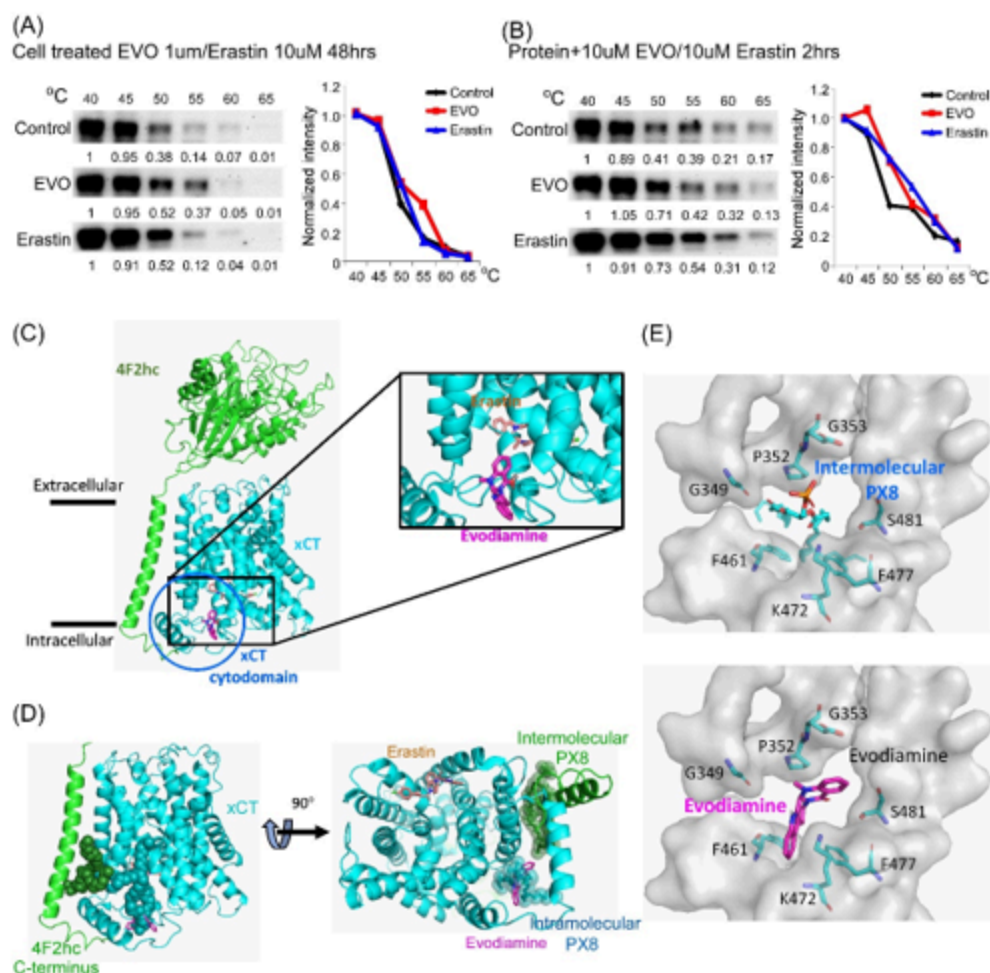
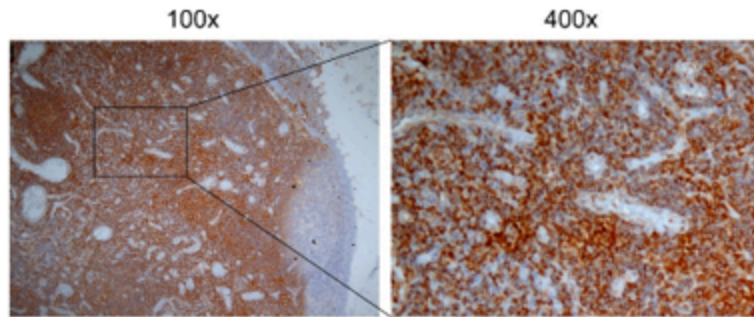


Figure 5.

Figure 5

EVO engages xCT in HNSCC. (A, B) Cellular thermal shift the assay (CETSA) confirmed that EVO targeted xCT. Cell treated EVO 1μM or Erastin 10μM for 48hrs in (A) and cell protein was extracted and incubated with 10μM EVO or 10μM Erastin for 2hrs in (B) before heat treatment. (C–E) Molecular docking of EVO within the erastin-bound 4F2hc-xCT complex. (C) EVO (magenta sticks) adopts a favorable binding pose (−7.5 kcal/mol) in the cytodomain of xCT (blue circle), overlapping the erastin-binding structure (orange sticks) located in the vestibule site. (D) EVO overlaps with intramolecular distearoyl phosphatidic acid (PX8). In the crystal structure, PX8 (dark green spheres) is positioned at the 4F2hc-xCT interface, while an additional PX8 molecule (dark cyan) binds the xCT cytodomain; the docked EVO overlaps with this intramolecular PX8 site. (E) Key cytodomain residues of xCT involved in interactions with PX8 (top) and EVO (bottom).

(A) HNSCC patient tissue



(B)

Buccal cancer, T0,1 vs T2,3

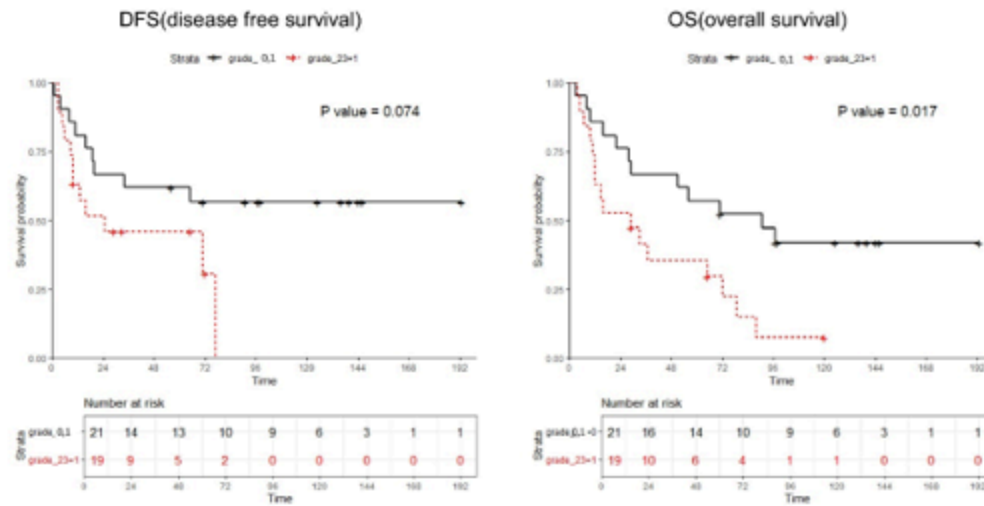


Figure 6

Figure 6

SLC7A11 expression was a prognostic factor in HNSCC patients. (A) SLC7A11 expression in HN cancer patient tissue (B) SLC7A11 expression correlated with poor overall survival in clinical buccal cancer.

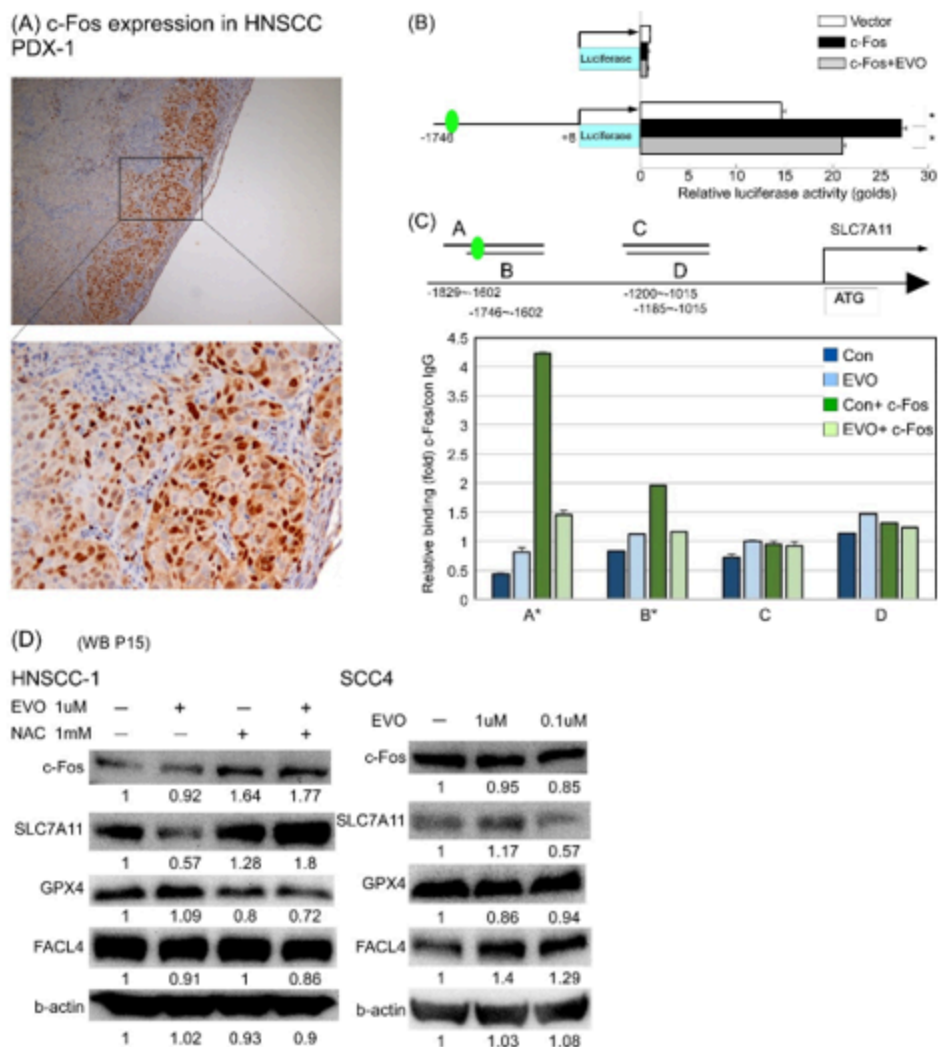


Figure 7.

Figure 7

c-FOS regulate SLC7A11 expression in HNSCC. (A) c-Fos expression in HNSCC PDX-1 (B) SLC7A11 promoter activity is increased by c-Fos overexpression and attenuated by evodiamine treatment. (C) ChIP-qPCR analysis showing that c-Fos binding to the SLC7A11 promoter is enhanced by c-Fos overexpression and attenuated by evodiamine treatment. (D) c-FOS protein expression correlated with SLC7A11 in HNSCC cell lines.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [QPCRforEVO.xlsx](#)
- [WBraw2.pdf](#)

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